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Assessment of ameliorative effect of *Moringa oleifera* leaves against fipronil induced biochemical responses in *Clarias batrachus*

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

[1]Research scholar, Department of Zoology, Guru Ghasidas Vishwavidyalaya, Koni, Bilaspur, Chhattisgarh, India. Email: chandrahasini.jak01@gmail.com

[2]Assistant professor, Department of Zoology, Guru Ghasidas Vishwavidyalaya, Koni, Bilaspur, Chhattisgarh, India.

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ABSTRACT

The present experiment examined the biochemical changes caused by sublethal dosage of fipronil and measured the ameliorative effects of *Moringa oleifera* leave as a dietary supplementation in the fish *Clarias batrachus*. In the experiment serum biochemical parameters were used to evaluate the toxic impact of fipronil on fish subjected to graded doses over 10, 20 and 30 days at concentrations containing a 1/30 of LC_{50} (0.05mg/l), 1/20 of LC_{50} (0.08mg/L) and 1/10 of LC_{50} (0.16mg/l). Findings showed that a pronounced, concentration and time-dependent increases in hepatic enzymes (AST, ALT and ALP), which reflects metabolic stress and hepatocellular injury. In the dietary intervention study, the high toxic sublethal dose of fipronil was chosen and experimental diets were made by the inclusion of 5% and 10% *Moringa oleifera* leave powder in them. Supplementation with *M. oleifera* demonstrated the dose response protection effect, whereas the inclusion of 10% supplementation achieved a significant protection on serum biochemical liver enzymes activity. The findings indicate that sublethal fipronil exposure induces significant biochemical disruption in *C. batrachus*, while dietary *M. oleifera* effectively counteracts these toxic effects through its antioxidant and hepatoprotective properties.

1. INTRODUCTION

The pollution of freshwater ecosystems with synthetic pesticides has become an important environmental issue worldwide. Among these agrochemicals, the insecticide Fipronil ($C_{12}H_4Cl_2F_6N_4OS$), a representative of the class of phenylpyrazole, is widely used due to its high effectiveness against various pests in the agriculture field and veterinary medicine. However, its perseverance in the environment and its significant toxicity to aquatic organisms pose significant risk to the environment (Chagnon *et al.* 2014). Fipronil's toxic mechanism of action is predominantly due to the suppression of g-aminobutyric acid (GABA) controlled chloride channels in the CNS leading to neuronal hyper excitability, physiological dysfunction and ultimately, death in exposed organisms. In addition to neurotoxicity, exposure to pesticides has been linked to major hematological and biochemical perturbations in fish. For example, exposure to Fipronil has been reported to cause a decrease in the number of red blood cells and hemoglobin levels, which often result in anemia and inability to transport oxygen (Tahir *et al.* 2020). Additionally, biochemical biomarkers of neurotoxicity are emerging as a new marker of exposure to pesticides in fish (Witeska, 2024).

The freshwater air-breathing catfish *Clarias batrachus* (Magur) is widely considered as an important bio-indicator species for the ecotoxicological research because of its ecological adaptability, high sensitivity to the changes in water quality and its socioeconomic importance as a food fish in South Asia. In catfish and other teleosts, exposure to pesticides is a common cause of oxidative stress, which is defined by a disproportion between production of ROS and the antioxidant screening mechanisms of an organism (Alarape *et al.* 2024). Elevated ROS production can be damaging to the cellular lipids, proteins and nucleic acids, which alter the activities of important antioxidant enzymes such as lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) (Barathinivas *et al.* 2022). Moreover, hepatic impairment due to xenobiotics is often manifested as increased serum hepatic enzymes level (alanine aminotransferase, ALT and aspartate aminotransferase, AST), which are good biochemical indicators of hepatocellular damage (Rohani *et al.* 2023).

In view of increasing chemical toxicity of aquaculture systems, plant-derived phytochemicals have attracted interest as sustainable and ecologically friendly therapeutic agents. *Moringa oleifera* (Drumstick tree) is popularly known for its powerful anti-inflammatory, antioxidant and immunomodulatory characteristics which is mainly attributed to its rich polyphenols, flavonoid, vitamin and essential micronutrients composition (Elahwl *et al.* 2025*;* El-Son *et al.* 2022). Dietary supplementation with *Moringa oleifera* leaf extract has been found to reduce oxidative stress and

biochemical disturbances caused by pesticides by increasing hepatic antioxidant capacity and free radical scavenging (Elahwl *et al.* 2025). Experimental evidence further proves that Moringa supplementation can restore antioxidant enzymes like SOD and CAT in fish exposed to environmental toxicants, thus enhancing cellular defense systems and physiological resilience (Azhar *et al*. 2023). Despite the high level of documentation of toxic effects of Fipronil and the protective potential of *Moringa oleifera*, studies testing the interaction between Fipronil and *Moringa oleifera* in *Clarias batrachus* are limited. Therefore, the present study aims to give an overall evaluation of biochemical responses in *Clarias batrachus* under fipronil toxicity and assessment of the efficacy of *Moringa oleifera* intervention effect in restoring physiological balance and biochemical homeostasis.

2. METHODOLOGY

The present experimental research was carried out in Department of Zoology, GGU, Bilaspur, C.G. Healthy females *Clarias batrachus* of almost equal size and weight (30±5gm) were obtained as certified local hatchery and acclimatized under controlled laboratory conditions after a duration of two weeks prior to experimentation. Fish were kept in aerated and clean glass fish aquaria using dechlorinated tap water under the optimum physicochemical conditions (temperature, pH and dissolved oxygen) and the natural photo period. Fish were being fed with a standard commercial pellet diet during the process of acclimatization. Following the acclimation period, the subjects were randomly divided into the control and experimental groups. Sublethal fipronil concentrations were chosen based on already determined LC_{50} values and three graded concentrations (1/30, 1/20 and 1/10 of LC_{50}) were made. Exposure of the 10, 20 and 30 days of exposure was done under semi-static conditions with periodic replacement of the test media to maintain the same concentration levels. The dietary intervention experiment used the fipronil sublethal concentration (1/10 LC_{50}) and the diet used in the experiment were prepared by adding the *Moringa oleifera* leave powder at 5 and 10 percent levels in the basal feed. The powdered leaves were well blended with the feed components, pellet, shade dried and kept under hygienic conditions until use. The respective treatment groups of fish were given the supplemented diets during the educational period, whereas control fish were fed with the basal diet only. The feeding was conducted twice a day at a constant percentage of body weight to achieve equality in intake and nutritional balance.

At the terminus of every exposure period (10, 20 and 30 days), fish were anesthetized and samples of blood were taken from the caudal vein by using sterile syringes. The blood that was collected, clotted and was centrifuged to isolate serum that was refrigerated till the time of biochemical analysis.

Analysis of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as alkaline phosphatase (ALP) in serum was performed using spectrophotometer. Data received were statistically processed on the one-way ANOVA and the means of the treatments were analyzed at a significance of 5%. All the values were presented in mean with standard error (SE).

3. RESULT AND DISCUSSION

3.1. Effect of different sublethal concentration of fipronil in *C. batrachus*

3.1.1. AST (Aspartate Aminotransferase)

AST activity significantly elevated in all treated groups relative to controls, with the maximum values observed at 30 days in the high-dose group IV (34.20 ± 0.60 U/L vs. 22.70 ± 0.65 U/L) presented in (Table 1). The rise in AST activity was statistically significant ($p < 0.05$), indicating dose-dependent hepatotoxicity.

The progressive increase of AST indicates hepatic cellular damage and increased membrane permeability. Transaminases are enzyme that play an important role in amino acid metabolism, are usually released into circulation in hepatocyte injury. Elevated AST level commonly recognized as a marker of liver damage in fish exposed to pesticides and other environmental contaminants (Mamboungou *et al.* 2024). Similar increases in transaminase activities were reported in *C. punctatus* exposed to organochlorine insecticides, confirming that fipronil induces hepatic stress and cellular injury. (Gupta *et al.* 2023).

3.1.2. ALT (Alanine Aminotransferase)

ALT activity followed a similar pattern to AST, showing a progressive increase with dose and duration. At 30 days, ALT reached 39.80 ± 0.57 U/L in the high-dose group IV compared to 32.12 ± 0.65 U/L in controls presented in (Table 2). Statistical evaluation confirmed significant differences among treatments at $p<0.05$.

ALT is primarily linked with liver and its elevated level indicates hepatocyte degeneration. The ALT surge suggests Prolonged toxic stress stimulates the conversion of amino acids into keto acids facilitates energy production through TCA cycle under induced toxicity (Heydarnejad *et al.* 2013). Many studies demonstrated that pesticide exposure to fish causes increase in ALT activity, indicates liver injury due to oxidative stress and disruption of cellular membranes (Chen *et al*. 2021, Samanta *et al.* 2014). The

increased ALT levels observed supports the finding that long-term exposure to fipronil causes hepatic damage in *C. batrachus*.

3.1.3. ALP (Alkaline phosphatase)

Exposure to different sublethal concentrations of fipronil significantly increased ALP level with increasing exposure time period. ALP level rises from 37.20 ± 0.64 U/L in controls to 51.30 ± 1.17 U/L in the high- dose group IV at 30 days, presented in (Table 3). Statistical evaluation confirmed significant differences among treatments at $p < 0.05$.

Elevated ALP level, a membrane-bound enzyme suggests impairment of the biliary system and disruption of membrane transport mechanisms. Fipronil induces ROS generation that damage hepatocyte membranes result in release of ALP from the liver sinusoids. Similar result was observed by Kumari & Mishra (2025) when fish were exposed to deltamethrin, indicating pyrethroid-induced hepatotoxicity. Rohani *et al.* (2023) has shown that fish subjected to toxic agents exhibit increased ALP activity, which suggests cholestatic injury and compromised cellular transport functions. The persistent elevation in ALP level aligns with alteration in ALT and AST level, reinforcing evidence of cumulative hepatic stress induced by fipronil exposure.

Table 1: Effect of different sublethal concentrations of fipronil on AST level in *C. batrachus*

Treatment Details	**Aspartate aminotransferase (U/L)**		
	10 DAYS	**20 DAYS**	**30 DAYS**
Group I- Control	21.90 ± 0.57	22.05 ± 0.59	22.70 ± 0.65
Group II- Low sublethal concentration (1/30) of LC_{50} of fipronil	22.45 ± 0.01	23.95 ± 0.08	25.70 ± 0.59
Group III- Median sublethal concentration (1/20) of LC_{50} of fipronil	23.70 ± 0.59	25.95 ± 0.65	31.10 ± 0.57
Group IV- High sublethal concentration (1/10) of LC_{50} of fipronil	24.80 ± 0.65	31.40 ± 0.18	34.20 ± 0.60
Sem (±)	0.52	0.45	0.60

CD (5%)	1.70	1.46	1.96
CV (%)	3.89	3.01	3.66
SD	0.785	0.650	1.040
Variance	0.814	0.604	0.514

Table 2: Effect of different sublethal concentrations of fipronil on ALT level in *C. batrachus*

Treatment Details	**Alanine aminotransferase (U/L)**		
	10 DAYS	**20 DAYS**	**30 DAYS**
Group I- Control	30.60 ± 0.07	31.40 ± 0.62	32.12 ± 0.65
Group II- Low sublethal concentration (1/30) of LC50 of fipronil	31.20 ± 0.57	32.10 ± 0.59	34.40 ± 0.62
Group III- Median sublethal concentration (1/20) of LC50 of fipronil	31.80 ± 0.60	33.60 ± 0.65	38.10 ± 0.59
Group IV- High sublethal concentration (1/10) of LC50 of fipronil	32.80 ± 0.07	36.90 ± 0.60	39.80 ± 0.57
Sem (±)	0.42	0.61	0.61
CD (5%)	1.35	2.00	1.97
CV (%)	2.28	3.17	2.90
SD	0.565	1.063	1.048
Variance	0.518	1.130	1.100

Table 3: Effect of different sublethal concentrations of fipronil on ALP level in *C. batrachus*

Treatment Details	**Alkaline phosphatase (U/L)**		
	10 DAYS	**20 DAYS**	**30 DAYS**
Group I- Control	37.10 ± 0.61	37.30 ± 0.65	37.20 ± 0.64
Group II- Low sublethal concentration (1/30) of LC50 of fipronil	37.80 ± 0.59	38.40 ± 0.59	41.10 ± 0.59
Group III- Median sublethal concentration (1/20) of LC50 of fipronil	38.30 ± 0.09	39.10 ± 0.61	46.20 ± 0.65

Group IV- High sublethal concentration (1/10) of LC50 of fipronil	40.20 ± 0.61	48.40 ± 0.60	51.30 ± 1.17
Sem (±)	0.52	0.61	0.80
CD (5%)	1.70	1.99	2.59
CV (%)	2.36	2.59	3.13
SD	0.818	1.058	1.315
Variance	0.817	1.120	1.896

3.2. Effect of dietary supplementation of *M. oleifera* in *C. batrachus*

3.2.1. AST (Aspartate Aminotransferase)

Dietary inclusion of *M. oleifera* significantly attenuated the elevation of aspartate aminotransferase (AST) induced by fipronil exposure, presented in (Table 4). After 30 days, AST activity in the 5% *Moringa* group was recorded at 28.90 ± 0.59 U/L, whereas the 10% supplemented group showed a lower value of 25.80 ± 0.61 U/L, approaching the control level of 22.78 ± 0.57 U/L. The reduction in AST levels was statistically significant different ($p < 0.05$). The greater reduction noted in the 10% group clearly indicates a dose- dependent hepatoprotective effect of Moringa supplementation. AST is a key transaminase enzyme released into circulation following hepatocellular injury.

Increased AST activity reflects loss of membrane integrity and cytoplasmic enzyme leakage due to oxidative stress. The AST decline via Moringa supplementation suggests stabilization of hepatocellular membranes and improved liver function. In a study of Salih *et al*. (2022), supplementation with *M. oleifera* leaves mitigated BPA effects by enhancing antioxidant defenses (SOD, CAT) and lowering oxidative stress indicators (LPO), preventing against mitochondrial damage. Similar hepatoprotective effects of *M. oleifera* against pesticide-induced toxicity have been documented in fish models (Elahwl et al. 2025).

3.2.2. ALT (Alanine aminotransferase)

Alanine aminotransferase (ALT) activity showed a comparable pattern of improvement after Moringa supplementation, as indicated in (Table 5). After 30 days, ALT levels reached 37.10 ± 0.63 U/L in the 5% supplemented group, while the 10% group recorded a lower value of 34.60 ± 0.65 U/L, approaching the control level of 32.18 ± 0.54 U/L indicating hepatoprotection. Statistical analysis indicated significant differences among the treatment groups at $p < 0.05$.

The declination in ALT level reflects substantial mitigation of fipronil induced hepatotoxicity, particularly at the higher supplementation level. Elevated ALT levels signify structural disruption of liver cells and leakage of intracellular enzymes into the bloodstream. Supplementation with *M. oleifera* trend toward normalization suggests restoration of hepatocyte integrity and strengthened antioxidant defense capacity. *M. oleifera* leave supplementation lowers elevated ALT levels by counteracting toxicant induced hepatic damage through potent antioxidant and membrane stabilizing mechanisms. Upregulation of antioxidant enzymes may contribute to minimize oxidative injury and prevention of cytoplasmic enzyme leakage (Elahwl *et al.* 2025).

3.2.3. ALP (Alkaline phosphatase)

M. oleifera leave supplementation showed ameliorative response against fipronil in *C. batrachus* presented in (Table 6). At 30 days of exposure to 0.16mg/l fipronil, ALP value was 37.26 ± 0.61 whereas ALP levels reached 45.80 ± 0.58 U/L in fish exposed to 0.16mg/l fipronil with 5% *M. oleifera* supplementation and the 10% supplemented group recorded a comparatively lower value of 42.10 ± 0.59 U/L. The variation in ALP activities was statistically significant at the 5% level ($p < 0.05$).

The dose related declination in ALP activity indicates a significant protective role of *M. oleifera*, with 10% inclusion demonstrating greater effectiveness in normalization of enzyme levels. The observed reduction in ALP levels in *M. oleifera* supplemented groups suggests recover of membrane stability and improved hepatic functional integrity. Fipronil induced ALP surges were attenuated via flavonoid mediated antioxidant upregulation (Yadav *et al*. 2020). *M. oleifera* leaves counteract this through their polyphenolic and flavonoid constituents that neutralize ROS, stabilizing biliary function and restore ALP concentration toward control value (Awad *et al*. 2025, Hamed *et al*. 2019).

Table 4: Effect of dietary supplementation of *M. oleifera* leaves on AST in *C. batrachus*

Treatment Details	Aspartate aminotransferase (U/L)		
	10 DAYS	**20 DAYS**	**30 DAYS**
Group I- Control	21.95 ± 0.08	22.13 ± 0.60	22.78 ± 0.57
Group II- High sublethal con. of fipronil+ 5% M. oleifera leaves	24.10 ± 0.12	26.40 ± 0.59	28.90 ± 0.59
Group III- High sublethal con. of fipronil+ 10%	22.90 ± 0.58	24.10 ± 0.58	25.80 ± 0.61

M. oleifera leaves			
Sem (±)	**0.346**	**0.591**	**0.592**
CD (5%)	**1.197**	**2.045**	**2.049**
CV (%)	**2.607**	**4.23**	**3.97**
SD	**0.447**	**1.024**	**1.025**

Table 5: Effect of dietary supplementation of *M. oleifera* leaves on ALT in *C. batrachus*

Treatment Details	**Alanine aminotransferase (U/L)**		
	10 DAYS	**20 DAYS**	**30 DAYS**
Group I- Control	30.64 ± 0.59	31.48 ± 0.60	32.18 ± 0.54
Group II- High sublethal con. of fipronil+ 5% M. oleifera leaves	32.80 ± 0.12	35.18 ± 0.57	37.10 ± 0.63
Group III- High sublethal con. of fipronil+ 10% M. oleifera leaves	31.50 ± 0.16	33.00 ± 0.59	34.60 ± 0.65
Sem (±)	**0.360**	**0.585**	**0.608**
CD (5%)	**1.246**	**2.025**	**2.105**
CV (%)	**1.970**	**3.05**	**3.04**
SD	**0.505**	**1.013**	**1.051**
Variance	**0.389**	**1.027**	**1.110**

Table 6: Effect of dietary supplementation of *M. oleifera* leaves on ALP in *C. batrachus*

Treatment Details	**Alkaline phosphatase (U/L)**		
	10 DAYS	**20 DAYS**	**30 DAYS**
Group I- Control	37.16 ± 0.56	37.38 ± 0.55	37.26 ± 0.61
Group II- Selected most toxic dose of fipronil+ 5% M. oleifera leaves	39.62 ± 0.13	43.10 ± 1.15	45.80 ± 0.58
Group III- Selected most toxic dose of	38.10 ± 0.57	40.20 ± 0.58	42.10 ± 0.59

fipronil+ 10% M. oleifera leaves			
Sem (±)	**0.468**	**0.808**	**0.596**
CD (5%)	**1.620**	**2.796**	**2.062**
CV (%)	**2.117**	**3.48**	**2.47**
SD	**0.727**	**1.315**	**1.032**
Variance	**0.657**	**1.958**	**1.065**

4. CONCLUSION

The present study demonstrates the ameliorative potential of *M. oleifera* leave against sublethal exposure to fipronil induced serum biochemical hepatic toxicity in fish *C. batrachus,* characterized by increases in enzymatic activities (AST, ALT and ALP), Reflecting metabolic stress and hepatocellular damage. Incorporating *M. oleifera* leaves as a supplement diet particularly at 10% level, successfully ameliorated biochemical impairments. The ammeliorative effects of *M. oleifera* are likely attributed through its antioxidant profile including flavonoids, quercetin, polyphenols and hepatoprotective as well as membrane-stabilizing qualities, which enhance cellular protection against oxidative stress. Overall, these findings suggest that fipronil exposure poses substantial physiological hazards to freshwater fish, dietary incorporation of *M. oleifera* could represent a promising natural therapeutic strategy to mitigate pesticide induced biochemical toxicity in aquaculture systems.

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