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Research Article

TOXICITY AND BEHAVIOURAL STUDIES OF CHLORPYRIFOS IN FRESHWATER FISHES

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ABSTRACT

Chlorpyrifos toxicity was evaluated by using bioassay method in freshwater fish *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton). The median lethal concentration (LC_{50}) values for 96 hr are 0.63 mg/L, 0.22 mg/L and 0.72 mg/L respectively. These values indicate that among these fishes *Cirrhinus* is resistant and *Labeo* is most sensitive. Common behavioural changes observed are erratic swimming, hypo activity, hyper excitability, imbalance in posture, increase in opercular movement, profuse mucus release from the body surface, loss in equilibrium in all the three species. Specific behavioural patterns like swimming vertically downwards in *Catla*; coughing type of mouth movements, backward swimming and darkening 3/4th of the body from anterior end in *Labeo*; whereas bent tail in *Cirrhinus* were observed individually

INTRODUCTION

Biodiversity can be defined as the richness of species within an ecosystem and resulting complexity of interactions among them. Biodiversity is affected by human activities from the last so many years resulting in ecological imbalance and finally species extinctions. The pollution is one of the main hazardous components for depletion of Biodiversity.

Indiscriminate use of different pesticides in agriculture increased over the years especially in the developing countries (Santhakumar and Balaji, 2000). Pesticides enter the aquatic environment through runoff water or may be applied directly, causing toxicity to non target organisms especially fishes (Nwani et al., 2013; Tiwari and Ansari, 2014; Sunanda et al., 2016). Exposure of toxicants causes fish poisoning which damage their vital organs, altering biochemical parameters and decline in reproductive ability. The major problem with these pesticides is that their action is unselective, i.e. they are poisonous not only to the insects they are designed to destroy, but also to non target animals. So, aquatic organism in general and the fish are being affected. Fishes are a sensitive indicator of the quality of the aquatic environment, since they are highly susceptible to low concentrations of pesticides in the aquatic medium (Leela siva parvathi, 1997). A major part of the world's food is being supplied from fish source. So, it is essential to secure the health of fishes (Tripathi *et al.*, 2002). Toxicity tests are the principal indicator to evaluate the effect of pesticides on aquatic fauna such as fishes. The effects of pesticides on fish population and other organisms depend on concentrations and exposure periods (Khare, 2015; Mishra and Verma, 2016). Toxicity is species- specific having different levels of responses to the same dose and time interval of a toxicant (Bridges and Semlitsch, 2000).

Behaviour can be defined as the sum of responses of an organism to internal and external stimuli. According to Mello (1975) and Mitchall *et al.* (1982), the behaviour of an organism represents the functional integrity of the central nervous system, but the activity of central nervous system can not be determined independent of behavioural analysis. Behavioural changes are physiological responses shown by the animal, which are often used as the sensitive measure of stress and syndrome in the organism experiencing it (Prabhakar *et al.*, 1988 and Rajamanickam and Karpagaganapathy, 1988). Previous research has characterized physiological mechanisms of toxicity in animals exposed to contaminants. In contrast, effects of contaminants on fish behaviour are less frequently studied, because behaviour links physiological function with ecological processes. Toxicant exposure often completely eliminates the performance of behaviours that are essential to fitness and survival in natural ecosystems, frequently after

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exposures of lesser magnitude than those causing significant mortality. Unfortunately, the behavioural toxicity of many xenobiotics is still unknown, warranting their future study (Graham *et al.*, 2004). Fish have proved to be of significance as bioindicators of the aquatic environment so-called ecological integrity (Faggio et al. 2014a, b; Gobi et al. 2018; Bartoskova et al. 2013). It can provide quantitative information on the ecological integrity and its health. Therefore, fishes are successful bioindicators.

Chlorpyrifos (CPF) [O, O–diethyl–O (3,5,6-trichlor-2-pyridyl) phosphorothioate] is the second largest selling organophosphorus agro chemical in India. According to the US Environmental Protection Agency, approximately 800 registered products on the market contain chlorpyrifos, and they are used for several purposes, including pest control for a variety of food crops, turf and ornamental plants, green houses, and sod; indoor pest control; structural pest control; and pet collars (NPIC 2017)

Present investigation was undertaken to determine toxicity and behavioural response to Chlorpyrifos, an organophosphate insecticide, using lethal and sub lethal concentrations in the freshwater fishes *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton). These three species of fishes together called as Indian major carps. Chlorpyrifos is very highly toxic to freshwater fish, aquatic invertebrates, and estuarine and marine organisms (US Environmental Protection Agency. 1989). Indian major carps are having high food value and economical importance in India. Hence, an attempt is made to study Chlorpyrifos toxicity effects on Indian major carps.

MATERIALS AND METHODS

The Indian major carps *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton)) were selected for the present investigation. The above species of fish seed at the fry stage were purchased from Sri Maha lakshimi hatchery, Palakoderu, West Godavari District, Andhra Pradesh, India and were immediately transported to culturing tanks. Utmost care is taken to avoid physical damage and to reduce stress due to transportation. The fish were immediately released into the stocking tanks by following acclimatization techniques.

Fishes of uniform weight (*Catla catla* 3.6 ± 0.2 gm, *Labeo rohita* 1.6 ± 0.2 gm and *Cirrhinus mrigala* 1 ± 0.1 gm) were taken from the stocking tanks and are shifted to suitable aquariums for toxicity tests according to biomass ratio as suggested by Doudroff *et al.* (1951) and were acclimatized for four weeks at room temperature 25 ± 2 °C. During the acclimatization, the fish were fed with standard diet (rice bran + ground nut oil cake in 2:1 ratio) @ 3% of their body weight in two divided doses. Feeding the fish was stopped 2 days prior to the experiments. If mortality exceeds 5%, in any batch during acclimatization, that entire batch of fish was discarded. The water quality characteristics were determined by using procedures, APHA, 1998.

The studies on toxicity were conducted as per the recommendations given in the report of the committee on methods of toxicity tests and aquatic organisms (Anon, 1975). Technical grade Chlorpyrifos insecticide supplied by Nagarjuna agrichem Ltd. was used for the toxicity studies. As Chlorpyrifos technical grade sample will not dissolve completely in water, aliquots are prepared with Acetone. Preliminary experiments using in Chlorpyrifos individually for all the three species of fish were conducted to find out the concentrations that resulted in 10% - 90% mortality. After the range finding tests, 20 fish each of *Labeo rohita, Catla catla and Cirrhinus mrigala* were exposed separately, in test chambers, to different concentrations of the insecticide Chlorpyrifos. Duplicates were also set up besides running the control simultaneously. Mortality was recorded for every one hour interval and the fish that did not respond to tactile stimulus were considered dead and removed (Tilak *et al.* 2003). The toxicity tests were conducted to calculate the 96 hours value of Chlorpyrifos.

LC_{50} values were calculated by following the Finney's probit analysis (Finney, 1971) & Dragstedt and Behren's equation (Carpenter, 1975)

Behavioural studies also carried out separately and in combination for all the above three species at sub lethal (1/10 LC_{50}) and lethal (LC_{50} x 10) exposure of insecticide by maintaining control which is exposed only to acetone. The amount of acetone added to the control is based upon the amount of acetone present in the stock solution added to the exposed fishes. During this experiment the behavioural changes were critically observed.

Table 1 Water quality parameters

Parameters	Value
Temperature	25 ± 2°C
pH	6.95
Dissolved oxygen	6.3 – 7.5 ppm
Turbidity	0 NTU
Total Hardness	176 ppm
TDS	220 mg/L
Nitrates NO^{3}-	0 mg/L
Phosphates PO_4	>1.0 mg/L
Chloride as Cl-	28.46 mg/L
Total Alkalinity	123 ppm
Ammonia	NIL
Coli form bacteria	NIL

RESULTS AND DISCUSSION

Behavioral studies

The behavioral changes were observed in control and Chlorpyrifos exposed fishes. The fish maintained in normal fresh water behaved in usual manner i.e. active with their well coordinated movements. They were alert at slightest disturbance, and they were covering an area of about one third of the bottom. But when exposed to Chlorpyrifos erratic swimming, hypoactivity and hyper exitability, imbalance in posture, increased opercular movement, zig zag, tilted, belly upward swimming, spreading of excess of mucus over the surface of the body were observed in all the three species. Similar results with different toxicants and in different species were reported earlier by Carpenter (1924, 1927), Anderson and Weber (1975), Durve and Jani (1980), Hymavathi (2001), Prasanth *et al.* (2005), Ganesh Wade *et al.* (2006), Thete *et al.* (2006) and Akinwande *et al.* (2007).

Table 2 Mortality of *Catla catla* in different concentrations of Chlorpyrifos at 24 h, 48 h, 72 h and 96 h of exposure. Mortality expressed both in percent and probit kill

Sl. No.	Concentration of insecticide (in ppm)	Log concentration	Number of fish					Percent kill				Probit kill			
			Exposed	Dead 24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
1	0.30	-0.52288	20	3	3	3	3	15	15	15	15	3.9636	3.9636	3.9636	3.9636
2	0.50	-0.30103	20	5	6	6	8	25	30	30	40	4.3255	4.4756	4.4756	4.7467
3	0.70	-0.15490	20	9	9	9	12	45	45	50	60	4.8743	4.8743	5.0000	5.2533
4	0.90	-0.04576	20	12	12	13	15	60	60	65	75	5.2533	5.2533	5.3853	5.6745
5	1.10	0.04139	20	16	17	18	18	80	85	90	90	5.8416	6.0364	6..2816	6..2816

Table 3 Mortality of *Labeo rohita* in different concentrations of Chlorpyrifos at 24 h, 48 h, 72 h and 96 h of exposure. Mortality expressed both in percent and probit kill

Sl. No.	Concentration of insecticide (in ppm)	Log concentration	Number of fish					Percent kill				Probit kill			
			Exposed	Dead 24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
1	0.1	-1.0000	20	4	5	5	6	20	25	25	30	4.1584	4.3255	4.3255	4.4756
2	0.2	-0.6990	20	8	8	8	9	40	40	40	45	4.7467	4.7467	4.7467	4.8743
3	0.3	-0.5229	20	11	11	13	15	55	55	65	75	5.1257	5.1257	5.3853	5.6745
4	0.4	-0.3979	20	15	15	15	17	75	75	75	85	5.6745	5.6745	5.6745	6.0364
5	0.5	-0.3010	20	17	17	18	19	85	85	90	95	6.0364	6.0364	6.2816	6.6449

Table 4 Mortality of *Cirrhins nrigala* in different concentrations of Chlorpyrifos at 24 h, 48 h, 72 h and 96 h of exposure. Mortality expressed both in percent and probit kill

Sl. No.	Concentration of insecticide (in ppm)	Log concentration	Number of fish					Percent kill				Probit kill			
			Exposed	Dead 24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
1	0.50	-0.3010	20	4	5	6	7	20	25	30	35	4.1584	4.3255	4.4756	4.6147
2	0.75	-0.1249	20	5	5	8	10	25	25	40	50	4.3255	4.3255	4.7467	5.0000
3	1.00	0.0000	20	12	12	17	17	60	60	85	85	5.2533	5.2533	6.0364	6.0364
4	1.25	0.0969	20	15.	16	18	19	75	80	90	95	5.6745	5.8416	6.2816	6.6449
5	1.50	0.1761	20	17	18	20	20	85	90	100	100	6.0364	6.2816	8.09	8.09

Table 5 The LC_{50} values of Chlorpyrifos to the Indian major carps for 96 hr. exposure

Species	LC 50 value (in ppm)
Catla catla	0.63
Labeo rohita	0.22
Cirrhinus mrigala	0.72

The fishes exposed to toxicant occupied more than twice the area than that of the control groups. The fish spread out and appeared to be swimming independent of one another. The jerking behavior while swimming was noticed earlier in *Catla catla* and later in *Cirrhinus mrigala.* Jumping to leap out of the trough was also noticed to be very high and earlier in *Catla.* But no such jumping behaviour was observed in *Cirrhinus mrigala.* Control fishes of *Catla catla* & *Cirrhinus mrigala* showed negative photo taxis, whereas fishes exposed to Chlorpyrifos did not respond to photo stimulus.

Labeo rohita and Cirrhinus mrigala even after loosing equilibrium they responded well, they are disturbed by knocking the aquarium. *Labeo rohita has* shown coughing type of movements of the mouth whereas it is absent in control. Granesh wade *et al.* (2006) also observed coughing behaviour in *Cyprinus carpio* when exposed to industrial effluents. *Catla catla* rotated along horizontal axis by swimming vertically down wards as if they want to penetrate into the bottom. Backward swimming was observed in Labeo *rohita. Catla* died with its mouth and gill covers wide open. Discoloration of body and later acquisition of dark colour of anterior region under lethal exposure was observed in *Labeo rohita* after more than 48 hr. *Cirrhinus mrigala* exposed to sub lethal concentration after more than 48 hr which was observed with a bent tail. Change in colour of gill lamellae from reddish to light brown with thin film of coagulation of mucus on the gill lamellae was seen in dead fishes. Similar observation was also reported by Venkatramiah, (1991) and Hymavathi, (2001).

The LC 50 values differ from species to species (Pickering *et al.* 1962). Signs of Chlorpyrifos intoxication are summarized by Hudson *et al.* (1984). From the Table 5, it is clear that among Indian major carps *Labeo rohita* (Hamilton) is very sensitive to Chlorpyrifos, followed by *Catla catla* (Hamilton) and *Cirrhinus mrrigala* (Hamilton) respectively. Similar results were also reported by Tilak *et al.* (2003).

Earlier reports revealed that there is a strong correlation between physiological activities, metabolic changes and behaviour of animal (Rice and Armitage 1974), Pavan kumar (1976). The most commonly observed links with behavioural disruption include cholinesterase (ChE) inhibition, altered brain neurotransmitter levels, sensory deprivation, and impaired gonadal or thyroid hormone levels (Graham *et al.* 2004). The thin film of mucus formed on the gill lamellae may be due to corrosive action of insecticide leading to inhibition of gaseous exchange. In toxic condition, energy requirements are generally high, as more energy will be diverted to damaged tissue for its repair. So, Oxygen requirements will be high but gaseous exchange through gills is lowered which leads to suffocation leading to increase in opercular movements. Quick expansion and contraction of opercular movement helps in cleaning of debris accumulated on the gills.

Hypo activity and hyper excitability, Jerking, Zig zag movements, Belly upward, swimming by keeping head down, bent tail in insecticide exposed fishes indicates the loss of equilibrium, probably due to the inhibition of the enzyme AchE. Results in accumulation of Ach at synapse and neuro muscular junctions as reported earlier by many authors (Agarwal and Balakrishanan, 1989, Narsimha Murthy *et al.*, 1986). Trying to leap out of water and profuse mucus release over the surface of body may be to avoid contact with insecticide and to protect from corrosive action and to reduce absorption of insecticide through body surface. Similar findings were reported by Sadhu (1993), Sabita *et al.* (1995), Leela Siva Parvathi, (1997), Thete, *et al.* (2005) and Prasanth *et al.*, (2006). Discoloration of the whole body indicates the adverse affects of Chlorpyrifos on the structural integrity of the tissues. The tendency of acquiring dark colour by the body surface may be one of the symptoms of toxicity in the fish under lethal exposure. This may be due to decrease in the blood supply to the body surface.

We conclude that multi disciplinary research is needed to know the significance and usefulness of behavioural indicators in aquatic toxicology.

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