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Biochemical changes in Air-breathing fish, Anabas testudineus (Bloch) exposed to the Pesticide Rogor (Dimethoate)

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Abstract
In this paper the study has been designed to determine the LC_{50} values of Rogor an insecticide and relative behavioral changes in fresh water fish *Anabas testudineus* (Bloch) exposed for 24, 48, 72 and 96 hrs. The LC_{50} values for *Anabas testudineus* 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.

Keywords: Biochemical changes, Bloch, Anabas testudineus, Dimethoate

Introduction
The pesticide application has greatly contributed to meet with the growing demand of food through enhancement of agricultural yields. Excessive use of broad spectrum or non-selective pesticides damages the ecosystem, sometimes irreversibly, contaminate soil surface and groundwater as well as fodd chains and thus compromises the health and well-beings of aquatic and surrounding terrestrial environments (Bhat *et al*, 2012; Rita *et al*, 2006; Veeraiah, 2012) [2, 12, 15]. The toxic discharge from industries, different mining, and agricultural developments and processing find their way to the aquatic environment and may have detrimental effects on the inhabitants (Hamilton and Mehrle, 1986). Pesticides are one of the dangerous pollutants which cause great harm to the animals present in the aquatic environment including fishes.
The toxicity of a pesticide is altered in commercial form and the fish susceptibility also frequently influenced by environmental factors. The knowledge about lethal concentration of any pesticide it is easier to protect its discharge above the limit. Also, toxicity of pesticide may be classified as extreme, high, moderate, slight and relatively harmless chemicals.
Organophosphate compounds comprise insecticides currently used worldwide for agricultural and household applications. These insecticides produce toxicity by inhibition of the enzyme Acetyl cholinesterase accumulates in the central and peripheral nervous system, which, in turn results into over-activation on post-synoptic cholinergic receptors and produce neurotoxicity (Barat and Bahadur, 2014; Muthukumarawel *et al.*, 2013; Pandey *et al*, 2014) [3, 10]. The organophosphates are more frequently used due to their high insecticidal property, low mammalian toxicity, less persistence and low biodegradation in the environment. Dimethoate is an organophosphate with effect through contact or systemic circulation. The fish serves as a bio-indicator as it responds with great sensitivity to changes in the aquatic environment and thus also had important role in the monitoring of water pollution (Ahmad, 2012; Binukumari and Basanthi, 2013) [1, 4]. The recent and notable work published on the effects of organophosphate on various aspects of fish are those of Das and Mukherjee (2003) [5]; Shrutis and Tantarpale (2016); Tripathi and Rajesh (2015) [14]; Rajani and Revathy (2015) [11].
This investigation will be carried to evaluate the acute toxicity of an organophosphate pesticide-Dimethoate for the carp fish, *Anabas testudineus* due to their easy availability in local waterbodies and their long survival in an aquarium. This is a common edible fish inhabited several type of reservoirs. The insecticide Dimethoate will be selected for study due to its wider application in crop fields. This pesticide is also highly soluble in water and can leach into nearby water sources to affect aquatic organisms. This is a low persistence pesticide with half-life of 4-16 days but lasting also variable with medium conditions.

The test fish will be procured and acclimatized in the laboratory. The water quality parameters will evaluate as methods of APHA (2012), and, lethal toxicity for different intervals as standard methods described in monographs.
The biochemical parameters like blood glucose, plasma protein, Serum cholestral, Glutamate Pyruvate Transaminase and Glutamate Oxaloacetate Transaminase will be evaluated using assay kits supplied by scientific emporium, Gorakhpur. The histo-pathological studies for estimation of Glycogen, Protein, Lipid and Cholestral (Pearse, 1995; Bancroft *et al.*, 1994) will be performed in liver, kidney, testes and ovary. The estimation of Alkaline and Acid phosphatase, Pyruvic acid and lactic acid will also be performed (Bancroft *et al.*, 1994).
The one way analysis of variance (ANOVA) was applied to test the significance of difference among the control and experimental values. The P values which were less than 0.5 considered statistically significant.

Materials and Methods
The live freshwater fishes *Anabas testudineus* were collected from river, 35 km away from Siwan district. 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 LC_{50} values were calculated according to the physico-chemical characteristics of test water have been analyzed during experimentation.

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, *Anabas testudineus* 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.

Table 1: LC_{50} values for freshwater *Anabas testudineus* 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'	Fiducial limit up to 95 % confidence
						Ml M2
24hrs	Y=11.2266373 X - 5.00094227	9	9	0.00026081	0.00572734	0.92232061 0.98562741
48hrs	Y=7.95149167 X - 5.00225297	8.31	8.3	0.00025651	0.14220305	0.87497733 0.96492473
72hrs	Y=9.24280544 X - 5.00811372	7.8	7.9	0.000433447	0.03248002	0.85734784 0.93896244
96hrs	Y=12.0156952 X - 5.01063729	7.1	7.2	0.00023104	0.11853853	0.82769215 0.88727624

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. Stimulation of peripheral nervous system which results into increased metabolic activities. Disruption of schooling behaviour of the fish, due to the lethal and sub lethal stress at the toxicant., results in increased swimming activity and entails increased expenditure of energy. The abrupt erratic and jerky swimming may be due to inhibition of the acetylcholinesterase in the brain and neuromuscular junctions.
The opercular movement in fish has been reported to increase following the exposure of toxins. Contrary to it., the opercular movement in fish showed a marked decrease on dimethoate.

Conclusion
In this Study 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.
In this Study 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. The erratic swimming, rapid jerky movements and convulsions before death were evident and the serve asphyxiation as indicated by gasping to death. This has been laid to the conclusion that from the present study fishes were highly sensitive to the insecticide Rogor.

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