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Neurotoxic effects of insecticide Dicofol 18.5 % (EC) in freshwater fish, *Channa punctatus* (Bloch)

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Abstract
Insecticides are reported to have the potency to cause neurotoxicity in non-target organisms as well. Organochlorine pesticides are widely used in agricultural practices. The present study is regarding the neurotoxic effect of Dicofol, a miticide on freshwater fish *Channa punctatus*. The neurotoxic effect of this insecticide was evaluated using various hematological and biochemical parameters after exposure to two different sublethal concentrations of Dicofol i.e., 35 µL and 50 µL in 15L of water at varying exposure periods of 3, 5, 10 and 15 days. Behavioural study of the fish specimen was also conducted along with analysis of various physicochemical parameters of water. During different exposure times variation in different parameters was observed. It may be inferred from the results obtained that Dicofol causes neurotoxicity in the fish, and is hence neurotoxic to other aquatic and terrestrial species.

Keywords: pesticide, neurotoxicity, dicofol, *Channa punctatus*, hematological, behavioral parameters

1. Introduction
For several toxic chemicals the nervous system is the most responsive organ that inhibits the neuron's electrophysiological processes. Toxicants can induce structural changes in the axon, sheath of myelin and body of cells. The changes caused by structural damage in the conduction of nerve impulses include the output of local circuit currents, blockage or delay in conduction, and repeated firing, which is termed as neurotoxicity [1, 2]. Insecticides used to destroy pests of high order, for example mosquitoes, often specifically attack the nervous system. Neurotoxic insecticides function through various pathways, either influencing the central or peripheral nervous system, or both. Dicofol (IUPAC Name: 2, 2, 2-trichloro-1, 1-bis (4-chlorophenyl) ethanol) is an organochlorine miticide that is used on a wide range of fruit, vegetable, ornamental and field crops [3, 4]. Dicofol is structurally similar to DDT. According to the World Health Organization [5], Dicofol produces stimulation of axonal transmission of nerve signals, believed to be related to inhibition of ATPases in the central nervous system (CNS).
In moderate intervals, the sub-lethal toxicity test is based on one-tenth or more of an LC50 dose. In sub-lethal toxicity, the respiratory, hepatic, hematopoietic, nervous, cardiovascular, and reproductive, and immune systems may be the organs or biological systems that may be affected by such exposure [6]**.** Insecticides can cause changes in the biochemical parameters of the blood and the hematological profile of fish that can be investigated as a biomarker in pollution monitoring [7-9]
Channa punctatus was chosen as the experimental specimen in the present analysis because it resides mainly in contaminated water and can withstand several stresses. Therefore any significant harm inflicted by it in response to a neurotoxin will certainly threaten the entire biological environment, particularly aquatic life. The sublethal concentrations for this study were calculated using Dicofol's LC50 value and the effects of sublethal concentrations on different hematological (TEC and TLC), biochemical (Specific Catalase activity), and behavioral parameters were observed in different exposure periods of 3, 5, 10, and 15 days.

2. Materials and Methods
For the experiment *Channa punctatus* (Bloch, 1793) of average length, 14 cm, and average weight 40 gms were used live, stable, and disease-free. The fishes were acclimatized in aquariums under ideal conditions for 2 weeks before use in the experiments. They had been served commercial fish pellets. Aerators were used in the aquariums to ensure a constant airflow. Commercial grade Dicofol was procured from the local market of 18.5 percent EC. Based on the LC50 value, two sublethal concentrations (namely the. low and high dose) were estimated using Finney's (1972) probit analysis to be 35 µL and 50µL respectively in 15L of water for fish exposure.
At 3,5,10 and 15 days the fishes were exposed to sublethal concentrations. Fish were randomly divided into three groups of 6 fish each for control, low dose, and high dose, for each setup. The fishes were subjected to experiments after each exposure cycle. An analysis of behavior in Glass aquariums was also performed. Hematological parameters, i.e., Total Erythrocyte Count and Total Leucocyte Count were calculated using the Wintrobe 1967 technique to use Neubauer's counting chamber. The brain's basic catalase activity was calculated using the Aebi method, 1974. A 3ml homogenate containing 1.95 mL of 50mM phosphate buffer (ph 7.0) and 1mL of 30mM hydrogen peroxide was added with 0.05 mL supernatant. The absorbance changes were then reported at intervals of 15 seconds at 240 nm for 30 seconds. For Physico-chemical water parameters, Winkler's' method used to estimate dissolved oxygen, alkalinity was estimated by titration process, and pH was calculated using an automated pH meter. Statistical data analysis was performed using ANOVA one way, followed by Turkey's Multiple Comparison Test using Graphpad Prism 6 software. The data were considered significant for $p<0.05$.

3. Results & Discussion

The behavioural responses of fish exposed to Dicofol sublethal concentrations in Table 1 showed they are under stress. Fish movements decrease as the dose of Dicofol increases. In the high dose lethargy is seen as full. According to [10] the effect on their nervous system is suggested by a decrease in certain locomotory activities. The loss of balance in Dicofol's high dose is representative of nervous system disturbance and its activities. Changing physical features primarily involves a change in body colour and mucous secretion during periods of Dicofol exposure. Rao *et al*., [11] stated that the most significant change in the physical characteristics of the fish exposed to Dicofol is the loss of body weight. There was a substantial decrease in working motion in fish exposed to the pesticide as opposed to control fish (Table 1). Binoy *et al*, [3] found that fish handled with Dicofol are showing a decreasing trend in food intake and foraging behaviour. In continuously moving water current, the potential for continuous swimming declined severe. Dicofol can have long-lasting effects on the fish population, including at its sub-lethal dosage.

Table 1: Behavioural changes during various exposure periods of Dicofol at different sub-lethal concentrations. '=' – no change '+'-increase in parameter '*'- decrease in parameter

Exposure periods	Dose	Colour change	Bodyweight	Opercular activity	Movement	Resting	Mucus secretion	Loss of equilibrium
3 days	Control	=	=	=	=	=	=	=
	Low	+	=	=	=	=	=	=
	High	+	*	*	*	+	+	+
5 days	Control	=	=	=	=	=	=	=
	Low	+	*	*	*	+	+	=
	High	++	*	*	*	+	+	+
10 days	Control	=	=	=	=	=	=	=
	Low	+	*	*	*	+	+	+
	High	++	**	**	**	++	++	++
15 days	Control	=	=	=	=	=	=	=
	Low	++	**	**	**	++	++	++
	High	+++	***	***	***	+++	+++	+++

Dicofol's neurotoxicity has been attributed to altering fish's haematological parameters. Dicofol's effect on the counting of erythrocytes was found to increase during the exposure time (Table 2). After 15 days of treatment, the overall erythrocyte count (TEC) was least dose-high. The large reduction in TEC in this study may be attributed to hemolysis and shrinking of blood cells attributed to the insecticide's toxic effect. Shrivastava [12], observed cellular and nuclear hypertrophy, shape shift, agglutination, and erythrocyte bursting at Fingerlings of *C. mrigala* treated with urea. Chauhan, [13], and Singh and Srivastava, [14] also observed similar findings in fish treated with pesticides and chemicals.

Table 2: Total Erythrocyte Count (TEC) and Total Leucocyte Count (TLC) variations during a different period of exposures. The values of TRC and TLC are represented as (mean ± SEM) × 10^6/ mm^3 and (mean ± SEM) × 10^3/ mm^3, respectively.

Days	Parameter	Dose		
		Control	Low	High
3 Days	TEC	3.44 ± 0.04	3.21 ± 0.04	2.98 ± 0.11*
	TLC	3.26 ± 0.06	3.46 ± 0.11	3.93 ± 0.04*
5 Days	TEC	3.40 ± 0.07	3.14 ±0.07	2.77 ±0.08*
	TLC	3.20 ±0.16	3.62 ±0.04	4.32 ±0.14*
10 days	TEC	3.42 ±0.032	2.93 ± 0.046**	2.46 ± 0.151**
	TLC	3.30 ±0.112	3.90 ±0.115	4.61 ±0.150*
15 days	TEC	3.41 ±0.096	2.58 ±0.050*	2.19 ± 0.062**
	TLC	3.24 ±0.13	4.20 ± 0.147**	4.90 ± 0.160***

Level of significance is represented by '*', where '*'= p <0.05; '**'= p<0.01 and '***'= p< 0.001.

Total Leucocyte count increased as regards normal counts [15] with a rise in exposure time and dosage due to immune system activity attempting to combat Dicofol's effect (Table 2). According to [9], increased WBC count formed leucocytosis which under chemical stress is considered to be of adaptive value to the tissue.

The study revealed a decreasing trend with a rise in the dose of Dicofol (Table 3) in the particular behaviour of catalase in brain tissue homogenate. This may be due to a decline in enzyme synthesis rates. The declining production of catalase in brain tissue has resulted in oxidative stress in the brain [16].

Table 3: Variation in the specific activity of Catalase during two different periods of exposure. The values are in the form of (mean ± SEM) u/mg Protein.

Dose↓	10 Days	15 Days
Control	2.825 ± 0.075	2.705 ± 0.105
Low	2.25 ± 0.05	1.85 ± 0.05
High	2.015 ± 0.005*	1.35 ± 0.05**

The level of significance is represented by '*', where '*'= p <0.05; '**'= p<0.01

During this analysis, the physical-chemical properties of water were investigated. During the calculation of dissolved oxygen in Fig 1 there was no alteration between the various exposure times. But the modification is negligible since they used aerators. The pH in Fig 2 was relatively normal, too. However, alkalinity varies between the various exposure times in Fig 3. These marginal changes showed that, during the experiment, water quality had the least effect on the fish.

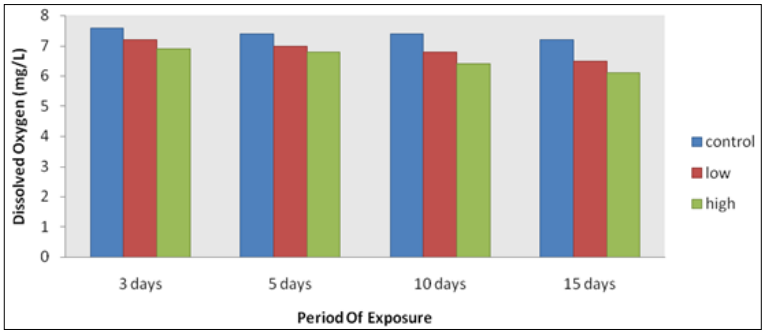

Fig 1: Graph showing dissolved oxygen variation after exposure to different sub-lethal concentrations of Dicofol for different period of exposures. The Values Are Represented In mg/L.

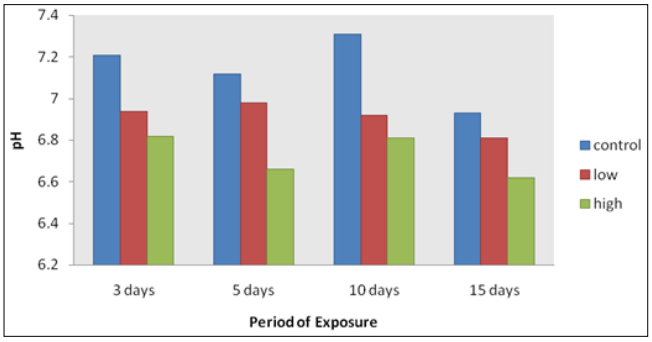

Fig 2: Graph showing the pH variation after exposure to different sub-lethal concentrations of Dicofol for different period of exposures.

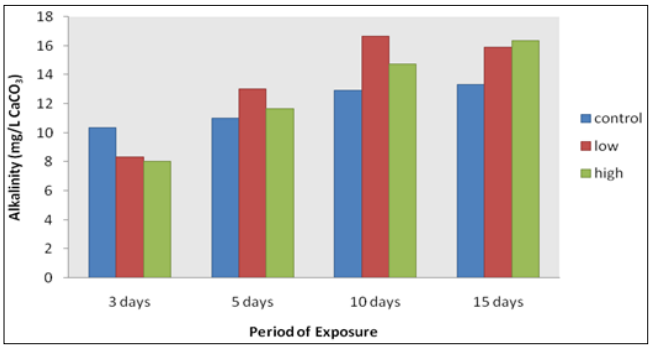

Fig 3: Graph showing the alkalinity variation after exposure to different sub-lethal concentrations of Dicofol for different period of exposures. The Values Are Represented In mg/L $CaCO_3$.

4. Conclusions

From this study, it can be inferred that Dicofol is a potent neurotoxin to *Channa punctatus* that is the organochlorine pesticide. The altered hematological, as well as basic brain catalase activity, demonstrated neurotoxicity. The changes in behavior often represent the effect Dicofol has on the fish. *Channa punctatus* can withstand a lot of tension since much of its habitat is polluted. But the substantial harm the pesticide shows indicates Dicofol's potential to cause damage to other species as well. But its widespread use should be reviewed, given the neurotoxicity shown by the insecticide, Dicofol.

5. References

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