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Acute Toxicity Bioassay of a Pyrethroid Pesticide Bifenthrin to the Asian Stinging Catfish, *Heteropneustes Fossilis* (Bloch)

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ABSTRACT

Bifenthrin is a type-I synthetic neopyrethroid pesticide having eight different stereoisomers.The current study aims to assess the short-term toxic effect of Bifenthrin to freshwater fish, *Heteropneustes fossilis*. The 24, 48, 72 and 96h LC_{50} values of bifenthrin to *H. fossilis* were 4.82, 4.47, 3.54 and 3.40 µg/l respectively. There was a significant variation ($p<0.05$) in the mortality of the treated fish exposed to bifenthrin with respect to the control at all the hours of exposure. A significant variation ($p<0.05$) between rate of mortality of H. fossilis and time slots (24-96h) was recorded for the final selected doses of bifenthrin except 4.0, 4.5 and 5.5 µg/l concentration of the toxicant. A vigorous mucous secretion and hyper-excitability was observed in the fish at the higher doses of bifenthrin specifically at 24h and 48h time slots. There was a profound loss in equilibrium of the treated fish particularly at the higher doses at 72h and 96h time slots.The gradual increase in dose of bifenthrin resulted in significant increase ($p<0.05$) in opercular movement of the fish with respect to the control. On the other hand, opercular movement showed a significant increase ($p<0.05$) with the advancement of time for all the treated doses. Therefore, bifenthrinis indicated to be very strongly toxic to fish.

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Introduction

Pesticides are being used indiscriminately to maintain a sustainable yield of various crops necessary to support the ever increasing animal population. They constitute a prime component of agricultural runoff which gets intermingled to the adjacent water bodies affecting thelife of differentnon-target organisms (Raina *et al*., 2009). The contamination of aquatic ecosystem by pesticides is a global problem (Hill, 1985; Sibley and Kaushik, 1991). Human beings are the worst victims of pesticide biomagnifications as they occupy the apex of the food pyramid (Sahai, 1992).

Bifenthrin is a type-I synthetic neopyrethroidhaving eight stereoisomers with the cis-isomer being the active ingredient (Khan *et al*., 2013). Pyrethroids act by exerting a time lag in the closing of sodium ion channelspresent on nerve cells even after an initial entry of sodium ions during the phase of depolarization of action potential (Saha and Kaviraj, 2008; Khan *et al*., 2013). This culminates in a sustained sodium ion flow. The absence of α-cyano-group assistsbifenthrin to bind to the sodium ion channels promoting the generation of after potentials followed bysustained firing of the axon, ineffective to the resting potential (Khan *et al*., 2013). Bifenthrinis also characterized by reduced environmental degradation and strong insecticidal effects (Mokry and Hoagland, 1989). It is also a popularstomach or contact insecticide and affects cellular ATPase production (Velisek *et al*., 2009; Roberts and Hutson, 1999). Though there are limited studies on the toxicity of Bifenthrin and its nanoencapsulated form to rainbow trout (Velisek *et al*., 2009), there is no report of its effects on air breathing fishes. Our study is the first of its kind and future chronic studies may be carried out to elucidate more detailed knowledge on the various aspects of toxicity to air breathing fishes.

The present research has the following objectives

- To determine the acute toxicity of the pesticide bifenthrin to *H. fossilis* in order to ascertain its safe permissible levels for the water bodies of our country.
- To determine the behavioural response and the alteration in respiratory rate of *H. fossilis* as a result of the toxic insult.

Materials And Methods

The Asian stinging catfish, *Heteropneustes fossilis* (Order: Siluriformes; Family: Heteropneustidae) was used as the test animal in the bioassay having a mean length of 11.7 ± 0.3 cm and a mean weight of 21.60 ± 0.7 g. It was procured from a nearby aquaculture farm followed by acclimatization under experimental ambience for three days prior to their use. During acclimatization the test fish were kept in the rectangular cemented tanks of 1000 litre capacity filled with unchlorinated water (pH 7.20 ± 0.35; temperature 26.53 ± 1.12°C) for 12 h each (dark and light cycle) (APHA, 2012). The food was supplied to the fish in the form of commercial pellets with 36% crude protein. The acclimatized fish were not fed 24h before the start of the bioassay for maintaining their normal metabolic activity (APHA, 2012).

The analytical grade Bifenthrin [IUPAC name: 2-methylbiphenyl-3-ylmethyl (Z)-(1RS, 3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclop ropanecarboxylate] (Brand Name: Marker, marketed by Dhanuka Agritech Limited) was used as the test chemical. It is a third-generation synthetic pesticide belonging to the pyrethroid family (Bansode and Patil, 2016).

Static replacement bioassay with the healthy, disease free fish (irrespective of sex) was conducted in 18l glass aquaria containing 10l unchlorinated water following standard protocols of American Public Health Association (APHA, 2012). The limnologicalnumeric of different water quality criterion for the experiment were:temperature 25.5 ± 0.65°C, pH 7.4 ± 0.55, free CO_2 10.3 ± 0.15 mg/l, DO 5.39 ± 0.23 mg/l, alkalinity 170 ± 9.11 mg/l as $CaCO_3$, hardness 116 ± 4.70 mg/l as $CaCO_3$. The experimental design comprised of four replicates along with a control. Each replicate comprised of twenty fish. Fish were not fed for 24h prior to the experiment.

A preliminary range finding test was conducted to demarcate the concentration range at which mortality of fish may occur. The final test concentrations of bifenthrin chosen to determine the 24, 48, 72 and 96h median lethal concentration (LC_{50}) values were 3.0, 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0 µg/l. During the bioassay, the dead organisms were removed from

the aquaria for avoiding any microbial decomposition. The count of dead fish was recorded after every 24h of experiment. Approximately, about 10% of the test water was replaced by newly prepared test water at every 12h interval to maintain a uniform concentration of the pesticide.

Toxicity factors of the test fish to bifenthrin at different time of exposure were assessed by dividing LC_{50} value at 24h by LC_{50} value at any other exposure time (Ayoola *et al*. 2011).

The safe level estimation for *H. fossilis* was obtained as a product of the 96h LC_{50} with different application factors (AF) based on Edwards and Brown (Edwards and Brown, 1966), Burdick (Burdick, 1967), Sprague (Sprague, 1971), Committee on Water Quality Criteria (CWQC, 1972), International Joint Commission (IJC, 1977), European Inland Fisheries Advisory Commission (EIFAC, 1983)and Canadian Council of Resources and Environmental Ministry (CCREM, 1971) besides the formula given by Hart *et al*. (Hart *et al*., 1948).

The probit program version 1.5 software was used to calculate the LC_{50} (with 95% confidence limit) from mean mortality data of *H. fossilis* obtained after 24, 48, 72 and 96h of bioassay (US EPA, 1999). Median lethal concentration (LC_{50}) was determined in MS Excel by plotting the test concentrations against the fish mortality within 24h, 48h, 72h and 96h after the bioassay (Finney, 1971). The relation between rate of mortality with exposure time and concentrations was evaluated using correlation analysis (US EPA, 1999; US EPA, 2006; Gomez and Gomez, 1984). The behavioral changes like restlessness, erratic swimming, and mucus secretion in the treated fish were also recorded during the bioassay (Saha *et al*., 2018; Dasgupta *et al*., 2010; Saha *et al*., 2020). Changes in the opercular movements in order to determine respiratory rate of *H. fossilis* exposed to selected doses of bifenthrin was also noted for the entire experimental period. Opercular movements of the fish per minute for both control and treatedsets were counted twice a day during the bioassay and their mean values per dose were plotted graphically. The statistical tool of analysis of variance (ANOVA) given in R-software (R Development Core Team., 2012) followed by multiple mean comparison using Duncan's Multiple Range Test (DMRT) was applied to the opercular movement data in order to find out significant difference within the average values at different doses of bifenthrin at 24, 48, 72 and 96h time slots.

Results

No test organism died during the acclimatization period. The acute toxicity of bifenthrin ($LC_{1,5,10,15,50,85,90,95,99}$) with 95% confidence limit to *H. fossilis* during the exposure period of 24, 48, 72 and 96h are shown in Table 1.The control set showed no mortality of fish during the entire test period.

Table 1: Acute lethal concentration (LC1,5,10,15,50,85,90,95,99) values with 95% confidence limits of bifenthrin to *H. fossilis* at 24, 48, 72 and 96h (Theoretical spontaneous response rate in control group = 0.000).

Points of Lethal Concentration	Dose values (µg/l)with 95% confidence limits in parentheses			
	24h	48h	72h	96h
LC_1	1.669 (0.721-2.299)	1.627 (0.754-2.223)	1.572 (0.863-2.071)	1.56 (0.860-2.051)
LC_5	2.277 (1.270-2.860)	2.188 (1.263-2.745)	1.994 (1.261-2.468)	1.961 (1.239-2.428)
LC_{10}	2.687 (1.714-3.220)	2.562 (1.661-3.077)	2.263 (1.543-2.712)	2.216 (1.505-2.658)
LC_{15}	3.005 (2.095-3.495)	2.85 (1.995-3.329)	2.466 (1.766-2.893)	2.406 (1.715-2.828)
LC_{50}	4.821 (4.361-5.539)	4.471 (4.034-4.979)	3.541 (3.078-3.863)	3.407 (2.933-3.724)

LC_{85}	7.734 (6.394-12.464)	7.014 (5.975-10.165)	5.085 (4.635-5.968)	4.824 (4.420-5.568)
LC_{90}	8.65 (6.926-15.262)	7.803 (6.461-12.213)	5.539 (4.975-6.790)	5.238 (4.741-6.291)
LC_{95}	10.208 (7.785-20.632)	9.137 (7.241-16.061)	6.288 (5.496-8.263)	5.917 (5.225-7.589)
LC_{99}	13.929 (9.671-36.406)	12.286 (8.941-26.927)	7.977 (6.578-12.029)	7.438 (6.217-10.880)
Slope ± SE	5.048±1.174	5.299±1.166	6.595±1.272	6.860±1.325
Intercept ±SE	1.550±0.773	1.553±0.761	1.378±0.795	1.347±0.819

There was a significant variation ($p<0.05$) in the mortality of the treated fish exposed to bifenthrin with respect to the control at all the hours of exposure. A significant variation ($p<0.05$) between rate of mortality of *H. fossilis* and time slots (24-96h) was recorded for the final selected doses of bifenthrin except 4.0, 4.5 and 5.5 µg/l concentration of the toxicant. The relationship between concentration of bifenthrin and fish mortality at 24 h was, $y = 27.75\ln(x) + 9.051$, $R^2 = 0.925$; it was $y = 29.37\ln(x) + 12.79$, $R^2 = 0.887$at 48h (Figure 1); at 72 h it wasy = $31.30\ln(x) + 31.16$, $R^2 = 0.912$ and at 96 h was $y = 30.22\ln(x) + 36.05$, $R^2 = 0.904$ (Figure 2).

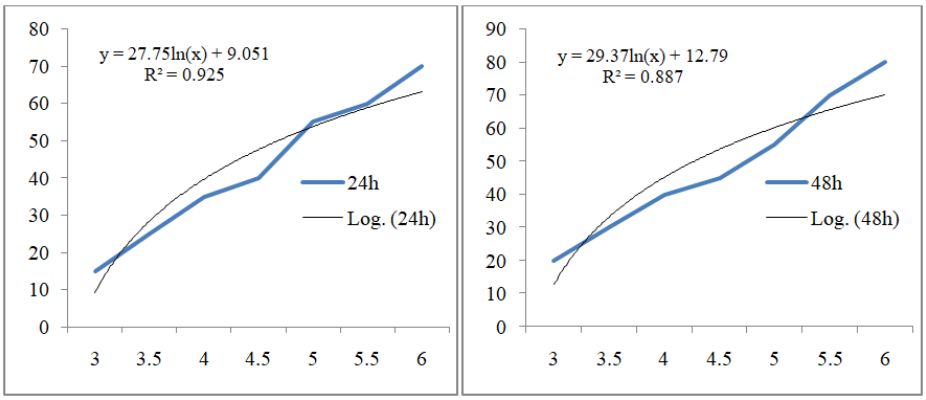

Fig. 1: Relationship between the concentrations of Bifenthrin and mortality of *H. fossilis* at 24h (left) and 48h (right)

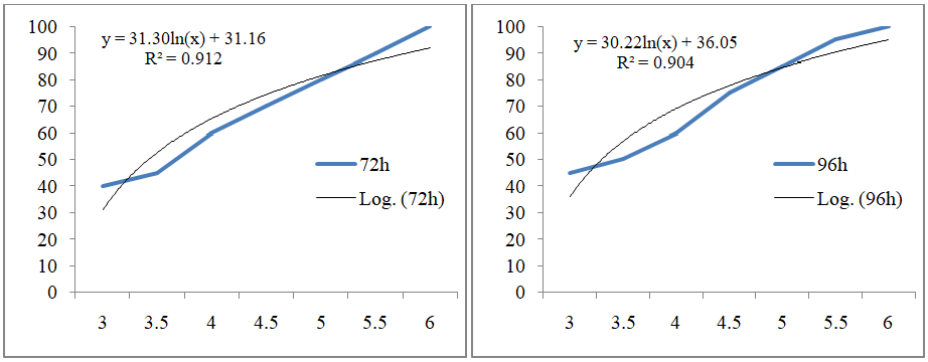

Fig. 2: Relationship between the concentrations of Bifenthrin and mortality of *H. fossilis* at 72h (left) and 96h (right)

The toxicity factors as calculated from median lethal toxicity (LC_{50}) values at different times of exposure are tabulated in Table 2. With the progress of time, the toxicity factor values for the tested fish species to bifenthrin increased gradually.

Table 2: Toxicity factors for *H. fossilis* exposed to bifenthrin at different time scale (24, 48, 72 and 96h)

Exposed time (h)	Toxicity factor value
24	1.000
48	1.078
72	1.361
96	1.415

The estimated possible safe level of bifenthrin for the fish isstated in Table 3. In the current experiment, safe level estimated for bifenthrin ranged from 0.0341 – 1.3628µg/l.

The ethological alterations noted in *H. fossilis* under exposure to various doses of bifenthrin are summarized in Table 4. The intensity of behavioral alterations increased with the increasing doses and progress of time of exposure (Table 4).

Table 3: Safe levels of bifenthrin to *H. fossilis* at 96 hours of exposure according to some International standard methods

Name of the test organism	96h LC_{50} value (µg/l)	Method	Application factor (AF)	Safe level (µg/l)
H. fossilis	3.407 (2.933-3.724)	Hart *et al*. (1948)*		0.1154
		Edwards and Brown (1966)	0.4	1.3628
		Burdick (1967), Sprague (1971) and EIFAC (1983)	0.1	0.3407
		CWQC (1972)	0.01	0.0341
		IJC (1977)	5% of 96h LC_{50}	0.1704
		CCREM (1991)	0.05	0.1704

(*C = 48h LC50 X 0.03/S2, where C= presumable harmless concentration; S = 24h LC_{50}/48h LC50)

Table 4: Impact of Bifenthrin on the behavioral parameters of *H. fossilis* (R: restlessness; ES: erratic swimming; MS: mucus secretion; NN: not noted due to death; –: none; +: mild; ++: moderate; +++: strong) at various doses during 24-96h time slots

Dose (mg/l)	24h			48h			72h			96h		
	Behaviour of *H. fossilis*											
	R	ES	MS	R	ES	MS	R	ES	MS	R	ES	MS
0.0	-	-	+	-	-	+	-	-	+	-	-	+
3.00	-	-	+	-	-	+	-	-	+	-	-	+
3.50	-	-	+	-	-	+	-	-	+	-	-	+
4.00	-	-	+	+	+	+	+	+	+	+	+	+
4.50	+	+	+	++	+	++	++	++	++	++	++	++
5.00	++	++	++	++	++	++	+++	++	++	++	+++	++
5.50	++	++	++	++	++	++	+++	++	+++	+++	+++	+++
6.00	++	+++	+++	NN	NN	NN	NN	NN	NN	NN	NN	NN

The gradual increase in dose of bifenthrin resulted in significant increase ($p<0.05$) in opercular movement of the fish with respect to the control. On the other hand, opercular movement showed a significant increase ($p<0.05$) with the advancement of time for all the treated doses.

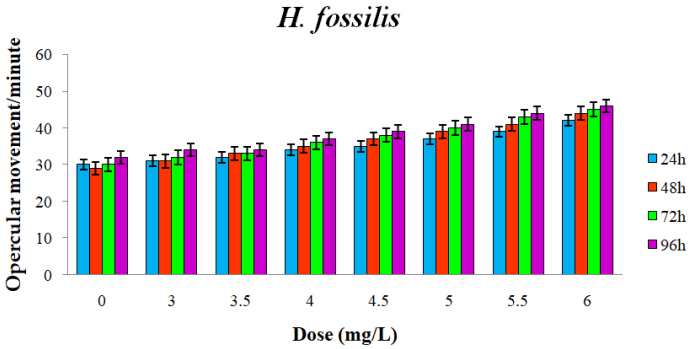

Fig. 3: Mean number (± SD) of opercular movement of *H. fossilis* exposed to bifenthrin

Discussion

Pesticides belonging to the group of pyrethroid present a risk for aquatic organisms, though they have low toxicity for aves and mammals (Bradbury and Coats, 1989). The current investigation shows the 96h median lethal concentration of bifenthrin as 3.407µg/l. The present value of 96h LC_{50} of bifenthrin to the exposed fish (3.407µg/l) is much higher in comparison to rainbow trout (1.47 µg/l), common carp (2.08 µg/l) and tilapia (0.80 µg/l) (Velisek *et al*., 2009, Liu *et al*., 2005). This variation may be because of difference in physicochemical parameters of the experimental water, age, size, health and species of fish (having accessory respiratory organ) used in the present study(Farah *et al*., 2004; Diedrich *et al*., 2015; Patra *et al*., 2015). Temperature of the test water may be a key factor in determining the degree of toxicity since lower temperature increases the toxic potential of bifenthrin to fish (Mauck *et al*, 1976).

Living organisms in response to an adverse surrounding exhibit its defensive nature by means of a vital parameter called tolerance (Enuneku and Ezemonye, 2012). Toxicity factor (TF) is an important index for tolerance assay (Ayoola *et al*. 2011). In the current investigation, the toxicity factor for the neopyrethroid, bifenthrin increases in fish with the length of period of exposure (Table 2). This is corroborated by the study of Ayoola *et al*. (2011). In our present investigation, the estimated possible safe level of bifenthrin to fish showed large variation (0.0341 – 1.3628 µg/l) due to different values of application factors (AFs) according to some International standard methods (Kennega, 1979) (Table 3).

Various changes in the ethological responses are the primary indicators of signs of toxicity to a given xenobiotic. Likewise, our study considered changes in ethology as an important tool to assess bifenthrin toxicity in fish (Table 4). Initially, hyperactivity was noted in the treated fish with respect to the control. With the advancement of time and gradual rise in concentration *H. fossilis* exhibited the symptoms of stress build up which was manifested as erratic swimming, restlessness, gasping for air, surface adherence etc. Besides, somersaulting pattern in fish also observed at the upper dose limit. Probably, this behaviour was symbolic of anescape reaction from bifenthrin (Saha *et al*., 2018). The vigorous mucus secretion in *H. fossilis* may be attributed as an evading mechanism to bifenthrin from entering the body. It is an outcome of stress and irritating effect similar to that of many other neurotoxicants. Some organ ophosphates also elicit a similar response in fish (Rao *et al*., 2005; Pandey *et al*., 2008). At all the exposures the death of fish was characterized by wheezing, repeated turning of the opercula, loss of balance, disruption in buoyant behaviour, enhanced rigor and momentary cessation of ventilation. Restlessness and erratic swimming of treated fish were probably attributed to the adverse impact of bifenthrin on the cerebrospinal nervous system (Velisek *et al*, 2009).

In fish, the opercular movements are directly related to respiratory rate, which is often the first physiological

response to be affected by the presence of toxicant in the aquatic environment (Dubeand Hosetti, 2010). In the present investigation, opercular movement in *H. fossilis* exposed to bifenthrin was found to increase significantly ($p<0.05$) in response to all doses of the pesticide (Figure 3). Gills are the principal respiratory organs of fish. The energy demand of different metabolic pathways is met up by the gills. Thus, any damage to this organ may culminate insevere respiratory ailments (Magare and Patil, 2000). The mechanism of toxicant uptake through gills probably occurs through simple diffusion (Opperhuizen *et al*., 1985). In the current investigation, gradual increase in the flapping of operculum of *H. fossilis* to bifenthrin may be a compensatory mechanism to overcome respiratory distress (Kumar *et al*., 2015).

Conclusion

This study reveals that bifenthrin is a potent toxicant and it may cause mortality in *H. fossilis* at very low concentration, even at short period of exposure. The results of the current experimental work may provide some insights onlaying out an action plan for management of this pyrethroid including the marking of maximum permissible limit for this xenobiotic before discharging them tothe aquatic ecosystem having a myriad of aquatic organisms in it. In future, more studies on the chronic toxicity of bifenthrin to aquatic organisms may open up new vistas in the field of aquatic toxicology.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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