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Original Research Paper

Acute Toxicity and Behavioural Changes in *Channa punctatus* (Bloch) Exposed to Rogor (An Organophosphorus Pesticide)

D. Sarma, J. Das and A. Dutta
Department of Zoology, Gauhati University, Guwahati-781 014, Assam, India

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ABSTRACT

Rogor is a commonly used pesticide in the agricultural field. The present study was undertaken to evaluate the lethal toxicity of Rogor, an organophosphorus pesticide, in freshwater fish *Channa punctatus* (Bloch) and relative behavioural changes after exposing for 96 hours. The regression equation calculated as y = 9.551x + 6.173 and the LC_{50} value calculated for *Channa punctatus* was at 0.75 mL/L. During exposure, fish showed faster opercular activity, erecting dorsal and ventral fin, huge secretion of mucus from whole body, etc.

INTRODUCTION

Human population growth and industrial development have been the major causes of water contamination around the world during recent years (Caussy et al. 2003). The ecological effects of pollutants in aquatic ecosystems and their bioavailability and toxicity are closely related to species distribution, both in the solid and the liquid phase of the aquatic ecosystem. (Ilavazhahan et al. 2010). The poisoning by pesticides from agricultural fields is a serious water pollution problem and its environmental long-term effect may result in the incidence of poisoning of fish and other aquatic life forms (Jothi & Narayan 1999). Pollution of aquatic environment from industrial, domestic and agricultural waste has exposed these important aquatic organisms to contaminants which not only threatened their lives but also eventually enter the food chain leading to serious public health hazards (Ilavazhahan et al. 2010). Unfortunately, the indiscriminate use of these pesticides to improve agricultural production and yield may have impacts on non-target organism, especially aquatic life and environment (Nwani et al. 2010).

The World Health Organization (WHO 1992) reported that roughly 3 million cases of pesticides poisoning occur annually, resulting in 220,000 deaths worldwide. The contamination of aquatic ecosystems by chemical pesticides has gained increasing attention and several recent studies have demonstrated the toxicity and effects to fish under field and laboratory conditions (Hassanein & Okail 2008, Nwani et al. 2010, Bhandare et al. 2011). Chronic exposure and accumulation of these xenobiotics by aquatic biota can result in biochemical and tissue burdens that produce adverse effects not only in the exposed organisms but also in human beings (IARC 1993). It seems essential to study the lethal toxicity and stress of such environmental pollutants so as to formulate the strategies for safeguarding aquatic organisms (Nwani et al. 2010).

Among different classes of pesticides, organophosphates are more frequently used, because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment (Bhandare et al. 2011). Many organophosphates are considered hazardous because of their ability to kill or immobilize various organisms in extremely low conditions. Indiscriminate use of different pesticides in agriculture to prevent the crop from pest peril has increased over the years, especially in the developing countries (Santhakumar & Balaji 2000).

Dimethoate, an organophosphate insecticide was first described by Hoegberg & Cassaday (1951) and introduced to the market in 1965 as common name Rogor (Bhandare et al. 2011). These insecticides produce toxicity by inhibition of the enzyme acetyl cholinesterase, which accumulates in the synapses of the central and peripheral nervous system. This in turn results into overactivation of postsynaptic cholinergic receptors and signs of cholinergic neurotoxicity (Srivastava et al. 2010). Dimethoate is highly soluble in water and can leach into nearby water sources and affect aquatic organisms. Rogor is used for the control of chewing and sucking pests of cereals, pulses, fibre crops, vegetables, oilseeds, fruits and plantation crops, particularly against aphids, jassids, thrips, whitefly, scale, leaf miner, leaf hopper, etc. It is a widely used insecticide by farmers of different agricultural fields.

Fishes are very sensitive to a wide variety of toxicants in water; various species of fish show uptake and accumulation of many contaminants or toxicants such as pesticides (Herger et al. 1995). Accumulation of pesticides in tissues produces many physiological and biochemical changes in the fishes and freshwater fauna by influencing the activities of several enzymes and metabolites (Nagarathnamma & Ramamurthi 1982). Toxicity studies have played an important role in man's efforts to monitor and modify the effects of his activities on the biota (Bhandare et al. 2011). In aquatic toxicology LC_{50} may be defined as the concentration of a compound that causes lethality of 50% of the exposed individuals (Wolf 1992). Fish serves as a bioindicator species as it responds with great sensitivity to changes in the aquatic environment and thus, has an important role in the monitoring of water pollution (Srivastava et al. 2010).

The present investigation is aimed to examine the acute toxicity and effects of organophosphorus pesticide (Rogor) on freshwater fish *Channa punctatus* by determining the LC_{50} values and analysing behavioural changes due to its toxic effects. The study will help further in estimating the safe level dose as well as strengthening the baseline data that could be used to find out comparative sensitivity of these pesticides.

MATERIALS AND METHODS

Healthy specimen of *Channa punctatus* (total length - 13.2 to 13.6 cm and weight - 37.2 to 37.9 g) were collected from the Urpod beel of Goalpara district, Assam. They were first treated with 0.5% $KMnO_4$ solution for two minute then transferred to an aquarium (952mm × 458mm × 405mm) for two weeks for acclimatization to laboratory conditions. During this period the fish were fed with boiled chicken eggs and mustered oil cake at the ratio of 1:2 on alternate days. No food was given at the time of toxicity test. To provide natural environment to the fish, all effort was made as far as possible in the laboratory.

To determine the probable range of concentration, a series of experiments were conducted prior to the acute toxicity test. Eight concentration of Rogor each with three replications plus one control were used for the test. The whole experiments were carried out in a set of nine plastic tubs (500 mm in diameter) with constant aeration (by two way aqua-pump). Test concentrations were prepared by diluting appropriate amount of test chemical with 20 litres of tub water (pond) 4 hours before the addition of fish. Before and during the experiments, certain important physico-chemical characteristics of test water *viz.*, temperature, pH, DO, FCO_2, alkalinity, acidity, hardness and chloride were measured frequently following the methods of Trivedy & Goel (1986) and APHA (1989).

Table 1: Results of probit analysis against the test concentrations.

Concentration (mL/L)	Log_{10} of Concentration	Total No.	No. of dead	(%) Mortality	Probit value
0.57	-0.244	10	1	10	3.72
0.63	-0.201	10	2	20	4.16
0.69	-0.161	10	4	40	4.75
0.76	-0.119	10	6	60	5.25
0.82	-0.086	10	7	70	5.52
0.88	-0.056	10	7	70	5.52
0.96	-0.018	10	8	80	5.84
1.04	0.017	10	10	100	-
0.00	Control	10	0	0	0

Table 2: Water quality parameters during pre and post experimental period.

S. No.	Parameter	Mean ± SD
1.	Temperature (°C)	28.3 ± 1.2
2.	pH	7.8 ± 0.2
3.	DO (mg/L)	9.86 ± 0.18
4.	FCO_2 (mg/L)	0.81 ± 0.13
5.	Total Alkalinity (mg/L)	108 ± 2.3
6.	Total Acidity (mg/L)	9.4 ± 0.3
7.	Total hardness (mg/L)	58 ± 0.9
8.	Chloride (mg/L)	7.02 ± 0.02

A set of 10 numbers of fish were exposed to different concentrations of test chemical under normal day/light condition. The experiment was conducted in the laboratory during March 2010. The range finding tests were also conducted at the same plastic tub, in the same month and with same water quality. Fish were exposed for 96 hours and frequent monitoring was made to observe mortality. A fish was considered dead when no response observed after prodding with a glass rod. Dead fish were immediately eliminated from the test tub. No mortality was found in control test tub. However, it was also proved that an increase in concentration of test chemical was required as a lethal dose for *Channa punctatus*. Total mortality of fish was recorded after exposure of 96 hours. Final result was calculated by taking mean mortality from a particular dose and its replicates. LC_{50} value was calculated from the data obtained in acute toxicity test by Finney's method (1971) of probit analysis and with computer statistical software.

RESULTS AND DISCUSSION

Acute toxicity data have been used to derive water quality guidelines for regulatory measures (Sunderam et al. 1994).

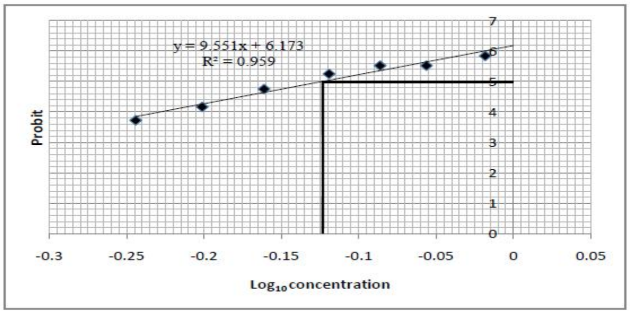

Fig. 1: The relationship between probit of kill and $\log_{10}$ concentrations of Rogor to find the LC_{50}.

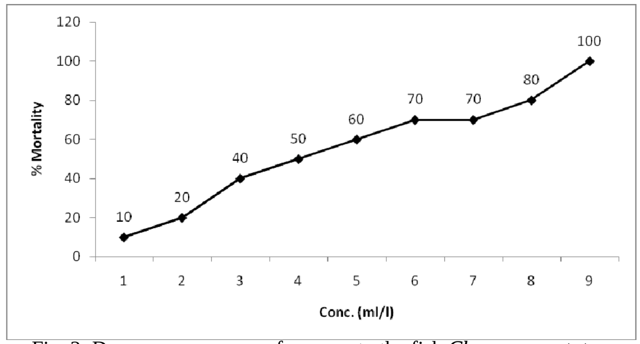

Fig. 2: Dose response curve for rogor to the fish *Channa punctatus.*

The mortality of *Channa punctatus* at different concentrations of Rogor along with the probit values are summarized in Table 1. After plotting the $\log_{10}$ concentration against each probit value, the regression equation observed as $y = 9.551x + 6.173$ (Fig. 1). Thus, the results of the LC_{50} (median lethal concentration) of the present study at 96 h was 0.75 mL/L for Rogor. In other two similar experiments, the LC_{50} value for freshwater fish *Puntius stigma* exposed to Rogor for 96 hrs was recorded at 7.1 ppm (Bhandare et al. 2011), at 11.34 mg/L for *Heteropneustes fossilis* (Srivastava et al. 2010). The value for monocrotophos on the juveniles of rohu and mrigal were found to be 46.34 and 42.33 ppm respectively (Sulekha et al. 1999). The LC_{50} of dimethoate for 96h exposure to the fish Nile tilapia (*Oreochromis niloticus*) was 40 mg/L (Sweilum 2006). Schimmel et al. (1976) concluded that it is difficult to compare the toxicity of individual insecticides to different species of fish because they are influenced by several factors like temperature, hardness, pH and dissolved oxygen content of the test water. The LC_{50} values obtained for metasystox on *Nemacheilus botia* exposed for 96 h was at 7.018 ppm (Nikam et al. 2011). The median lethal concentration (LC_{50}) values of Diazinon 60 EC on *A. testudineus, C. punctatus* and *B. gonionotus* were reported at 6.55, 3.09 and 2.72 ppm for 96 hrs of exposure (Rahman et al. 2002).

It is important to consider the physico-chemical characteristics of test medium along with biotic factors to know the mechanisms affecting LC_{50} concentrations of fish in toxicity tests (Ilavazhahan et al. 2010). The water used for the experiments was analysed during pre and post experimental period. The overall fluctuation ranges of water quality are summarized in Table 2. The water temperature varied from 28.5°C to 29.0°C, pH 7.9 to 8.0, while dissolved oxygen ranged from 9.83 mg/L to 10.06 mg/L. The free CO_2 concentration ranged from 0.99 mg/L to 1.1 mg/L, total alkalinity values varied from 110 mg/L to 120 mg/L, total acidity ranged from 9.9 mg/L to 11.0 mg/L, while total hardness varied from 59 mg/L to 61 mg/L during the experimental period. On the other hand, chloride value was observed between the ranges of 7.06 mg/L and 7.10 mg/L. All the test fish were found live during the experimental period.

All the parameters were within the permissible limit of fish survival (Saha 1996). Though temperature, hardness, pH, alkalinity, sex, age and other physiological status of the test animals were reported to have profound effects on the toxicity of agro-chemicals (Rand & Petrocelli 1985), it is suggested that the parameters did not seem to alter the toxicity of the insecticide to the test fish. Increase in water temperature enhanced the uptake of metals by the aquatic organisms (Witeska & Jezierska 2003). Water-borne metals generally exhibit their greatest toxicity to aquatic organisms in soft waters of low pH (Ilavazhahan et al. 2010).

Behavioural changes as a result of stress are further accepted as the most sensitive indication of potential toxic effects (Nwani et al. 2010). In the present investigation, fish were observed moving fast and trying to escape or avoid the toxic water. They frequently came to the upper surface and sometimes jumped. Faster opercular activity, erecting dorsal and ventral fin were observed. Hassanein & Okail (2008) observed loss of buoyancy and balance, with an initial increase in the opercular ventilation rates which then decreased significantly in *Ctenopharyngodon idella* after exposure to neem biopesticide. They were found to secret a huge amount of mucus from the whole body forming a thick layer. Similar symptoms were also seen by Pandey et al. (2005) and Chandra (2008) in fishes exposed to various pesticides.

Body pigmentation was decreased. Kopruçu et al. (2006) also observed lightening of skin colour in fingerling of European catfish exposed to organophosphorus pesticide diazinon. Similar findings were also made in three fish exposure to Diazinon 60 EC for 96 hrs (Rahman et al. 2002). Sometimes, they rubbed each other though bending the whole body. After few minutes, movement decreased with sudden jumping. According to Fulton & Key (2001), the restlessness and hyperactivity in fish may occur due to the inactivation of acetylcholinesterase, leading to accumulation of acetylcholine at synaptic junctions. Later, they were almost paralysed and observed lying horizontally at the bottom with head downwards. Finally, they settled on the bottom of the tub and died.

The results of the present study showed that Rogor (dimethoate) was more toxic to *Channa punctatus* (Bloch) which induced acute toxic effects in the form of behavioural changes in fish. The study will also help in formulating the safe level dose of such type of organophosphorus pesticide. Thus, the use of Rogor should be strictly controlled and regulated by forming suitable legislation to prevent its bioaccumulation in the environment which will reduce negative impacts on aquatic organisms.

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