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Acute toxicity of a pyrethroid pesticide Bifenthrin to Oreochromis mossambicus (Peters, 1852)

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ABSTRACT

In present investigation, acute toxicity of the pyrethroid pesticide Bifenthrin was carried out under experimental condition to adult *Oreochromis mossambicus* (Peters, 1852). The 96 h LC_{50} with 95% confidence limits of *Oreochromis mossambicus* is 2.423 (1.981-2.748)µg/l. None of the unexposed control fish died during the bioassay. Mortality rate of the exposed fish to the toxicant significantly ($p<0.05$) varied over the control at all concentrations during every 24h time interval. Significant relationship ($p<0.05$) was observed between mortality rate and exposure times (24, 48, 72 and 96 h) at all concentrations. Significant variation was recorded between mortality rate of fish at all the exposure doses at all the exposure times ($p<0.01$). The exposed fish exhibited abnormal ethological responses depending on dose of bifenthrin and duration of experiment. The opercular movement of the fish increased significantly ($p<0.05$) over the control with increasing concentration but decreased significantly ($p<0.05$) with the progress of time of exposure at all concentrations. The bifenthrin-based Marker pesticide was therefore classified as strongly toxic to fish.

Keywords: Bifenthrin, *Oreochromis mossambicus*, acute toxicity,behavioral responses, opercular movement

INTRODUCTION

The pesticides and some of its ingredients are highly toxic to non-target organisms and the aquatic ecosystem [1].Many laboratory and epidemiological studies have revealed that pesticides are responsible for carcinogenesis, neurotoxicity, behavioral alterations, reproductive diseases, endocrine abnormalities, developmental disabilities and respiratory dysfunction on non-target organisms [2]. Pesticides come to the adjacent water bodies as agricultural runoff and affect different non-target species such as fish which have an economic importance to man [3].Contamination of surface waters by pesticides used in agriculture field is a worldwide problem [4, 5]. Through food chain and biomagnifications pesticides manifest their toxic effects in the aquatic organisms and ultimately affect on human beings [6].

Bifenthrin [2-methylbiphenyl-3-ylmethyl (Z)-(1RS, 3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate] is a synthetic pyrethroid insecticide [7].It is characterized by strong environmental persistence and has high insecticidal activity [8]. It is effective as a gut or contact insecticide that affects the nervous system of vertebrates and invertebrates [9]. Bifenthrin acts on sodium channels at the nerve cell endings to depolarize the pre synaptic terminals [9]. It also affects cellular ATPase production [10]. The reports on the toxicity of bifenthrin on fish are scanty [7]. There is no report on the toxicity of bifenthrin on *O. mossambicus*. The aims of the present investigation were thus to evaluate the acute toxicity, ethological responses and alteration of respiratory responses of *O. mossambicus* exposed to bifenthrin.

MATERIALS AND METHODS

Adult fresh water fish, *Oreochromis mossambicus* (mean length 70.50±3.46 mm, mean weight 20.50±3.43g) belonging to Class Teleostomi and Family Cichlidae was used in the bioassays as the test organism. The fish were collected from the local unpolluted private fish farm at Barasat and allowed to acclimatize to the test condition prior to 96h of the experiment. While acclimatization the test organisms were kept in the rectangular cement tanks of 1000 litre capacity filled with unchlorinated, well aerated water (pH 7.10 ± 0.45; temperature 26.63 ± 1.22 °C) under 12 h each dark and light cycle) [11]. During these periods, food was supplied to the fish in the form of commercial pellets with 38% crude protein.

The commercial grade Bifenthrin [2-methylbiphenyl-3-ylmethyl (Z)-(1RS, 3RS)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate] was used as the test chemical which is a third-generation synthetic pesticide belonging to the pyrethroid group [7].

Static replacement bioassay test with the healthy, disease free fish (irrespective of sex) was conducted in 15l glass aquaria containing 10l un-chlorinated water following the methods outlined in American Public Health Association[11].The physico-chemical values of different parameters of water used in the experiment were as follows: temperature 26.5 ± 0.15 0C, pH 7.9 ± 0.25, free CO_2 10.7 ± 0.45 mg/l, DO 5.69 ± 0.33 mg/l, alkalinity 171 ± 11.21 mg/l as $CaCO_3$, hardness 118 ± 4.10 mg/l as $CaCO_3$.Each bioassay was accompanied by four replicates with control. Each replicate was provided with ten fish randomly. They were not fed for 24h prior to commencement of the experiment.

Rough range finding experiments were conducted initially to determine range of concentrations at which the mortality of fish may occur. The selected test doses of bifenthrin finally used to determine the 24, 48, 72 and 96h median lethal (LC_{50}) values were 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 µg/l. During the experiment, the dead fish were removed quickly from the aquaria to avoid any microbial decomposition causing decrease the level of dissolved oxygen and the number of dead fish was recorded at every 24h interval. The 10% of the test water was replaced by newly prepared test water at every 24h interval to maintain a fixed concentration.

Toxicity factors of the tested organism to bifenthrin at different time of exposure was assessed after Ayoola et al. [12] by multiplying LC_{50} value at 24h with LC_{50} at any other exposure time.

The safe level estimation for *O. mossambicus*was calculated by multiplying the 96h LC_{50} with different application factors (AF) based on Edwards and Brown [13], Burdick [14], Sprague [15], Committee on Water Quality Criteria [16], International Joint Commission [17], European Inland Fisheries Advisory Commission [18] and Canadian Council of Resources and Environmental Ministry [19] and also on the basis of formula developed by Hart et al.[20].

Mean mortality of *O. mossambicus*after 24, 48, 72 and 96h of bioassay was used to calculate the LC_{50} values (with 95% confidence limit) through the statistical software Probit program version 1.5 [21]. The lethal concentration (LC_{50}) was determined in MS Excel by plotting the test doses against the fish mortality within 24 hr, 48 hr, 72hr and 96h after the experiment [22]. The values of percentile mortality of the fish were subjected to analysis of variance (ANOVA) using R-software [23] succeeded by Duncan's Multiple Range Test (DMRT) to find out the significant difference within the mean values at different doses of bifenthrin at 24, 48, 72 and 96h of exposure. The relation between mortality rate with exposure time and doses was evaluated using correlation analysis [21, 24].

The behavioral alterations like restlessness, erratic swimming, and mucus secretion in the exposed fish were also recorded during the experiment [25].Changes in the opercular movements to determine respiratory rates of the fish exposed to different concentrations of the toxicant were also recorded during 96h bioassay condition. Opercular movements of the fish per minute for both control and exposed were counted twice a day at every 24h during the bioassay and their mean values per concentration were plotted graphically.

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 *O. mossambicus*during the exposure period of 24, 48, 72 and 96h are given in **Table 1**. No mortality was also observed in the control group during the test.

Table 1 Acute lethal concentration ($LC_{1,5,10,15,50,85,90,95,99}$) values with 95% confidence limits of bifenthrin to *O. mossambicus*at 24, 48, 72 and 96h (Control group theoretical spontaneous response rate = 0.000)

Lethal Concentration parameters	**Concentration values with 95% confidence limits (µg/l)**			
	24h	**48h**	**72h**	**96h**
LC_1	1.314 (0.726-1.772)	0.837 (0.345-1.272)	0.919 (0.500-1.275)	0.781 (0.379-1.132)
LC_5	1.882 (1.243-2.331)	1.285 (0.675-1.749)	1.278 (90.803-1.646)	1.088 (0.619-1.457)
LC_{10}	2.279 (1.652-2.706)	1.614 (0.963-2.077)	1.523 (1.031-1.889)	1.299 (0.804-1.669)
LC_{15}	2.594 (1.997-3.000)	1.883 (1.223-2.336)	1.715 (1.221-2.075)	1.463 (0.958-1.831)
LC_{50}	4.480 (4.033-5.116)	3.612 (3.140-4.092)	2.831 (2.434-3.152)	2.423 (1.981-2.748)
LC_{85}	7.739 (6.405-11.093)	6.929 (5.714-10.120)	4.675 (4.164-5.578)	4.013 (3.584-4.712)
LC_{90}	8.808 (7.079-13.446)	8.083 (6.434-12.828)	5.264 (4.608-6.552)	4.521 (3.986-5.538)
LC_{95}	10.668 (8.198-17.911)	10.157 (7.648-18.285)	6.275 (5.321-8.367)	5.396 (4.617-7.109)
LC_{99}	15.281 (10.765-30.753)	15.586 (10.527-35.712)	8.725 (6.908-13.353)	7.517 (5.999-11.518)
Slope ± SE	4.365±0.758	3.663±0.683	4.759±0.748	4.731± 0.797
Intercept ±SE	2.156±0.470	2.956±0.408	2.848±0.420	3.181±0.429

Significant relationship ($p<0.05$) between mortality rate of *O. mossambicus*and exposure times (24, 48, 72 and 96h) was recorded at all doses of the toxicant except 3.5, 4.5 and 5.0 µg/l concentrations of the toxicant. The relation between concentration of bifenthrin and fish mortality at 24 h was, $y = 30.70\ln(x) - 3.671$, $R^2 = 0.876$; it was $y = 29.22\ln(x) + 11.21$, $R^2 = 0.941$ at 48h (figure 1); at 72 h it was$y = 32.36\ln(x) + 22.29$, $R^2 = 0.893$ and at 96 h was $y = 28.18\ln(x) + 36.57$, $R^2 = 0.939$ (figure 2).

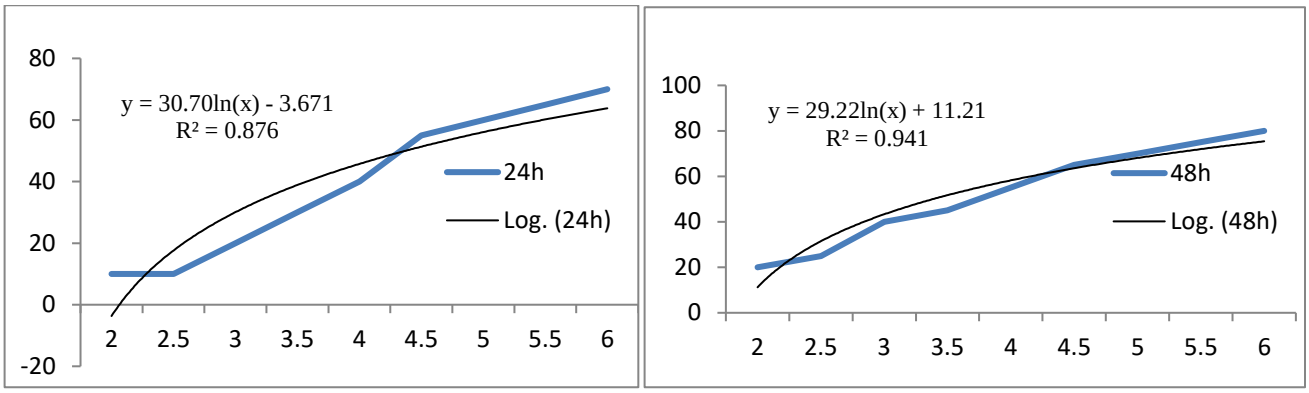

Figure 1 Relationship between the concentrations of Bifenthrin and mortality of *O. mossambicus* during 24h (left) and 48h (right)

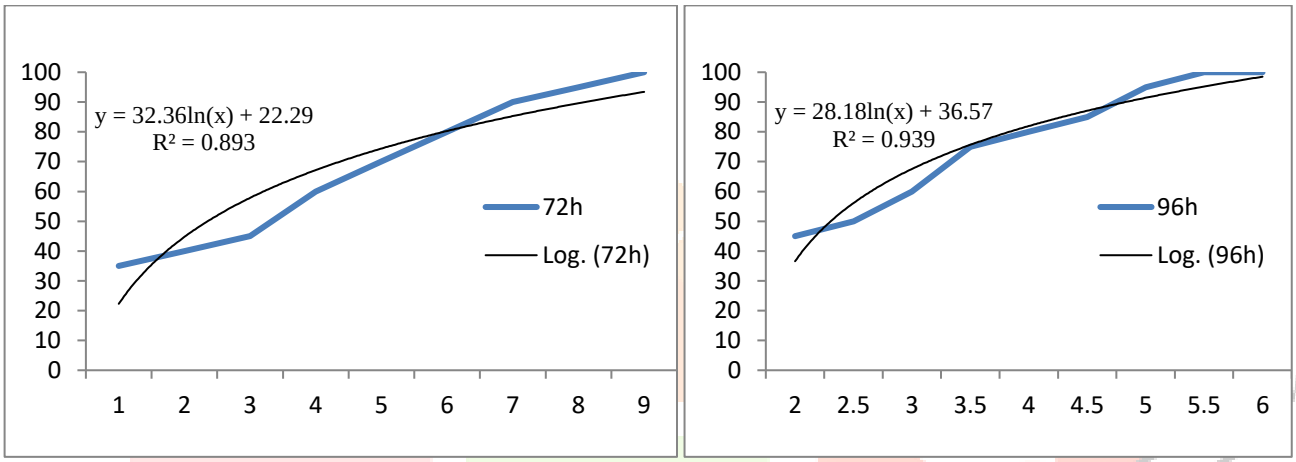

Figure 2 Relationship between the concentrations of Bifenthrin and mortality of *O. mossambicus* during 72h (left) and 96h (right)

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

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

Exposed time (h)	Toxicity factor value
24	1.000
48	1.240
72	1.582
96	1.849

The estimated possible safe level of bifenthrin for the fish as calculated by multiplying their 96h LC_{50} values with different application factors and formula are recorded in **Table 3**. In the present study, the safe level was estimated for the toxicant at 0.070 – 0.969 µg/l**.**

Table 3: Estimate of safe levels of bifenthrin to *O. mossambicus* at 96h ofexposure time

Name of the test organism	96h LC_{50} value (µg/l)	Method	Application factor (AF)	Safe level (µg/l)
O. mossambicus	2.423	Hart et al. (1948)*	-	0.070
		Edwards and Brown (1966)	0.4	0.969
		Burdick (1967), Sprague (1971) and EIFAC (1983)	0.1	0.242
		CWQC (1972)	0.01	0.024
		IJC (1977)	5% of 96h LC_{50}	0.121
		CCREM (1991)	0.05	0.121

(*C = 48h LC_{50} X $0.03/S^2$, where C is the presumable harmless concentration and S = 24h LC_{50}/48h LC_{50})

The ethological alterations observed in the test organisms exposed to various lethal concentrations 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). At higher doses somersaulting of fish observed. Probably this was an early indication of their avoidance reaction from the test chemical (Saha et al., 2018).

Table 4 Impact of Bifenthrin on the behavioral parameters of *O. mossambicus* (R: restlessness; ES: erratic swimming; MS: mucus secretion; x: not recorded due to death; –: none; +: mild; ++: moderate; +++: strong) at various concentrations during different hours of exposure.

Dose (µg/l)	24h			48h			72h			96h		
	Behaviour of *O. mossambicus*											
	R	ES	MS	R	ES	MS	R	ES	MS	R	ES	MS
0.00	-	-	+	-	-	+	-	-	+	-	-	+
2.00	-	-	+	-	-	+	-	-	+	-	-	+
2.50	-	-	+	-	-	+	-	-	+	-	-	+
3.00	-	-	+	+	+	+	+	+	+	+	+	+
3.50	+	+	+	++	+	++	++	++	++	++	++	++
4.00	++	++	++	++	++	++	+++	++	++	++	+++	++
4.50	++	++	++	++	++	++	+++	++	+++	+++	+++	+++
5.00	++	++	++	+++	++	+++	+++	+++	+++	x	x	X
5.50	++	++	++	+++	+++	+++	x	x	x	x	x	X
6.00	++	+++	+++	x	x	x	x	x	x	x	x	X

Significant ($p<0.05$) time and dose dependent relationship in respect of opercular movement in the exposed *O. mossambicus* over their control group was observed (Figure 3). The opercular movement of the fish increased significantly ($p<0.05$) over the control with increasing concentrations at all exposure time. Similarly, it was significantly ($p<0.05$) increased with progress of time at all treatments.

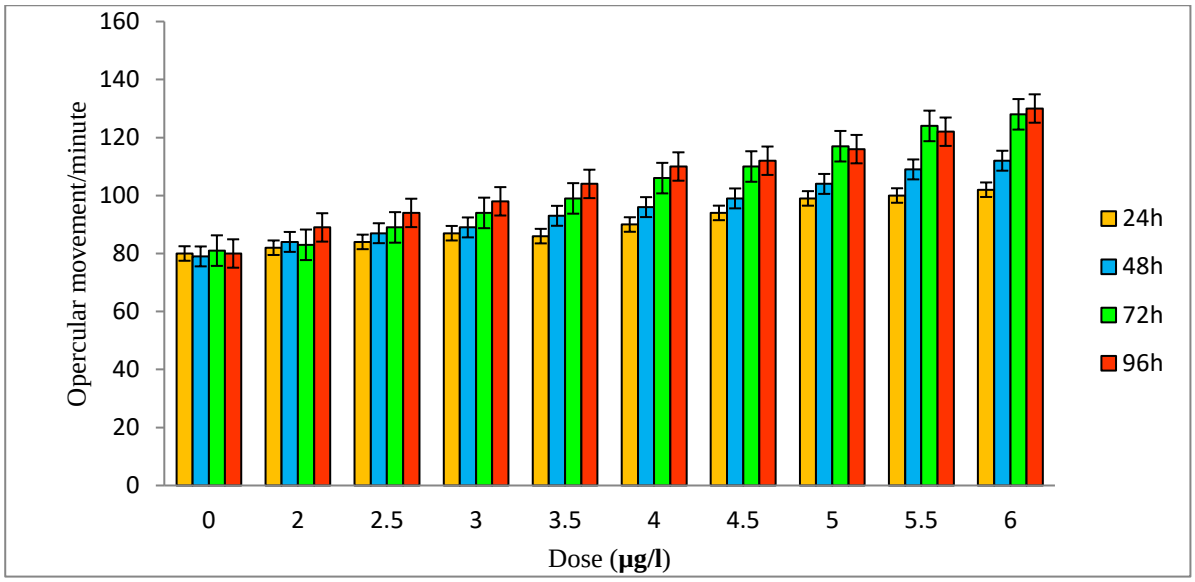

Figure 3: Mean opercular movement (no. /minute) of *O. mossambicus* exposed to bifenthrin.

DISCUSSION

Indiscriminate and increased use of pesticides by human activities to control different pests may cause high risk to non-target organism's especially aquatic organisms [7]. The surface water contamination by pesticides used in agriculture is a worldwide problem [4, 5]. Various earlier workers reported different effects of pesticides on the aquatic organism as well as aquatic ecosystem[26, 27]. Pesticides belonging to the group of pyrethroids present a risk for aquatic organisms, thoughthey have low toxicity for mammals and birds[28]. The present study indicates that the bifenthrin is toxic to *O. mossambicus*. The 96h LC_{50} value of bifenthrin to the tested fish (2.423 µg/l) is much higher than the findings of the earlier workers (Liu et al., 2005; Velisek et al., 2009)[9, 29]. Velisek et al. reported 1.47 µg/l of bifenthrin as 96h LC_{50} value to rainbow trout. Liu et al. stated the 96h medial lethal toxic values of 2.08 µg/l and 0.80 µg/l for common carp and tilapia (Tilapia spp.) respectively. The differences in our result may be associated with differentness in limnological parameters of the test medium and also with age, size, health and species variation[30, 31, 32, 33]. It was reported that bifenthrin is more toxic at lower temperatures, and thus more toxic to cold than warm water fish, but the toxicity of pyrethroids is little affected by pH or water hardness [34].

The toxicity study is essential to find out toxicant limit and safe concentration as such there will be minimum harm to aquatic fauna [7]. Toxicity factor (TF) at different time of exposure may be explained by the degree of tolerance of the tested organism to the toxicant [35].With the progress of time of exposure, the toxicity factor for bifenthrin increases to the worm in the present study (Table 2). This is in conformity to the some earlier records on fish exposed to other toxicant [36, 37].This is probably in accordance with the degree of decreased uptake, increased excretion or redistribution of the metal to less sensitive target sites [38].

The estimated possible safe level for bifenthrin as recorded in the present study (**Table 3**) showed large variation (0.070 – 0.969 µg/l) due to adoption of different application factors (AFs) and its dependence on LC_{50} value [39].So the findings indicate that it is difficult to decide the acceptable concentration as "safe" for the toxicant to *O. mossambicus*[40, 41, 42].The comment is in conformity to some earlier workers in different aquatic species exposed to various pesticides [43, 44, 45].

The observation on the ethological responses of the fish in the present study (**Table 4**) may be an indicative parameter for assessing the toxicity of bifenthrin in the ecosystem.

The opercular movements in fish 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 [46]. In the present study, opercular movement in *O. mossambicus* exposed to bifenthrin was found to be increased significantly in response to all concentrations of the pesticide tested (**Table 5**).Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply and damage to this vital organ may lead to respiratory distress[47].A mechanism of toxicant uptake through gills probably occurs through simple diffusion [48]. In the present study gradual increase in the opercular movement of the exposed fish to bifenthrin may be indicative of the sequence of the type of compensatory mechanism to overcome the load of stress[49].

CONCLUSION

This experimental work reveals that bifenthrin is a potent toxicant and may cause mortality in *O. mossambicus* at very low concentration, even at short period of exposure. The value of 96h LC_{50} of bifenthrin to *O. mossambicus* may be used for determining the safe dose of the pesticide prior to release to the aquatic environment.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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