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Assessment of Acute Toxicity Test of Fipronil in *Clarias batrachus* (Linnaeus, 1758) and *Heteropneustus fossilis* (Block, 1794)

Chandrahasini Nirnejak*[1] and Neha Jain[2]

[1,2]Department of Zoology, Guru Ghasidas Vishwavidyalaya, Koni, Bilaspur - 495009 (India)
[1]Email: chandrahasini.jak01@gmail.com
[2]Email: drnehajain206@gmail.com

Abstract

Fipronil, a phenylpyrazole insecticide with a broad range of applications, is extensively utilized in both agriculture and public health sectors. Determining its median lethal concentration (LC_{50}) is crucial for assessing its ecotoxicological effects on non-target aquatic and terrestrial species. This research seeks to ascertain the LC_{50} value of fipronil by conducting acute toxicity bioassays on *Clarias batrachus* and *Heteropneustus fossilis*. The organisms were subjected to varying concentrations of fipronil (0mg/l, 1.0mg/l, 1.2mg/l, 1.4mg/l, 1.6mg/l, 1.8mg/l, 2.0mg/l, and 2.2mg/l) over a 96-hour period in a controlled laboratory condition. Throughout the experiment, the physicochemical properties of the water were monitored. Mortality rates were recorded at regular intervals, and probit analysis was used to calculate the LC_{50} value with 95% confidence intervals. The LC_{50} value for *Clarias batrachus* was found to be 1.6mg/l, while for *Heteropneustus fossilis*, it was 1.46mg/l. These findings contribute to environmental risk assessment frameworks and aid in establishing regulatory guidelines for the safe use of pesticides. Our results also highlight the necessity for further research on sub-lethal and chronic effects to ensure thorough environmental safety evaluations.

Key words : Fipronil, insecticide, LC_{50}, *Clarias batrachus, Heteropneustus fossilis.*

Worldwide, including India, there is an increase in the indiscriminate application of agricultural pesticides to eradicate pest which is increased 24,232.62 MT in the year of 2021[25]. During the 1960s, the Green Revolution promoted increasing crop production through all means possible, including the use of chemical fertilizers, pesticides[40]. According to Jayaraj *et al*.,[19] crop losses in India are estimated to be more than INR 60 billion a year due to a variety of factors, including "weeds (33 %), diseases (26 %), insects (20

[1]Research Scholar, [2]Assistant Professor,

%), birds (10 %), rodents, and others (11%). The use of pesticides is recommended to control the pest for qualitative and quantitative growth of crops. According to Ram *et al*.,[27] there are three main routes of exposure exist for fish and other non-target species to pesticides that is dermal, inhalation and direct absorption. Chemicals such as pesticides are produced by human activity and agricultural practices and they can leak into water sources either directly or indirectly. Such pesticides enter the trophic levels through the food chain and affect human beings. Phenylpyrazole is widely utilized pesticide classes for pest management and they negatively impact aquatic wildlife. Low concentrations of these pesticides are typically sufficient to kill fish and reduce their population.

Toxicity is classified into acute and chronic and assessment of the median lethal concentration (LC_{50}) is the considered as a preliminary step for examining the degree of acute or chronic toxicity. However, the LC_{50} value varies with different pesticides and fish species. Thus, Carbendazim has LC_{50} value of 11.4 µg/l in *C. chanos*[24], while in *Clarias gariepinus*, the LC_{50} values of endosulfan, heptachlor and dieldrin were calculated as 0.004 mg/l, 0.056 mg/l, and 0.006 mg/l, respectively[18]. Similarly, 96h acute toxicity of bifenthrin and carbosulfan in *Gara mullya* (sykes) was determined as 1.2537 and 6.9807 ppm, respectively[4]. Whereas, the LC_{50} value for malathion and carbendazim was estimated as in fish *Hyphessobrycon erythrostigma* 252 and 3690 µg/l, in *Paracheirodon axelrodi* 247 and 1648 µg/l, *Nannostomus unifasciatus* 111, 4138 µg/l and in *Otocinclus affinis* 1067 and 4238 µg/l, in *Colossoma macropomum* 1507, 4162 µg/l respectively[28]. The median lethal concentration with 95% confidence limits at 96 h of triazophos, carbofuran, carbaryl and profenophos were 6.64 mg/L, 1.40 mg/l, 8.24 mg/l and 0.32 mg/L in *Labeo rohita*, respectively[22].

Fipronil is a phenylpyrazole insecticide inhibit GABA receptors in CNS[5] and reported as a highly toxic insecticide affect non target aquatic life by altering their behaviour response[30,38], biochemical parameters[2,9,11,26,30], hematological parameters[9,11,26,30,37], genotoxicity[3,11,13,30,37], oxidative stress[3,30,37,38], neurotoxicity[14,15,35], histopathology[2,12,26]. The effects of pollutant exposure on organisms are ascertained through toxicity tests.

Fish are the primary targets of toxicants among aquatic organisms and provide a valuable model for experimentation in evaluating the aquatic ecosystem's quality. This study evaluated the acute toxicity tests of pesticide fipronil to fish *Clarias batrachus* and *Heteropneustus fossilis.*

Preparation of test chemicals : Technical grade Fipronil (5% SC) were purchased from local market. Different concentration of pesticides ranging between 0% to 100% mortality was prepared by using Charles's equation (C1 V1 = C2 V2).

Collection and acclimatization of fish : The active and healthy fish *Clarias batrachus* and *Heteropneustus fossilis* with mean body weight (35±5) and length (15±5) were collected. For avoidance of any skin infection, the glass aquaria were cleaned with 1% potassium permanganate ($KMnO_4$) and therefore sun dried and fish were treated with

0.05% $KMnO_4$ solution before the exposure tests. Fish were acclimatized for 15 days in aerated glass aquarium and aquarium water were replenished on daily basis to maintain the physiochemical properties of water at optimum condition. Commercial food was used to fed fish once a day at a rate of 3% of their body weight. No mortality occurred during this period.

Experiment design : To determine median lethal concentration (LC_{50}) of fipronil, *Clarias batrachus* and *Heteropneustus fossilis* were exposed to different concentration of fipronil solution *i.e.*, 0mg/l, 1.0mg/l, 1.2mg/l, 1.4mg/l, 1.6mg/l, 1.8mg/l, 2.0mg/l, 2.2mg/l for 96 hrs. Fish were monitored throughout the experiment and mortality were recorded at 24h, 48h, 72h and 96h and physiochemical properties of water like temperature, pH, dissolve oxygen, free carbon dioxide, ammonical nitrogen were monitored throughout the experiment by using multimeter.

Estimation of safe level : Safe level of fipronil were estimated according to Hart *et al.*[16] by using formula: 48 h $LC_{50} \times 0.03 \times S^2$, where S= 24 h LC_{50} /48h LC_{50}

Statistical analysis : The LC50 value of fipronil with 95% confidence limits were estimated by using Finney[10] probit analysis method.

Determination of median lethal concentration (LC_{50})*:*

Acute toxicity test was conducted to determine median lethal concentration at different exposure periods. The mortality rate of test fish for different concentration of fipronil *i.e.*, 0mg/l, 1.0mg/l, 1.2mg/l, 1.4mg/l, 1.6mg/l, 1.8mg/l, 2.0mg/l, and 2.2mg/l were examined at 24h, 48h, 72h and 96h exposure period. 0% mortality resulted during acclimatization and control group during experiments. Mortality rate of test fish was increased significantly with increasing concentration of fipronil. Mean %mortality at different concentration and exposure time is presented in Table-1 and Table-2. The LC_{50} value for 96h was calculated 1.6mg/l and 1.46mg/l for *Clarias batrachus* and *Heteropneustes fossilis* respectively results in 50% fish mortality presented in Table-3.

Table-1. Mean mortality of fish *Clarias batrachus* exposed to different concentration of fipronil for 96hrs.

Concentration (mg/l)	Number of fish	Duration of exposure				Mean %Mortality
		24h	48h	72h	96h	
Control	09	00	00	00	00	00
1.0 mg/l	09	00	00	00	00	00
1.2 mg/l	09	00	00	00	01	11
1.4 mg/l	09	00	00	01	02	22
1.6 mg/l	09	00	01	02	03	33
1.8 mg/l	09	00	01	03	05	55
2.0 mg/l	09	01	03	06	07	77
2.2 mg/l	09	02	04	07	09	100

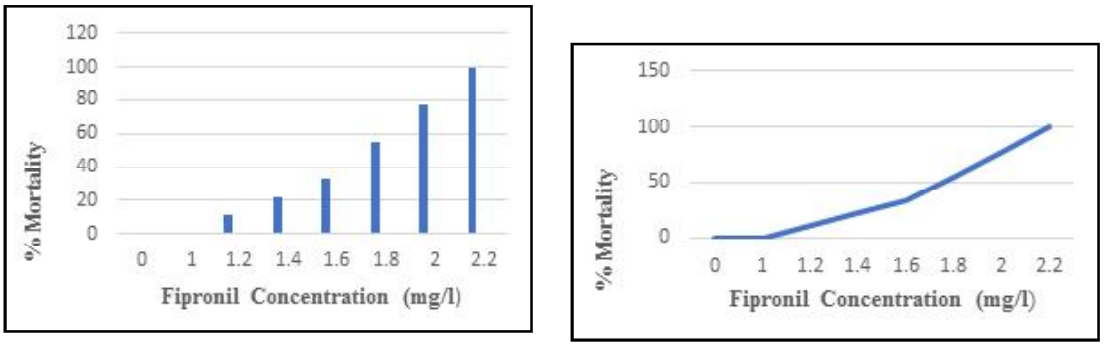

Fig. 1. Dose response relationship between fipronil concentration and percent mortality of *Clarias batrachus*

Table-2. Mean mortality of fish *Heteropneustes fossilis* exposed to different concentration of fipronil for 96hrs.

Concentration (mg/l)	Number of fish	Duration of exposure				Mean %Mortality
		24h	48h	72h	96h	
Control	09	00	00	00	00	00
1.0 mg/l	09	00	00	00	01	11
1.2 mg/l	09	00	00	01	02	22
1.4 mg/l	09	00	01	02	03	33
1.6 mg/l	09	01	02	03	04	44
1.8 mg/l	09	01	02	04	05	55
2.0 mg/l	09	02	04	05	08	88
2.2 mg/l	09	03	05	07	09	100

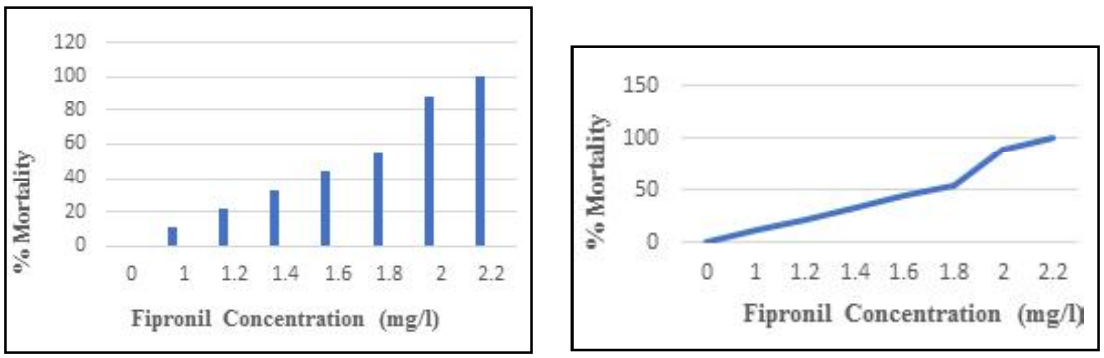

Fig. 2. Dose response relationship between fipronil concentration and percent mortality of *Heteropneustes fossilis*

Table-3. Median lethal concentration (LC_{50}) of fipronil for *Clarias batrachus and Heteropneustes fossilis*

Fish species	Exposure period	LC_{90} Value (mg/l)	LC_{50} Value (mg/l)	LC_{10} value (mg/l)	Safe Value
Clarias batrachus	24 h	3.27	2.74	2.30	0.10mg/l
	48 h	2.43	2.11	1.83	
	72 h	2.20	1.88	1.61	
	96 h	1.97	1.6	1.29	
Heteropneustes fossilis	24h	2.70	2.25	1.88	0.07 mg/l
	48h	2.38	1.99	1.67	
	72h	2.26	1.82	1.46	
	96h	1.94	1.46	1.32	

Physiochemical properties of water :

The physiochemical properties of water during experiment at different concentration of fipronil are listed in Table-4. pH ranges from 6.62 to 6.82, temperature varies from 28.40 to 29.20 °C. dissolved oxygen, free CO_2 and ammonical nitrogen ranged from 7.12 to 6.84mg/l, 0.5 to 0.66mg/l and 2.14 to 2.30mg/l respectively.

Table-4. Physiochemical properties of water

Fish species	Concentra-tion	pH	Tempe-rature (°C)	Dissolve O_2 (mg/l)	Free Co_2 (mg/l)	Ammonical nitrogen (mg/l)
Clarias batrachus	Control	6.62±0.04	28.40±0.10	7.12±0.05	0.5±0.03	2.14±0.25
	1.0 mg/l	6.63±0.28	28.40±0.22	7.10±0.04	0.51±0.04	2.15±0.20
	1.2 mg/l	6.66±0.23	28.60±0.13	6.98±0.02	0.52±0.01	2.18±0.22
	1.4 mg/l	6.69±0.33	28.60±0.16	6.95±0.03	0.54±0.02	2.19±0.24
	1.6 mg/l	6.73±0.25	28.78±0.18	6.93±0.01	0.57±0.02	2.20±0.18
	1.8 mg/l	6.76±0.28	28.84±0.15	6.88±0.02	0.62±0.04	2.22±0.22
	2.0 mg/l	6.80±0.18	29.20±0.13	6.86±0.04	0.62±0.05	2.25±0.24
	2.2 mg/l	6.82±0.18	29.20±0.20	6.84±0.03	0.64±0.04	2.29±0.18
Heteropneustes fossilis	Control	6.62±0.04	28.40±0.12	7.12±0.02	0.51±0.03	2.14±0.21
	1.0 mg/l	6.62±0.22	28.41±0.18	7.12±0.05	0.51±0.01	2.15±0.26
	1.2 mg/l	6.64±0.18	28.48±0.23	6.99±0.04	0.53±0.05	2.18±0.16
	1.4 mg/l	6.65±0.23	28.56±0.21	6.95±0.01	0.54±0.02	2.21±0.22
	1.6 mg/l	6.68±0.20	28.59±0.23	6.92±0.05	0.57±0.03	2.21±0.28
	1.8 mg/l	6.69±0.19	28.65±0.18	6.90±0.03	0.62±0.02	2.25±0.23
	2.0 mg/l	6.73±0.21	28.76±0.20	6.87±0.03	0.63±0.05	2.26±0.20
	2.2 mg/l	6.75±0.16	28.93±0.16	6.84±0.04	0.66±0.03	2.30±0.25

Table-5. Safe level of fipronil for *Clarias batrachus* and *Heteropneustes fossilis*

Fish species	96h LC_{50} value	Method	Application factor	Safe level
Clarias batrachus	1.6 mg/l	Hart *et al.* (1948)[16]	48 h LC_{50} × 0.03 x S^2	0.10mg/l
		CWQC (1972)[7]	0.01	0.016 mg/l
		Sprague (1971)[34]	0.1	0.16 mg/l
		CCREM (1991)[6]	0.05	0.08 mg/l
		IJC (1977)[17]	5% of 96h LC_{50}	0.08 mg/l
Heteropneustes fossilis	1.46 mg/l	Hart *et al.* (1948)[16]	48 h LC_{50} × 0.03 x S^2	0.07 mg/l
		CWQC (1972)[7]	0.01	0.014mg/l
		Sprague (1971)[34]	0.1	0.146mg/l
		CCREM (1991)[6]	0.05	0.073 mg/l
		IJC (1977)[17]	5% of 96h LC_{50}	0.073 mg/l

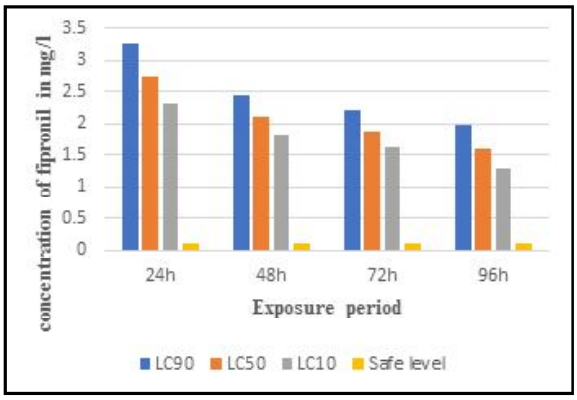

Figure 3. Comparison of LC_{90}, LC_{50}, LC_{10} and safe value of fipronil for fish *Clarias batrachus*

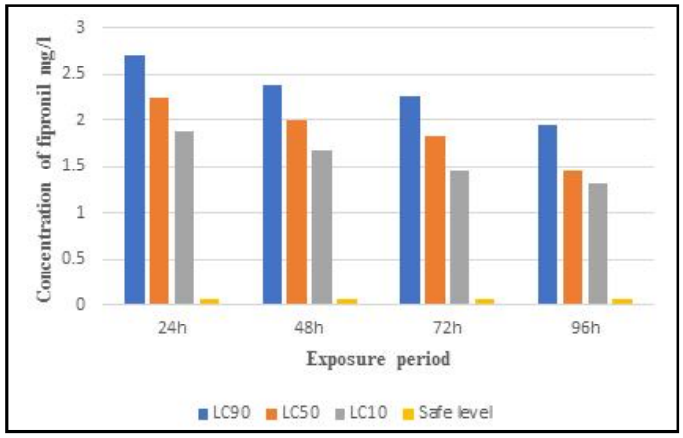

Figure 4. Comparison of LC_{90}, LC_{50}, LC_{10} and safe value of fipronil for fish *Heteropneustes fossilis*

Pesticide toxicity of waterways is a serious issue that causes numerous health issues in developing nations. This experiment was conducted to assess the acute toxicity (LC_{50} value) of the insecticide fipronil on *C. batrachus* and *H. fossilis* due to their ecological as well as economical significance in aquaculture. The calculated 96-hour LC_{50} value provides critical insight into the vulnerability of this species to fipronil exposure under controlled laboratory conditions.

Our result indicates that fipronil demonstrates significant toxicity toward *C. batrachus* and *H. fossilis*, with an LC_{50} value of 1.6mg/L and 1.46mg/l respectively. This level of toxicity is consistent with previous studies that have demonstrated the high sensitivity of freshwater teleosts to phenylpyrazole-based insecticides.

The present study's determination of the 96h LC_{50} value of fipronil for *Clarias batrachus* and *Heteropneustes fossilis* is less than 3.0mg/l for fish *Oreochromis mossambicus*[30] and more than 64.6 µg/L for *Tor khudree*[8], 0.172mg/l for *H. eques*[21], 0.246 mg/L for *Oncorhynchus mykiss*[37], 0.22 mg/L for post-larvae and 0.33mg/l for *Colossoma macropomum* fingerling[29], 597 µg/L for *Danio rerio*[20].

In contrast, LC_{50} value of fipronil for *clarias batrachus* and *Heteropneustes fossilis* was greater than 0.42 µg/L for *Chironomus crassicaudatus* (midge larvae), 0.42 µg/L for *Glyptotendipes paripes* (midge larvae), 0.43 µg/L for *Aedes taeniorhynchus* (mosquito larvae)[1], 0.43 µg/L for *Chironomus tepperi* (midge larvae)[36], 0.24 µg/L for *Simulium vittatum* (black fly larvae)[23]. Compared to other fish species, *Clarias batrachus* appears moderately sensitive to Fipronil, although further interspecies comparison studies are needed to validate this. The use of *C. batrachus* and *H. fossilis* as a model species is supported by its wide distribution, ease of maintenance, and ecological relevance, especially in South and Southeast Asia.

Various factors may influence the LC_{50} value like acute toxicity test method, toxicant's purity percentage health status and size of test fish. For example, Wang *et al.*,[39] reported Tebuconazole 96h LC_{50} value of 2.7 mg/L for embryo, 1.1 mg/L for larvae, 3.5 mg/L for juvenile and 4.8 mg/L for adult Zebra fish and Bifenthrin 96h LC_{50} value of 0.41 mg/L for embryo, 0.18 mg/L for larvae, 0.013 mg/L for juvenile, 0.015 mg/L for adult Zebra fish. LC50 value of Malathion were reported as 1.51mg/l for Fingerling[28] and 15.8mg/l for Juvenile[32]. In addition to fish sizes, the LC_{50} of fishes also depends on temperature and characteristics of the water quality, such as temperature, pH, dissolve O_2 and CO_2. Cypermethrin 96h LC_{50} value at 15±1°C, 20±1°C and 25±1°C was 1.54 mg/L, 1.49 mg/L and 1.30 mg/L respectively[31]. Barrage 96h LC_{50} value was 3.34 mg/L, 1.34 mg/L and 0.43 mg/L at pH 6.0, 7.5 and 9.0 respectively[33] Our result state that temperature, pH, free Co_2, and ammonical nitrogen slightly increased whereas dissolved oxygen slightly decreased with increasing concentration of fipronil in 96h fipronil exposed test water and similar result is also reported by Mustafa *et al.*,[22].

The resulted LC_{50} value state that *C. batrachus* and *H. fossilis* is highly susceptible

to the toxic effect of fipronil. Consequently, exposure of these pesticides may induce various physiological alteration in fish, endangering the fish population within the aquatic ecosystem.

A dose response relationship, the lethal concentration of fipronil to catfish *C. batrachus* and *H. fossilis* is evaluated in this experiment which showed that the mortality rate was directly correlated with the pesticide concentration. The study establishes the acute toxicity thresholds of fipronil for two important freshwater fish species, highlighting its potential risk to non-target aquatic organisms. The LC_{50} values obtained are essential reference points for environmental surveillance and regulatory actions. These results highlight the need for rigorous pesticide management strategies and emphasize the importance of further research into the prolonged ecological impacts of fipronil exposure.

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