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Mortality and behavioral changes in *Channa punctatus*, a freshwater air-breathing fish, exposed to Rogor

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Abstract
Rogor is a highly soluble and low persistent pesticide of organophosphate group. The aim of the current experiment was to investigate the toxicity and behavioral alterations in *Channa punctatus* when exposed to different concentrations. First mortality was observed in 48 hours when fishes were exposed to concentration of 0.6ml/20L. 100% mortality in *Channa punctatus* was observed after 96 hours of exposure at concentration of 1ml/20L. Some behavioural alterations such as erratic movement, hyperactivity, loss of pigmentation and equilibrium, and increased surface activity were recorded in the fishes exposed to different concentrations of Rogor. LC_{50} value of Rogor was determined as 0.74ml/20L. These behavioural alterations were due to the toxic effect of Rogor or may be due to stress or inactivation of specific enzyme acetyl cholinesterase activity.

Keywords: Concentration**,** *Channa punctatus*, Mortality, LC_{50}, Rogor, Behaviour alterations

Introduction
Water pollution is a global issue and one of the most critical challenges facing humanity today. The contamination of water bodies primarily occurs due to the discharge of industrial effluents, domestic waste, and various pesticides (Pandey *et al*., 2011) [12]. The advancement of human civilization, coupled with rapid population growth, has increased the demand for food, comfort, and infrastructure. This has led to significant industrial and agricultural expansion, as well as relentless urbanization (Singh, 2007) [15]. Chemicals such as pesticides, generated through agricultural activities, are often released directly or indirectly into water bodies. The environmental impact of these chemicals is a worldwide concern (Khan and Law, 2005) [9].
In India, around 70% of agricultural chemical formulations are thought to negatively affect non-target organisms and ultimately end up in freshwater systems (Bhatnagar *et al*., 1992) [1]. Pesticides are widely used globally to control agricultural pests. According to the National Institute of Health Sciences, a pesticide is any substance used to destroy, repel, control, or prevent plants and animals considered pests (NIEHS, 2021) [19]. Common types of pesticides include fungicides, herbicides, insecticides, rodenticides, and bactericides, each designed to target specific pests. However, over 98% of insecticides and 95% of herbicides do not reach their intended targets, as they are dispersed over large agricultural areas (Miller, 2004) [17].
Pesticides pose significant risks not only to the environment but also to human health. Research has established links between pesticide exposure and various health issues, including cancer, Alzheimer's disease, ADHD, and birth defects (Kalita, 2022) [8]. Many pesticides used in agriculture are washed into water bodies, degrading water quality and causing structural and functional changes in aquatic life. Water quality and habitat conditions are critical factors affecting fish survival and productivity. The toxicity of pesticides to fish is particularly concerning, as contaminated fish can have adverse effects on human health (Chaudhari & Saxena, 2021) [2].
Fish are considered bioindicators due to their high sensitivity to changes in aquatic environments, making them essential for monitoring water pollution (Srivastava *et al*., 2010) [14]. As secondary consumers in the food chain, fish play a vital role, especially since they are a major food source for higher trophic levels.

Fish are highly susceptible to a wide range of waterborne toxicants, with many species absorbing and accumulating contaminants such as pesticides (Herger *et al*., 1995) [7].
The current study focuses on evaluating the acute toxicity and effects of Rogor, an organophosphate pesticide, on the freshwater fish *Channa punctatus*. The study aims to determine the LC_{50} value (lethal concentration) and observe behavioral changes in the fish exposed to this pesticide.

Materials and Methods

Experimental Chemicals

Rogor with product name Roguish (Agrichem India Pvt. Ltd.), was purchased from the local market and used for this experiment. It is pesticide of organophosphate Dimethoate group. It is highly soluble and low persistent pesticide in water and can reach into nearby water bodies to affect aquatic organisms.

Experimental Animals:

For the study live healthy specimens of fresh water air breathing fish *Channa punctatus* (Total length 13.5 – 16.5cm and body weight 30-40g) were collected from local fisher man of Bhagalpur, Bihar and brought to the laboratory. *Channa punctatus* is a snake headed freshwater air breathing fish belong to the family Channidae and order Channiformes. It is locally known as Garai, and are mostly found in the fresh water pools & wetlands like ponds, ditches chaurs and swampy areas of Northern India. These fishes can survive in oxygen deficient water bodies (Choudhary and Azad, 2024 & Ghosh and Munshi, 1987) [5]. The collected fishes were first treated with 0.5% $KMnO_4$ solution for two minutes then transferred to the tank. Fishes were acclimatized for two weeks under laboratory condition. All the effort was made to provide natural environment to the fishes as far as possible in the laboratory. During this period fishes were fed boil chicken eggs and chopped parts of small fishes @ 5% of body weight approximately on alternate days (Pandey *et al*., 2011) [12].

Experimental Design

The experiment was conducted in Biodiversity laboratory of Univ. Dept. of Zoology, TMBU, Bhagalpur, and Bihar during the month of August 2024.After acclimatization fishes were divided into control and experimental group. A group of ten healthy fish of average length (13.5-16.5 cm) and average weight 30-40g were selected for the present experiment/study. The experimental group of fishes were exposed to different concentrations of Rogor (0.50, 0.60, 0.70, 0.80, 0.90 & 1.0ml) for the period of 96 hours. After exposing the fishes to different concentrations of Rogor as mention above, the survival and behaviour of the fishes were recorded. Water from each tank was replaced with tap water every day during the experiment period. No food was given at time of toxicity test. Fishes were regularly monitored to observe the survival and behavioural alteration if any. A fish was considered dead when no response observed after touching with glass rod. Dead fishes were immediately eliminated from the tanks. Total mortality and abnormal behavioural alterations were recorded regularly between 8a.m.-5p.m. Every observation and data was collected properly to draw better inferences. The range-finding tests were conducted during the same month under consistent water quality conditions. Fish mortality was recorded after a 96-hour exposure period. The final results were analyzed using probit analysis and the statistical tools available on the O.P. Sheoran website.

Results and Observations

Acute Toxicity of Rogor

Table 1 presents the mortalities observed in the different treated groups after 96 hours of exposure. As shown in Figure 1, the number of mortalities increased with higher concentrations of the insecticide. The 96 hours LC_{50} value of Rogor was 0.74ml/20L (0.037ml/L). First mortality was observed in 48 hours when fishes were exposed to concentration of 0.6ml (Table1). 100% mortality in *Channa punctatus* was observed after 96 hours of exposure at concentration of 1ml/20L.

Behavioural Changes

Fish exposed to Rogor exhibited various behavioral changes, including jumping, increased respiratory frequency, erratic swimming, and loss of balance, hyperactivity, heightened surface activity, mucus secretion, bulging eyeballs, and discoloration. Initially, the fish appeared healthy and active. However, as the experiment progressed, they attempted to avoid the toxic water by swimming rapidly, jumping, and displaying random movements to escape the effects of Rogor.
At higher concentrations of Rogor, the fish displayed sudden, irregular swimming patterns with jerky movements and signs of hyperexcitation. Excessive mucus secretion was observed, covering the buccal cavity. Over time, the fish's skin showed reduced pigmentation. These conditions impaired their ability to uptake oxygen efficiently, leading to an increased breathing rate and frequent visits to the water surface to gulp air in response to oxygen deficiency. Eventually, the fish experienced loss of equilibrium, exhaustion, and the appearance of red spots on their bodies. In the final stages, they assumed a vertical position with their mouths near the water surface and tails pointing downward, struggling to breathe. Shortly after, they became passive, settling at the bottom of the tank, and eventually turned belly-up.

Table 1: Mortality and behavioural alterations in the fishes exposed to different concentrations of Rogor

Period in hours	No of Fishes	Concentrations of doses in ml						Behavior Observed
		0.5	0.6	0.7	0.8	0.9	1.0	
		Tank A	Tank B	Tank C	Tank D	Tank E	Tank F	
		Mortality in every tank						
24	10	-	-	01	02	03	03	N. & L.A.
48	10	-	01	02	03	02	03	S.F.A., L.P., L.E. &L.S.
72	10	-	01	01	01	02	02	F.O.A., L.F.A, R.S. I.O.A & E.B.O.
96	10	-	-	-	-	-	02	E.B.O & R.S
Total Mortality		0	2	4	6	7	10	

Abbreviations

L.A-Less Active, S.F.A-Slow Feeding Attempts, F.O.A-Fast Opercular Activity, N.-Normal, L.P-Less Pigmentation, L.S-Less swimming, L.F.A.-Less Feeding Attempts, I.O.A-Increase Opercular Activity, L.E-Loss of Equilibrium, E.B.O.-Eyeball bulged out & R.S.-Red Spot

Table 2: Maximum likelihood estimation of LC50

Conc.	Tot No fish	No Kills	%, Mort.	Log(Dose)	Exp. Prop	Emp Probit	Exp Probit	Work. Probit	Weight	wx	wy	wx2	wy2	wxy	Y'
0.500	10.000	0.000	0.025	-0.301	0.036	-1.960	-1.800	-1.938	1.800	-0.542	-3.488	0.163	6.762	1.050	-1.800
0.600	10.000	2.000	0.200	-0.222	0.163	-0.842	-0.983	-0.832	4.444	-0.986	-3.697	0.219	3.075	0.820	-0.983
0.700	10.000	4.000	0.400	-0.155	0.385	-0.253	-0.292	-0.253	6.172	-0.956	-1.562	0.148	0.395	0.242	-0.292
0.800	10.000	6.000	0.600	-0.097	0.621	0.253	0.307	0.253	6.152	-0.596	1.556	0.058	0.394	-0.151	0.307
0.900	10.000	7.000	0.700	-0.046	0.798	0.524	0.835	0.486	4.920	-0.225	2.393	0.010	1.164	-0.110	0.835
1.000	10.000	10.000	0.975	0.000	0.904	1.960	1.307	1.723	3.335	0.000	5.745	0.000	9.898	0.000	1.307

Table 3: Working probit regression line

Parameter	Estimate	Std. Error	T-Cal	P-Value
Intercept	1.307	0.342	3.823	0.012
Beta	10.321	2.290	4.508	0.006

Table 4: Estimation of probit line, LC_{50} and fit statistics

Component	Estimate
Mean X	0.753
Mean Y	0.035
Intercept	1.307
Beta	10.321
LC_{50}	0.747
LL	0.623
UL	0.896
Log(LC50)	-0.127
Log LL	-0.205
Log UL	-0.048
Chi-Square ML	1.336
Sign (Chi-Square)	0.855

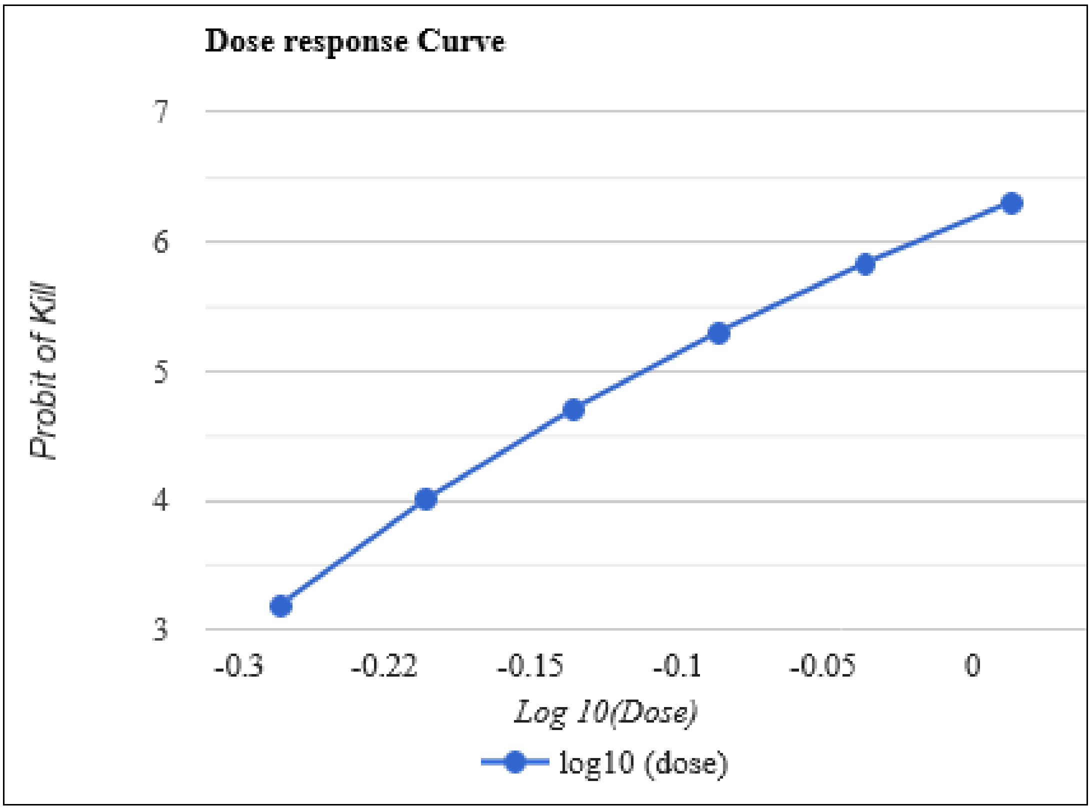

Fig 1: Graph of optimum solution converged in 6 iterations

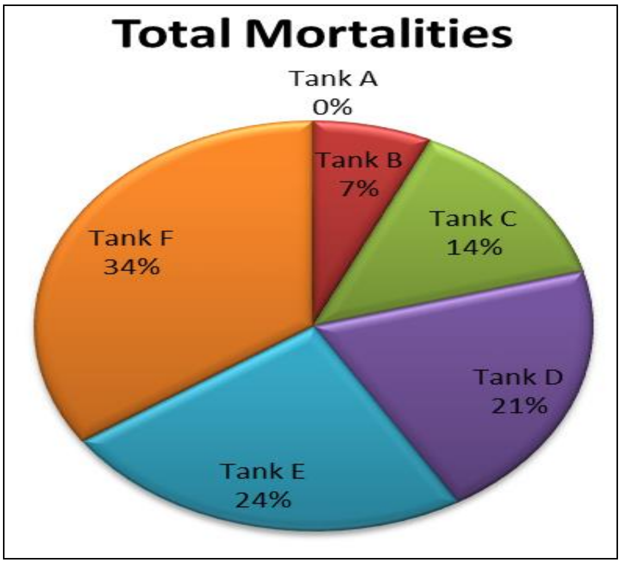

Fig 2: Pie chart showing the% mortality of the fishes treated with Rogor with different concentrations

Discussions

Fish are highly suitable subjects for studying the behavioral effects of exposure to various toxic chemicals because they are in constant and direct contact with their aquatic environments, where chemical exposure occurs across their entire surface (Chaudhari & Saxena, 2021) [2]. They are particularly sensitive to a wide range of waterborne toxicants. The accumulation of pesticides in their tissues leads to numerous physiological, biochemical, and behavioral changes by affecting the activity of various enzymes and metabolites (Nagarathnamma & Ramamurthi, 1982; Sarma *et al.*, 2013) [18, 13]. Behavior serves as a critical link between an organism's physiology, its ecology, and its environment (Little & Brewer, 2001) [10]. It is also shaped by adaptations to environmental factors. Behavioral studies provide direct insights into how fish respond to their surrounding environment or to chemicals present in the water (Pandey *et al.*, 2011) [12]. According to Warner *et al.* (1966) [22] and Radhaiah *et al.* (1987) [20], behavioral abnormalities in organisms reflect the ultimate outcome of complex biochemical and physiological processes.

In the present study exposure to different concentration of Rogor caused alterations in the behaviour of *Channa punctatus*. At the concentration of 1ml/20L of water or above, fishes became restless showed abnormal movement and died. Most of the fishes under observation were found less active and expressed erratic swimming movement. It may be the effect of stress caused due to toxicity of Rogor or aquatic environment (Srivastava *et al.*, 2010) [14]. Loss of pigmentation were also decreasing gradually in almost all the treated fishes may be due to dysfunction of the pituitary gland under stress causing changes in the number and distribution of chromatophores (Yadav *et al.*, 2007; Ramesh & Saravanan, 2008) [23, 21]. Bad water quality or changes in physico-chemical properties of water as well as the unfavourable habitat of the fish tank may also cause stress or may influence the behavioral pattern of fishes including loss of equilibrium, increase rate of opercular activity and less feeding attempts, may be the another reasons. Hassanein & Okail (2008) [6] had also observd loss of buoyancy and balance with an initial increase in the opercular ventilation rate which then decreased significantly in *Ctenopharyngodon idella* (grass carp) after exposure to the biopesticide. According to Fulton & Key (2001) [4], the restlessness and hyperactivity in fish may occur due to the stress or inactivation of acetylcholinesterase, enzyme leading to accumulation of acetylcholine at synaptic junctions. Finally, fishes were almost paralysed and settled on the bottom of the tank and died.

The fish in the control group exhibited no behavioral changes, confirming that Rogor was solely responsible for the observed behavioral alterations and mortality (Tiwari & Singh, 2003) [16]. The current study clearly demonstrated that Rogor is highly toxic to *Channa punctatus*, causing acute effects manifested as behavioral changes. Therefore, the use of Rogor should be strictly controlled and regulated through appropriate legislation to prevent its bioaccumulation in the environment, thereby minimizing its adverse effects on aquatic organisms. This research will also contribute to establishing safe dosage levels for such organophosphorus pesticides.

Conclusion

The findings of this study clearly demonstrate that even low levels of exposure to Rogor can lead to alterations in the behavior and physical structure of *Channa punctatus*. Additionally, the use of pesticides on agricultural fields not only targets and eliminates specific pests but also harms non-target species, including aquatic organisms like *Channa punctatus*. This raises significant concerns about the potential risks to human health, particularly for individuals who consume this fish as part of their diet, as pesticide residues may accumulate and pose long-term health hazards.

Authors Contribution

First author designed & conducted the whole experiment. The second author analysed the data and prepared the manuscript.

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Competing Interests

Authors have declared that no competing interest exist

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