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Neurotoxicity of sublethal concentrations of Dicofol 18.5 % (EC) in fresh water fish, *Channa punctatus* (Bloch)

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Abstract
A number of pesticides can cause neurotoxicity. Insecticides, which kill insects by targeting their nervous system, have neurotoxic effect in other organisms as well. The present work is regarding the neurotoxic effect of Dicofol, a miticide on fresh water fish *Channa punctatus*. The neurotoxicity was evaluated on the basis of hematological (Total Erythrocyte Count and Total Leucocyte Count), biochemical (Catalase activity) parameter and behavioural study of the fish and physicochemical parameters of the water after exposure to two different sublethal concentrations of Dicofol i.e., 35 µL and 50 µL in 15L of water in different exposure periods of 3, 5, 10 and 15 days. Alteration in various parameters was observed during different periods of exposure. From the results obtained it is concluded that the pesticide Dicofol induces neurotoxicity in the fish and thus is neurotoxic to other aquatic and terrestrial organisms also.

Keywords: Pesticide, Neurotoxicity, Dicofol, *Channa punctatus*, Hematological, Behavioral Parameters

1. Introduction
The nervous system is the most sensitive organ for many toxic chemicals which interrupt the electrophysiological mechanisms of neuron. Toxicants also cause structural changes in the axon, myelin sheath and cell body. The changes in the conduction of nerve impulses due to structural damage include production of local circuit currents, blockage or delay in conduction and repetitive firing. This phenomenon is called neurotoxicity [1, 2]. Pesticides used to kill high order pests, such as insects, often target the nervous system directly. Neurotoxic pesticides work through multiple mechanisms, affecting either 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 used on a wide variety 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).
Sub-lethal toxicity testing is based on one tenth or more of LC_{50} dose in moderate periods. In sub-lethal toxicity, the organs or biological systems which may be affected at such exposure can be respiratory, hepatic, hematopoietic, nervous, cardiovascular, and reproductive and immune systems [6]. Insecticides may lead to changes in the blood biochemical parameters and hematological profile of fish which can be investigated as biomarker in pollution monitoring [7-9].
In the present study *Channa punctatus* was chosen as the experimental specimen, as it resides mostly in polluted water and can overcome lots of stresses. Thus any significant damage shown by it in response to a neurotoxin will surely threaten the entire biological life specially the aquatic life. The sublethal concentrations for the present study was determined using the LC_{50} value of Dicofol and the effects of the sublethal concentrations were observed on various hematological (TEC and TLC), biochemical (Specific Catalase activity) and behavioural parameters in different exposure periods of 3, 5, 10 and 15 days.

2. Materials and methods
Live, healthy and disease free, *Channa punctatus* (Bloch, 1793) of average length 14 cm and average weight 40 gms were used for the experiment. The fishes were acclimatized in aquariums for 2 weeks prior to use in the experiments under optimum conditions. They were fed with commercial fish pellets. Aerators were used to ensure continuous air flow in the aquariums. Commercial grade Dicofol 18.5% EC was procured from the local market. Based on LC_{50} value, calculated using Finney's (1972) probit analysis two sub lethal concentrations (viz. Low dose and high dose) were determined to be 35 µL and 50µL respectively in 15L of water for exposure of fishes.
The fishes were exposed to the sublethal concentrations for 3,5,10 and 15 days. For each setup, fishes were randomly divided into three groups of 6 fishes each for control, low dose and high dose. After each exposure periods, the fishes were subjected to experiments. Behavioral study was done in Glass aquariums. Haematological parameters i.e., Total Erythrocyte Count and Total Leucocyte Count were determined by using Neubauer's counting chamber using methodology of Wintrobe 1967. Specific activity of catalase of brain was determined according to the method of Aebi, 1974. Supernatanat 0.05 mL was added to a 3ml homogenate that contain 1.95 mL of 50mM phosphate buffer (ph 7.0) and 1mL of 30mM hydrogen peroxide. The changes of absorbance were then recorded at 240nm for 30 seconds at 15 seconds interval. For physico-chemical parameters of water, Estimation of dissolved oxygen was done by Winklers' method, Alkalinity was determined by titration method & pH was determined using digital pH meter. Statistical analysis of data was carried out using one way ANOVA followed by Turkey's Multiple Comparison test using Graphpad Prism 6 software. For $p<0.05$ were considered significant.

3. Results & Discussion
The behavioral responses of fishes exposed to sub lethal

concentrations of Dicofol in Table 1 showed that they were under stress. Movement of the fishes decreases with increase in dose of Dicofol. Lethargy is seen maximum in the high dose. According to [10], decrease in such locomotor activities indicates the affect on their nervous system. Loss of equilibrium in the high dose of Dicofol indicates the disruption of nervous system and its activities. Alteration of physical feature mainly includes change of body colour and mucous secretion over periods of exposure to Dicofol. Rao *et al*, [11] have reported that the most notable alteration in physical feature of the fishes exposed to Dicofol is the loss of body weight. Significant reduction in opercular movement was observed in fishes exposed to the pesticide as compared with control fish (Table 1). Binoy *et al*, [3] have observed that Dicofol treated fish exhibited a declining trend in food intake and foraging behavior. The ability for sustained swimming in constantly flowing water current declined drastically. Dicofol even in its sub-lethal concentration can produce long lasting effects on fish population.

Table 1: Behavioural changes during various exposure periods of Dicofol at different sub-lethal concentrations

Exposure periods	Dose	Colour change	Body weight	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	+++	***	***	***	+++	+++	+++

'=' – no change, '+'-increase in parameter, '*'- decrease in parameter

The neurotoxicity of Dicofol has been related to alteration of haematological parameters of fishes. The influence of Dicofol on erythrocyte count was observed to increase during the increase in period of exposure (Table 2). Total erythrocyte count (TEC) was least in high dose after 15 days of exposure. The significant decrease in TEC in the present study might be due to haemolysis and shrinkage of blood cells by the toxic effect of insecticide. Shrivastava [12], observed cellular and nuclear hypertrophy, change in shape, agglutination and bursting of erythrocytes in C. mrigala fingerlings 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 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 of Exposure→	3 days		5 days		10 days		15 days	
Dose↓	TEC	TLC	TEC	TLC	TEC	TLC	TEC	TLC
Control	3.44 ± 0.04	3.26 ± 0.06	3.40 ± 0.07	3.20 ± 0.16	3.42 ± 0.032	3.30 ± 0.112	3.41 ± 0.096	3.24 ± 0.13
Low	3.21 ± 0.04	3.46 ± 0.11	3.14 ± 0.07	3.62 ± 0.04	$2.93 \pm 0.046^{**}$	3.90 ± 0.115	$2.58 \pm 0.050^{*}$	$4.20 \pm 0.147^{**}$
High	$2.98 \pm 0.11^{*}$	$3.93 \pm 0.04^{*}$	$2.77 \pm 0.08^{*}$	$4.32 \pm 0.14^{*}$	$2.46 \pm 0.151^{**}$	$4.61 \pm 0.150^{*}$	$2.19 \pm 0.062^{**}$	$4.90 \pm 0.160^{***}$

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

Total Leucocyte count increased, with respect to the normal counts [15] with the increase in period of exposure and dose due to activity of immune system trying to counteract the effect of Dicofol (Table 2). According to [9], increased WBC count established leucocytosis, which is considered to be of an adaptive value for the tissue under chemical stress.

In the study, the specific activity of Catalase in brain tissue homogenate showed a decreasing trend with increase in dose of Dicofol (Table 3). It may be due to reduction in the rate of synthesis of the enzyme. According to [16], decreasing activity of Catalase in the brain tissue resulted in oxidative stress in the brain. Thus oxidative stress preferentially damage neuronal population in the brain.

Table 3: Variation in specific activity of Catalase during two different period 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	$2.015 \pm 0.005^{*}$	$1.35 \pm 0.05^{**}$

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

The physicochemical properties of water were investigated during this study. Little alteration among different period of exposure was observed during estimation of dissolved oxygen in Fig 1. But the alteration is negligible as aerators were used. The pH was also relatively normal in Fig 2. However, alkalinity varies among different period of exposure in Fig 3. These negligible alteration showed that the water quality has least effect on the fish during the experiment.

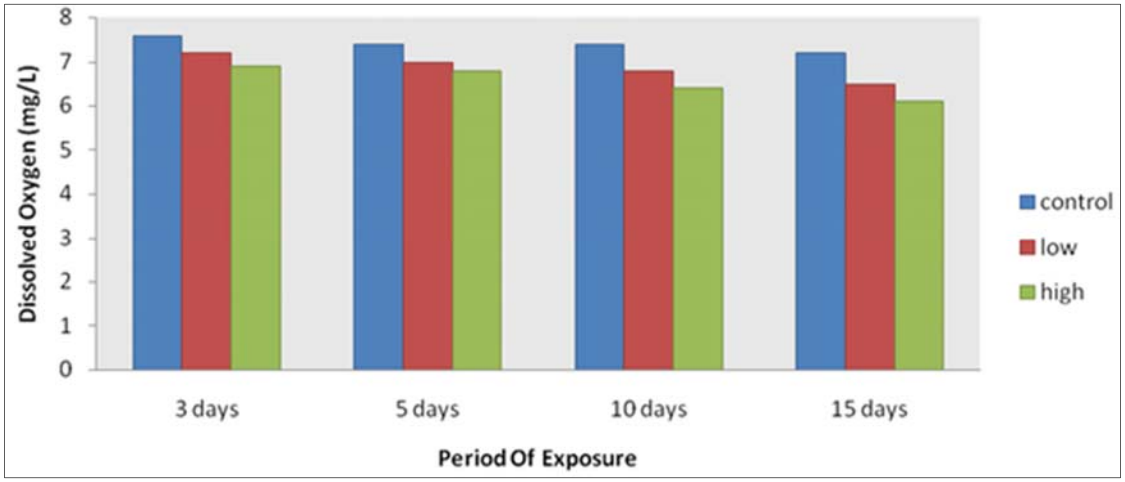

Fig 1: Graph showing dissolved oxygen variation after exposure to different sub-lethal concentrations of Dicofol for different period of exposures. The Values Are Represented In mg/L.

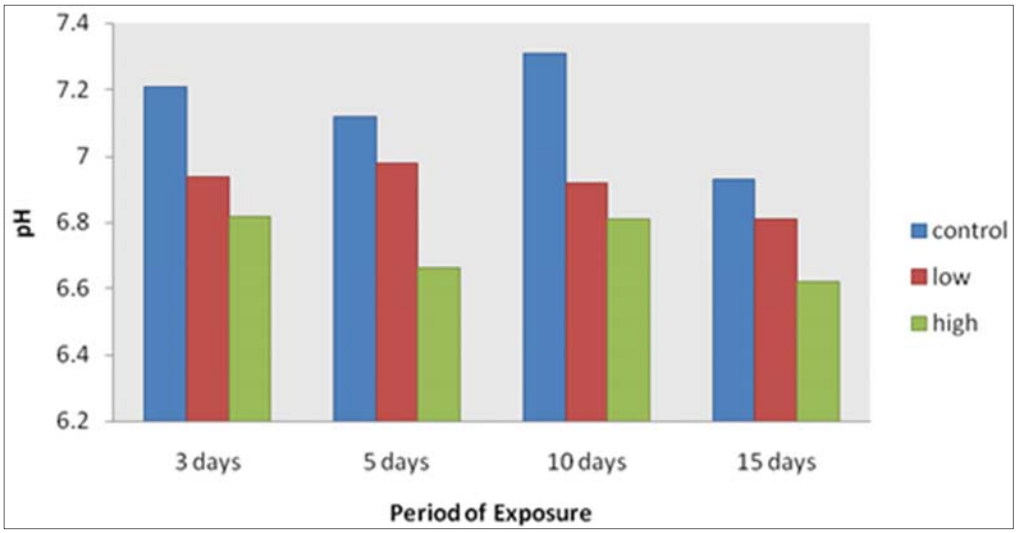

Fig 2: Graph showing the pH variation after exposure to different sub-lethal concentrations of Dicofol for different period of exposures.

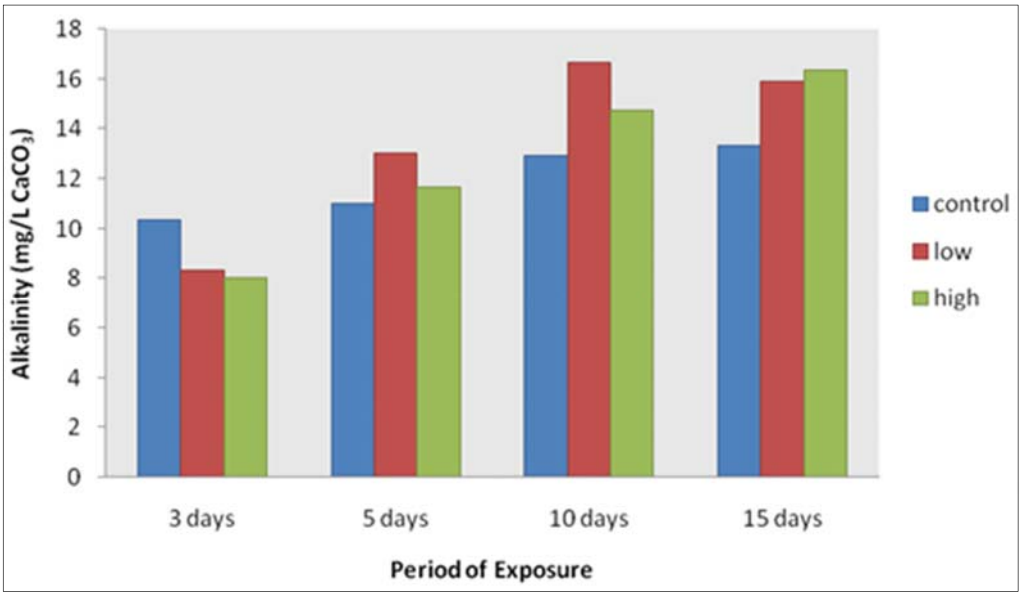

Fig 3: Graph showing the alkalinity variation after exposure to different sub-lethal concentrations of Dicofol for different period of exposures. The Values Are Represented In mg/L $CaCO_3$.

4. Conclusions

From the present study it can be concluded that the organochlorine pesticide, Dicofol is a potent neurotoxin to Channa punctatus. The neurotoxicity was shown by the altered hematological as well as specific catalase activity of the brain. The behavioural changes also depict the effect of Dicofol on the fish. Channa punctatus can overcome lots of stress as its habitat is mostly polluted one. Thus, the significant damage shown by the pesticide predicts the ability of Dicofol to cause damage to other organisms as well. So, in view of the neurotoxicity shown by Dicofol, its widespread use should be checked.

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