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Toxicity and Behavioral Changes in Fresh Water Fish *Puntius stigma* Exposed to Pesticide (Rogor)

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Abstract: The present study has been designed to determine the LC_{50} values of Rogor an insecticide and relative behavioral changes in fresh water fish *Puntius stigma* exposed for 24, 48, 72 and 96 hrs. The LC_{50} values for *Puntius stigma* were found at 9ppm, 8.31ppm, 7.8ppm and 7.1ppm respectively. Fish show increased opercular moment, loss of equilibrium, increased surface activity, over secretion of mucous, irregular swimming activity, rapid jerky movements and aggressiveness were observed.

Key words: Toxicity · Rogor · Behaviour · Puntius stigma · Sukhana River

INTRODUCTION

Indiscriminate use of different pesticides in agriculture to prevent the crop from pest peril has increased over the years especially in the developing countries [1]. These pesticides even when applied in restricted areas are washed and carried away by rains and floods to large water bodies like ponds and rivers and there by alter the physicochemical properties of water [2] this proved to the highly toxic, not only to the fishes but also to other organism which form food of the fishes [3].

Toxicity studies have long played an important role in man's efforts to monitor and modify the effects of his activities on the biota. The toxicity studies are especially useful in determining the sensitive species of an ecosystem that can be used as indicator species, for a particular type of pollution. The results of toxicity are generally reported in terms of median lethal concentration LC_{50} or median tolerance limit (TLM).

The major groups of insecticides in common use are the organophosphorous compounds. Many of organophosphates are considered hazardous because of their ability to kill or immobilize various organisms at extremely low conditions [4]. The pesticides are found to be highly toxic not only to fish but also to other organisms which constitute food of the fishes. According to WHO estimate nearly three million cases of pesticide poisoning occur annually. Organophosphates (OP) are one of the most preferred pesticides due to their effectiveness and low persistence in the environment. OP pesticides directly inhibit acetylcholinesterase enzyme activity in fishes and invertebrates [5-7]. Dimethoate, an organophosphate insecticide was first described by Hoegberg and Cassaday in 1951 and introduced to the market in 1956. The OP compounds are esterase inhibitor neurotoxicants, with acute cholinergic effect preceded by inhibition of acetelcholinesterase.

Several workers have investigated the toxicity of organoposphorous pesticides in fish and other organisms [8 to 14].

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. Organophosphates accounted for 70% of the total insecticides used in US in the year 2001 (URL2). Dimethoate (IUPAC Name- 0, o dimethyl S- (N methyl carbamoylmethyl) phosphoro-dithioate), CAS No. 60-51-5, is an organophosphate available in the market by the trade name of rogor. It is a systemic insecticide used for control of a wide variety of insects pest of fruits, vegetables and crop plants. Dimethoate is highly selective

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as insecticide because relative rate of degradation of toxicant by enzymes (esterase and amidases) are very low in insects as compared with those of mammals [15]. Like other organophosphates, rogor is also an acetycholinesterase inhibitor (URL3), therefore, works primarily as nerve poison which is reflected in uncoordinated abnormal behavior of the soon after exposure to pesticide. Dimethoate is acutely toxic and is classified as a possible human carcinogen by USEPA based on occurrence of tumors in mice (URL4). In the WHO acute hazard ranking this is rated as moderately hazardous (URL5). USEPA has registered dimethoate as a systemic organophosphate insecticide but in 2006 it released Interim Re-registration Eligibility Decision (IRED) document for dimethoate in accordance with FQPA requirements (url6).

The present paper deals with the toxicity and behavioural changes in *Puntius stigma* exposed to lethal concentration of Rogor.

MATERIALS AND METHODS

The live freshwater fishes *Puntius stigma* were collected from Sukhana river, 35 km away from Aurangabad district (M.S) India and brought to the laboratory. The fishes were fed with live pieces of Earthworm every alternate day and allow acclimatizing in the laboratory conditions in large aquaria for 15 days prior to the experimentation. Water was renewed every day to provide fresh water rich in oxygen.

The fishes were (average weight 10 gm and length 9.5 cm.) selected for LC_{50} determination. Preliminary experiment at different concentration of Rogor was conducted to find concentration (ppm) that resulted in 50% mortality in given time. The organophosphate pesticide Rogor used in the present investigations manufactured by Sanjay insecticides Pvt. Ltd dist. Jalna (M.S). The LC_{50} values were calculated according to [16]. The physico-chemical characteristics of test water have been analyzed during experimentation [17].

A batch of 10 fishes was also maintained along with experimental fishes as control group. The changes in behaviour of *Puntius stigma* exposed to Rogor pesticide were critically observed during experiment.

RESULTS AND DISCUSSION

The results revealed that the water used for experiment did not contain any toxic substance. Initially no mortality was observed in control group. Fishes exposed to lethal concentrations of Rogor for a short-term exposure were studied in terms of behavioural, rate of survival and mortality.

The LC_{50} values of freshwater fish, *Puntius stigma* exposed to Rogor for 24, 48, 72 and 96hrs have been recorded at 9 ppm, 8.31ppm, 7.8 ppm and 7.1ppm respectively. The LC_{50} values, regression results have calculated to support present observations in Table 1.

In the present investigation LC_{50} values were decreased at 96 hrs and found to be increased during the exposure period of 24 hrs. So the values were found at the highest towards 24 hrs and the lowest at 96 hrs of exposure.

The LC_{50} values were determined using different concentrations of rogor for fishes. The 24, 48, 72 and 96 hrs. LC_{50} of rogor to *Puntius stigma* was 9ppm, 8.3ppm, 7.8 ppm and 7.1 ppm respectively. The LC_{50} values of Dimethoate for certain air-breathing fishes are reported to be very high, as in *Clarias batrachus*, [18]. It is 65 mg/l for 96 h, whereas in Heteropneustes fossilis [19]. In *Channa punctatus* it is 17.9 mg/l for 96 h. Very low LC_{50} value for 24, 48, 72 and 96 h dimethoate exposure is recorded as 3.38, 3.23, 3.08 and 2.98 mg/l [20]. The LC_{50} values observed in *Channa punctatus* was 8.5 ppm and for 96 h LC_{50} it was found 5.248ppm [21].

When first set of solution was started, the 50% fishes were killed in 24 hrs. of the exposure. While in second phase of solution the 50% fishes have been killed in 48 hrs. of exposure. Similarly in the third stage of set about 50% fishes were killed in 72 hrs of exposure.

Table 1: LC_{50} values for freshwater fish *Puntius stigma* after exposure to insecticide Rogor for a period of 24, 48, 72 and 96 hrs

Exposure period in hrs.	Regression equation	LC_{50} values in % concentrations	Calculated LC_{50} values in % concentrations	Variance	Chi-square X^2	Fiducial limit up to 95 % confidence	
						M1	M2
24 hrs	Y=11.2266373 X -5.00094227	9	9	0.00026081	0.00572734	0.92232061	0.98562741
48 hrs	Y=7.95149167 X -5.00225297	8.31	8.3	0.00025651	0.14220305	0.87497733	0.96492473
72 hrs	Y=9.24280544 X -5.00811372	7.8	7.9	0.000433447	0.03248002	0.85734784	0.93896244
96 hrs	Y=12.0156952 X -5.01063729	7.1	7.2	0.00023104	0.11853853	0.82769215	0.88727624

Where as at the fourth set of the solution 50 % mortality were found in 96 hrs. of exposure respectively. In general the insecticide could affect the behavior of exposed fish, minimize survival and store in the tissues of the test animals.

The exposure of fish to different concentrations of rogor shows altered behavioral responses, The restlessness and hyperactivity in fish may occur due to the inactivation of acetylcholinesterase, leading to accumulation of acetylcholine at synaptic junctions [5]. Stimulation of peripheral nervous system which results into increased metabolic activities. Disruption of schooling behaviour of the fish, due to the lethal and sublethal stress at the toxicant, results in increased swimming activity and entails increased expenditure of energy [22]. The abrupt erratic and jerky swimming may be due to inhibition of the acetylcholinesterase in the brain and neuromuscular junctions [6-7].

The opercular movement in fish has been reported to increase following the exposure of toxins [23-25]. Contrary to it, the opercular movement in fish showed a marked decrease on dimethoate [20].

Fishes exposed to lethal concentration of Rogor at 9 ppm for 24 hrs, at first fishes showed increased opercular movement. This could be due to clearance of the accumulated mucus debris in the gill region for proper breathing. At the inception the fishes suffocate and used to come at the surface for gasping the air. The fishes were avoiding toxic water with fast swimming and jumping faster. Presence of over secretion of mucous on the body was found and irregular swimming activity was noticed.

The behavioral changes are the obvious of the motivational biochemical, physiological and environmental influence state of the fishes. An erratic swimming of the treated fishes showed loss of equilibrium. It is prove that the region in the brain associated with the maintenance of equilibrium should have been affected [26]. The erratic swimming, rapid jerky movements and convulsions before death were evident and the serve asphyxiation as indicated by gasping to death [27]. This has been laid to the conclusion that from the present study fishes were highly sensitive to the insecticide Rogor.

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