

Comparative Toxicity Analysis of Fenpropathrin with Its Two Commercial Formulations on Developing Zebrafish Embryos

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Abstract

Fenpropathrin displays enduring effects on aquatic environment which can be highly toxic and accumulative to non-targeted aquatic organisms like fish. While most of the previous studies focused on the toxicity of analytical grade of Fenpropathrin, insufficient attention has been paid to compare toxicity of the active ingredient (a.i) with its commercial formulation. Therefore, the present study is an attempt to evaluate the lethal as well as sublethal toxicity along with cardiac, morphological, behavioral and neurotoxic biomarker responses induced by Fenpropathrin and its commercial formulation Meothrin and Danitol on zebrafish embryos. The study reveals 96-hour (h) LC₅₀ values that are 0.156(0.121-0.202), 0.953(0.736-1.248) and 1.168(0.913- 1.503) mg a.i/L; 96 h EC₅₀ values are 0.016, 0.152 and 0.369 mg a.i/L and Teratogenic Index (TI) ratio of 9.75, 6.26 and 3.16 for Fenpropathrin, Meothrin and Danitol respectively (Fenpropathrin>Meothrin>Danitol). The reduction in toxicity in commercial formulations may be attributed to Control Release Systems (CRS). The study also reveals that intensity of malformations, teratogenic potential, behavioral abnormality, cardiotoxicity and neurotoxicity are more prominent in Fenpropathrin when compared to its two formulations.

Introduction

Pesticides are playing a key role in securing an uninterrupted food supply by taking care of the pest problem in global agriculture (Wang et al., 2020). However, with their increasing use, pesticides enter into different strata of an aquatic ecosystem through surface runoffs, spray drift, and agricultural returns which may harm the non-targeted aquatic organisms leading to a decrease in aquatic biodiversity (Wang et al., 2020). Pyrethroids, a class of pesticides have been increasingly used in agriculture and household pest control because of their outstanding potency and environmental stability (Amweg et al., 2005). Moreover, they are also used in aquaculture for the eradication of aquatic insects (Tang et al., 2018). Due to their extensive use, their residues

are detected ubiquitously in the aquatic environment in many regions of the world and can cause significant damage to the environment (Li et al., 2017).

Fenpropathrin(α -cyano-3-phenoxybenzyl-2,2,3,3-teramehycyclopropanecarboxylase, (Fen), a relatively new, synthetic pyrethroid pesticide, has not been classified into the traditional classification of pyrethroids (Xiong et al. 2014). Meothrin and Danitol are their two important formulations (NCBI, 2022). Residues of Fenpropathrin (FEN) were detected in the environment and food from various places of the world. In Turkey, it was detected in pepper at 0.008 mg/kg despite a governmental ban on its application (Yildirim & Cifteci 2022). Fenpropathrin was detected in fish at 0.17 μ g/Kg from Chilka lake, India (Nag et al., 2020). In agricultural soil, 37.6 ng/gm was detected in the Yangtze

River delta, China (Yu et al., 2020), while in Litani river and Qaraoun lake, Lebanon it was detected up to 220.1 ng/L (Kouzayha et al., 2013).

As fenpropathrin has characteristics like quick metabolism and elimination from the body, it was initially considered to be non-toxic to mammals (Bradberry et al., 2005). However, a study with mice for 120 days of exposure to Fenpropathrin showed dopaminergic degeneration (Xiong et al., 2014). Mohammad et al. (2019) reported testicular damage, apoptosis, and genomic DNA impairment in adult rats exposed to this pesticide. Fenpropathrin displays long-lasting effects in the aquatic environment which can be highly toxic and accumulative to non-targeted aquatic organisms like fish (Kanawi et al., 2013).

Zebrafish embryos have been extensively used in ecotoxicological studies as *in vitro* test models due to their sensitive nature to toxicants upon their exposure (Muthulakshmi et al., 2018). Prior studies on Fenpropathrin toxicity to Zebrafish and its embryos were restricted to its active ingredient (Wang et al., 2020; Yu et al., 2022). However, a comparative assessment of the active ingredient toxicity to its commercial formulations is missing. Therefore, the present study evaluates and compares lethal (LC₅₀), and sublethal toxicity (EC₅₀) along with morphological, behavioral, cardiac, neurotoxic, and teratogenic effects induced by Fenpropathrin along with its two commercial formulations - Meothrin & Danitol on developing Zebrafish embryos.

Materials and Methods

Chemicals

Fenpropathrin with 99.9% purity used during the study was procured from Sigma-Aldrich (Sigma-Aldrich Private Limited, Bengaluru, India). Its commercial formulations Meothrin (30% e.c, 300g/L) and Danitol (10% e.c, 100g/L) were obtained from the online shopping website bighaat.com, India. Egg water was prepared as defined in the OECD TG 203 ANNEX-2 (OECD, 1992) with a final concentration containing: 294.0 mg/L CaCl₂ .2H₂O, 123.3 mg/L MgSO₄.7H₂O, 64.7 mg/L NaHCO₃, and 965.7 mg/L KCl.

Fish Collection, Maintenance, and Production of Eggs

A total of 50 pairs of Zebrafish wild-type were obtained from Fish Farm, College of Fisheries, Assam Agricultural University, Raha. Collected fishes were thoroughly examined for any abnormalities and infection before transferring them into the stock glass aquaria (250 L capacity each). The fishes were acclimatized to laboratory conditions for 21 days in large aquaria (100L capacity) containing dechlorinated water maintaining a temperature of 26±2°C, Dissolve oxygen= 6.7±1.2 mg/L and Total Ammonia=0.12±0.03 mg/L before exposure. The aquaria were provided with

continuous aeration and a 10:14 h light-dark cycle was maintained throughout the experimental period. The fish were fed ad libitum three times a day with commercial feed, supplemented with brine shrimp.

The gravid stock was then selected for spawning and transferred to small aquaria. The breeding protocol for the production of the embryo was as per Adu and Thomsen (2011), with slight modification. Fertilized eggs with no apparent irregularity were then transferred into a Petri dish for further analysis. The plate act as a predecessor for the test solution of varying concentration and controls.

Bioassays with Zebrafish Embryos

The test was initiated 3-4, hours post fertilization (hpf) at the gastrulation period and ended at 96 hpf, as this covers the entire organogenesis (Adam et al., 2021). The zebrafish embryo toxicity test was performed in 24-well cell culture plates covered and sealed with parafilm (Sarmah et al., 2020).

The stock solution of Fenpropathrin, Danitol, and Meothrin was prepared by dissolving them in the required quantity of egg water and further subsequent concentrations were prepared from the stock solution. The exposure mediums were then transferred to 2ml per well maintaining positive, internal, and negative plate control. The plates were covered and sealed with parafilm to avoid evaporation. The exposure medium, as well as egg water, was changed after every 24 hours. After multiple range-finding tests, the concentrations of Fenpropathrin, Meothrin, and Danitol considered for testing were 0.8 mg/L to 0.025 mg/L, 4 mg/L to 0.13 mg/L, and 5 mg/L to 0.156 g/L with a spacing factor of two of each chemical respectively. Plates were incubated at 26±1°C with a photoperiod of 14h light:10 h dark and the experimental setup was repeated 3 times until the occurrence of lethal and sublethal endpoints (OECD, 2013). The endpoint indicators of LC₅₀ value were coagulation, somite formation, and lack of heartbeat whereas for EC₅₀ value other adverse effects were taken into consideration.

Other experiments like cardiotoxicity, behavioral toxicity, hatching rate, morphological alterations, and AChE enzyme activity in whole Zebrafish embryos were performed at the calculated EC₅₀ values for the above-mentioned chemicals.

Cardiotoxic Measurements

Heart Morphology and Rhythm: The heart rate in the Zebrafish embryos at 72hpf and 96hpf was measured according to Ahmad et al. (2015) and Maharajan et al. (2018) from the three treatment groups anesthetized with 0.16% tricaine (Himedia). The heart size was also measured following Ahmad et al. (2015) at 72 hpf and 96 hpf using ImageJ.

Behavioral Response

Spontaneous Coiling Movement: The occurrence of spontaneous coiling movement was observed according to Kalueff et al. (2013) at 24 hpf and 48 hpf after exposure. Before measurements, 5 randomly selected embryos for each treatment group were transferred into a 94-well plate in 2 ml of solution. After a short acclimatization period, the number of spontaneous coiling movements by embryos in each well for 1 min was monitored with the help of an inverted microscope (Zeiss, Germany). Experiments were repeated three times (i.e. three independent experiments were conducted) with five embryos for measurement per condition in every repetition. The average number of spontaneous movements per minute per individual was calculated for each replicate.

Touch response: Touch response was also evaluated at 72 hpf and 96 hpf according to Ma et al. (2019). The response was observed under the stereo zoom microscope (Zeiss, Germany), and touch response was evoked when the dorsal tail and the head region were touched using an eyelash probe. The tail region and head were recorded individually for each organism. If body bending or swimming behavior occurred after the initial touch, it was considered a positive response. Experiments were repeated three times (i.e. three independent experiments were conducted) with five embryos used for measurement per each condition in every repetition.

Hatching

The hatching rate was observed at 48 hpf, and 72 hpf using a stereo zoom microscope (Zeiss, Germany), and the images were documented according to Raja et al. (2020). The percentage of hatched embryos, recorded at 48, and 72 hpf was calculated as (number of embryos hatched / the number of incubated embryos) x 100.

Body Length Assessment

The growth of embryos was evaluated by measuring body length during the exposure period at 72 hpf and 96 hpf based on Ma et al. (2019) using EC₅₀ concentrations of test chemicals in a separate experiment. Zebrafish larvae were carefully collected, positioned on microscopic slides under a stereo zoom microscope (Zeiss, Germany), and photographed using a Tusen camera. Body length was measured using ImageJ software.

AChE Enzyme Activity

The AChE activity was studied following Ellman et al. (1961). After 96 hpf, 10 individuals from each treatment and control were homogenized in potassium phosphate buffer 0.1 M (pH 7.2) and centrifuged at 3000

g for 4 min at 4°C. Following that, 50 µL of supernatant was collected and added to 250 µL of the reaction solution (1 mL 10mM 5,5-dithiobis-2-nitrobenzoic acid solution with sodium hydrogen carbonate, 0.2mL of 0.075 M acetylcholine solution and 30 ml of 0.1 M phosphate buffer) and the absorbance was measured at 414 nm in nanodrop spectrophotometer (Micro Digital).

Statistical Analysis

The lethal experiments were performed in 3 runs with 3 replications each run. To determine the LC₅₀ value of the chemicals, probit regression analysis with 95% CI was used in SPSS software v22(IBM). The EC₅₀ values were calculated using AAT Bioquest (Sunnyvale, CA, USA) EC₅₀ calculator (<https://www.aatbio.com/tools/ec50-calculator>). GraphPad Prism v7 was used to analyze one-way as well as bifactorial analysis of variance (ANOVA) according to the concentration, the time of exposure, and their interaction with recommended posthoc tests using a least significant difference calculation at P<0.05(*). The results were expressed as ±SEM. The graphs were drawn and designed in GraphPad Prism V7.

Results

The water quality parameters were monitored at 12 hours intervals following APHA (2018). The results of the analyzed water quality parameters were Water Temperature = 26.2±1.2°C, Dissolved Oxygen = 6.9±0.5mg/L, pH = 7.9±0.2, Alkalinity = 159.5±6.2 mg/L, Hardness = 78.8±5.8 mg/L, and Total Ammonia=0.24±0.04 mg/L. They were under the permissible limit for carrying out Zebrafish embryo toxicity test guideline 236 as per OECD (2013).

All three test chemicals induced lethal toxicity in a concentration and time-dependent manner. No mortality was observed in the negative control, Internal plate control and solvent control. Positive control showed recommended mortality (>50%). Initial concentrations of Meothrin induced no mortality while that of Fenpropathrin and Danitol caused minimal mortality. The final concentrations of all the pesticides induced 100% mortality within 96hpf. The trend of lethal effects was observed in increasing order with the ascending concentration. In the case of Fenpropathrin, mortality was recorded up to 24 hpf and a decreasing trend was observed thereafter, no mortality was observed after 72 hpf. Mortality was recorded until 96 hours in the case of Meothrin and Danitol. The LC₅₀ values of Fenpropathrin, Danitol, and Meothrin at 96 hpf were found to be 0.156(0.121-0.202), 0.953(0.736-1.248), and 1.168(0.913- 1.503) mg/L respectively at 95% CI. The comparative percentage mortality vs concentration curve is shown in Figure 1. The study chemicals displayed variance lethal toxic effects on developing zebrafish embryos as, Fenpropathrin>Meothrin>Danitol.

The malformations in this study are presented in Table 1 and Figure 2. Formation of edema, delayed development, delayed hatching, missing pigmentation, accumulation of RBC, reduced yolk absorption, yolk deformation, impaired fin development, lordosis, scoliosis, and kyphosis were considered an indicator of malformations. Lordosis, scoliosis, and kyphosis were observed in delayed hatched embryos exposed to all the study chemicals. The number of embryos affected was concentration-dependent. The calculated EC₅₀ value for Fenpropathrin, Meothrin, and Danitol at 96 hpf were 0.016, 0.152, and 0.369 mg/L. Effective concentration indicators followed a contrasting trend to lethal concentration ones; sub-lethal effects were not detected till 24 hpf, but after 48 hpf the effects started appearing slowly and were prominent till 96 hpf in all the test chemicals.

The distance between the concentration-response curves for a 96 hpf period at the 50% affected concentration for embryotoxicity and malformation is considered a measure of the specific teratogenic, non-embryotoxic potential of the compound. This is also demonstrated by Teratogenic Index (TI) values, which is the ratio of LC₅₀/EC₅₀ indicating that the substances may have strong teratogenic potential. The TI values of

Fenpropathrin, Meothrin, and Danitol are 9.75, 6.26, and 3.16. Fenpropathrin with the highest TI may pose a high risk of teratogenicity to non-targeted organisms while the commercial formulations Meothrin and Danitol showed less teratogenic potential in comparison to Fenpropathrin.

The EC₅₀ value-based concentrations (Fenpropathrin = 0.016, Meothrin = 0.152, and Danitol = 0.369 mg/L) were considered for comparison of cardiac (heart rate and heart size), behavioral, morphometric, hatching, and neurotoxic analysis.

Cardiac Analysis

Heart rate and heart size of 72 hpf and 96 hpf embryos were measured for all the chemicals. The heartbeat and heart size of the control group was normal. The two-way ANOVA Post-analysis results showed a marginal decrease in heart rate at 72 hpf for all the chemicals. However, at 96 hpf the heartbeat of embryos decreased significantly (P<0.05) for all the chemicals with respect to control as shown in (Figure 3a). There is a decreasing trend in all the chemicals exposed to embryos in the heartbeat.

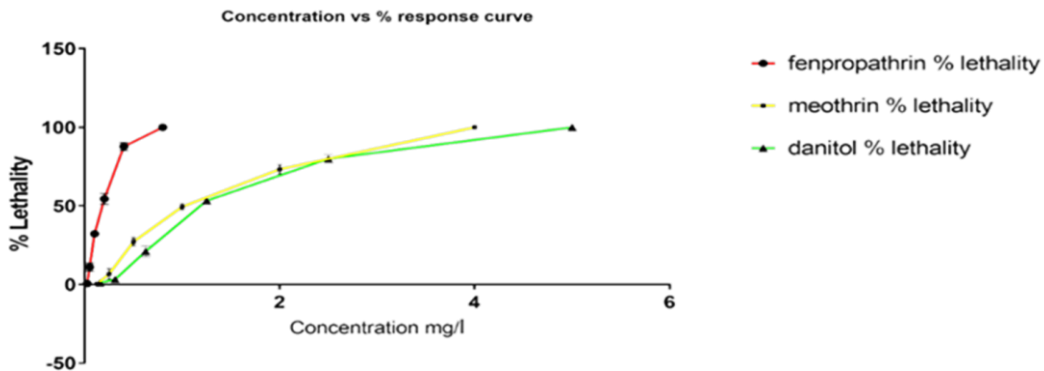


Figure 1. Concentration (mg/L) Vs Response Curve of zebrafish embryos exposed to Fenpropathrin, Meothrin and Danitol for 96hpf.

Table 1. Table showing Lethal and Sub lethal Indicators of Fenpropathrin, Meothrin and Danitol at 24, 48, 72 and 96hpf.

		24h			48h			72h			96h		
		Fenpropathrin	Meothrin	Danitol	Fenpropathrin	Meothrin	Danitol	Fenpropathrin	Meothrin	Danitol	Fenpropathrin	Meothrin	Danitol
Lethal effects	Coagulation	+++	++	++									
	Lack of tail formatin	+++	++	++									
	Lack of somite formation				+++	+++	+++						
	Missing Blood flow							++	+	+		+	+
	Lack of Heartbeat							+	+	+		+	+
Sublethal effects	Accumulation of RBC							+			+	+	+
	Formation of odema							+++	+++				
	Delayed development				+	+		++	++	+++	++	++	++
	Delayed Hatching							+++	+++	+++	+++	++	
	Missing pigmentation							++	+		++	+	+
	Reduced yolk absorption							+++	+	+	++	+	+
	Yolk Deformation							+++	++	++	++	+	++
	Impaired fin development							+	+		+++	+	+
	Lordosis							+++	+++	+++	+++	+++	+++
	Scoliosis							+++	+++	+++	+++	+++	+++
Kyphosis							++	+	+	++	+	+	

*+ indicates mild, ++ indicates moderate, +++ indicates severe

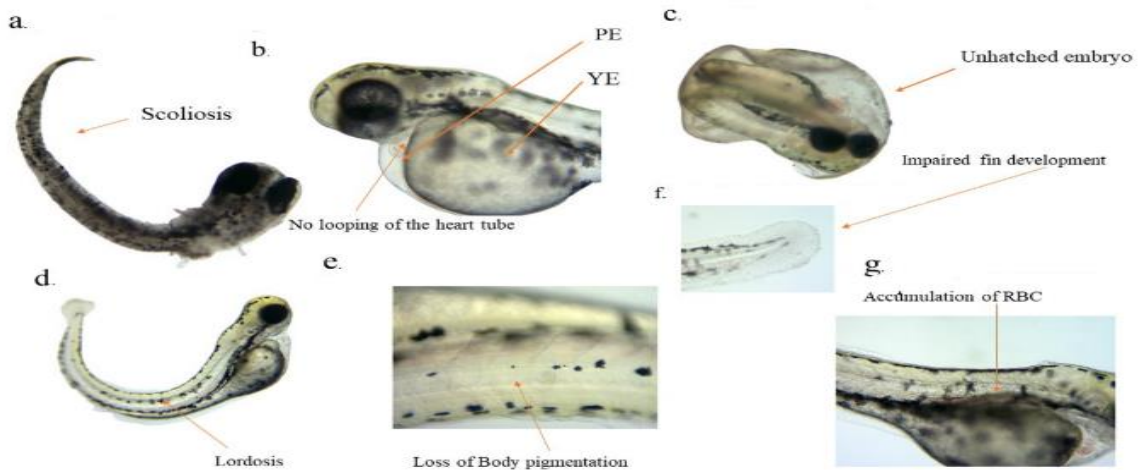


Figure 2. Microscopic image showing morphological alterations in zebrafish embryos exposed to a), Fenpropathrin 0.016 mg/L at 72 hpf; b), Danitol 0.369 mg/L at 72 hpf; c), Meothrin 0.152 mg/L at 72 h; d), Danitol 0.369 mg/L at 96h; e), Fenpropathrin 0.016 mg/L at 96 h; f), Meothrin 0.152 mg/L at 96 h; g), Fenpropathrin 0.016 mg/L at 96 hpf. PE-Pericardial edema, YE-Yolk sac edema.

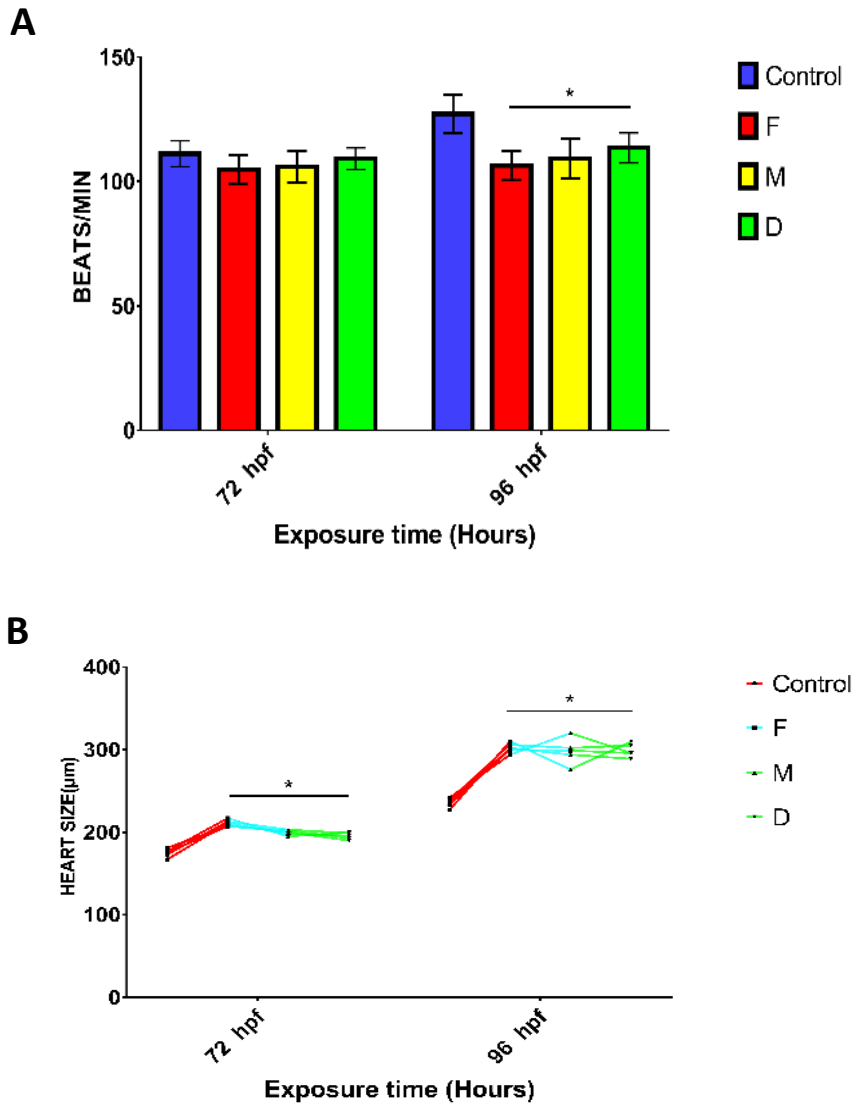


Figure 3. Represents the A) Heart beats B) Heart size of zebrafish embryos at different time points (72 and 96 hpf) exposed to Fenpropathrin (0.016mg/L), Meothrin (0.152 mg/L) and Danitol (0.369 mg/L). Two-way ANOVA followed Sidak’s post analysis was used at $P < 0.05$ (*).

The elongation of the heart was prominent during 72 hpf and 96 hpf exposures to all the chemicals. The result showed a significant ($P < 0.05$) increase in heart size for all the exposed embryos at 72 and 96 hpf with respect to control as shown in Figure 3b. In contrast to the heartbeat, an increasing trend of heart size observed in the treated embryos.

Behavioral analysis

After exposure to all three chemicals, the behavioral parameters like spontaneous coiling movement (24 and 48 hpf), head touch response (72 and

96 hpf), and tail touch (72 and 96 hpf) response were measured in the embryos as shown in Figure 4. Except for head touch response, all the parameters showed an observable reduction compared to control from 24 to 48 hpf in spontaneous movement and 72 to 96 hpf in tail touch response. In spontaneous movement and tail touch response rate, Danitol exposed embryos were observably low at 24 and 72 hpf respectively, while it was significantly ($P < 0.05$) low at 48 hpf (spontaneous movement) and 96 hpf at all other parameters. Fenpropathrin and Meothrin-exposed embryos were significantly low ($P < 0.05$) at (24 and 48 hpf) and (72 and 96 hpf) in all the parameters.

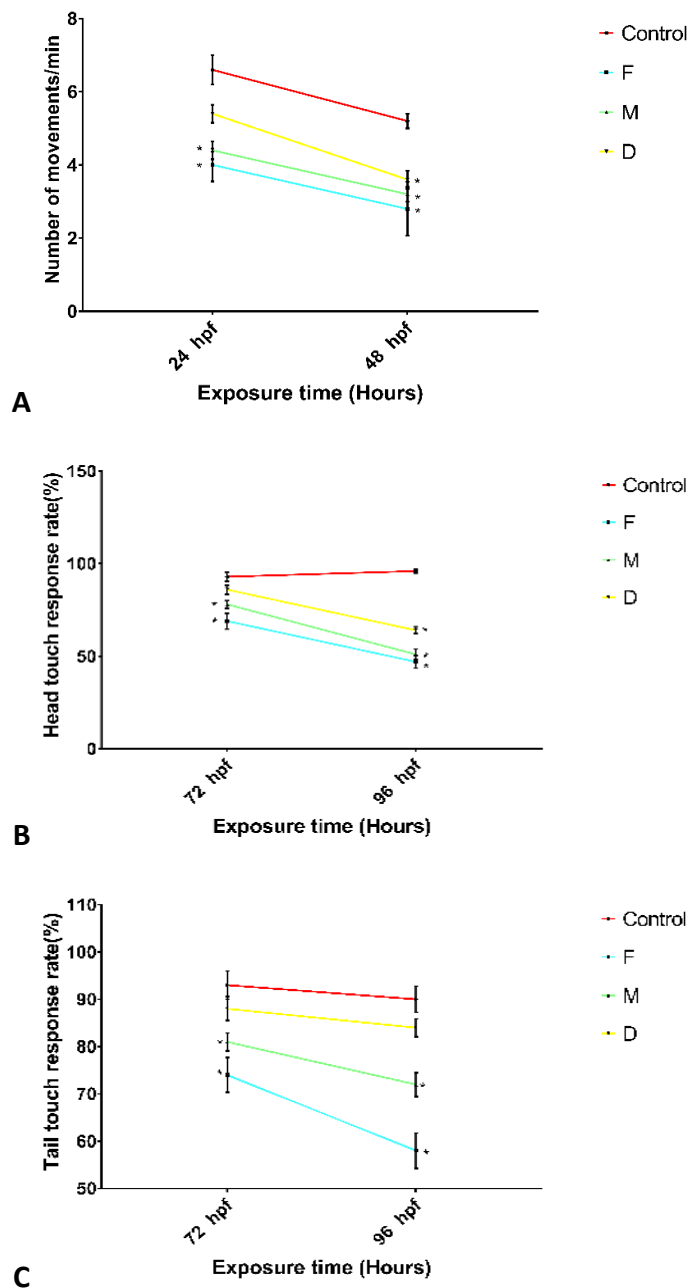


Figure 4. Represents the a) Spontaneous Movement b) Head Touch response rate c) Tail Touch response rate of zebrafish embryos at different time point exposed to Fenpropathrin (0.016mg/L), Meothrin (0.152 mg/L) and Danitol (0.369 mg/L). Two-way ANOVA followed Sidak's post analysis was used at $P < 0.05$ (*).

Hatching Delay, Morphometric, and AChE

Delayed hatching, growth retardation, and reduction of neurotoxic enzyme activity were observed during the current study as seen in Figure 5. The hatching rate significantly declined ($P<0.05$) during 48 hpf in all the exposed groups. However, at 72 hpf only Fenproprathrin exposed group showed a significant

($P<0.05$) decline when compared with the control. Significant ($P<0.05$) reduction in body length was observed in all the exposed groups at 72 hpf, however at 96 hpf significant reduction was observed only in Fenproprathrin exposed groups. The AChE activity was measured at 96 hpf. Findings revealed that the activity was significantly ($P<0.05$) reduced in all the exposed groups.

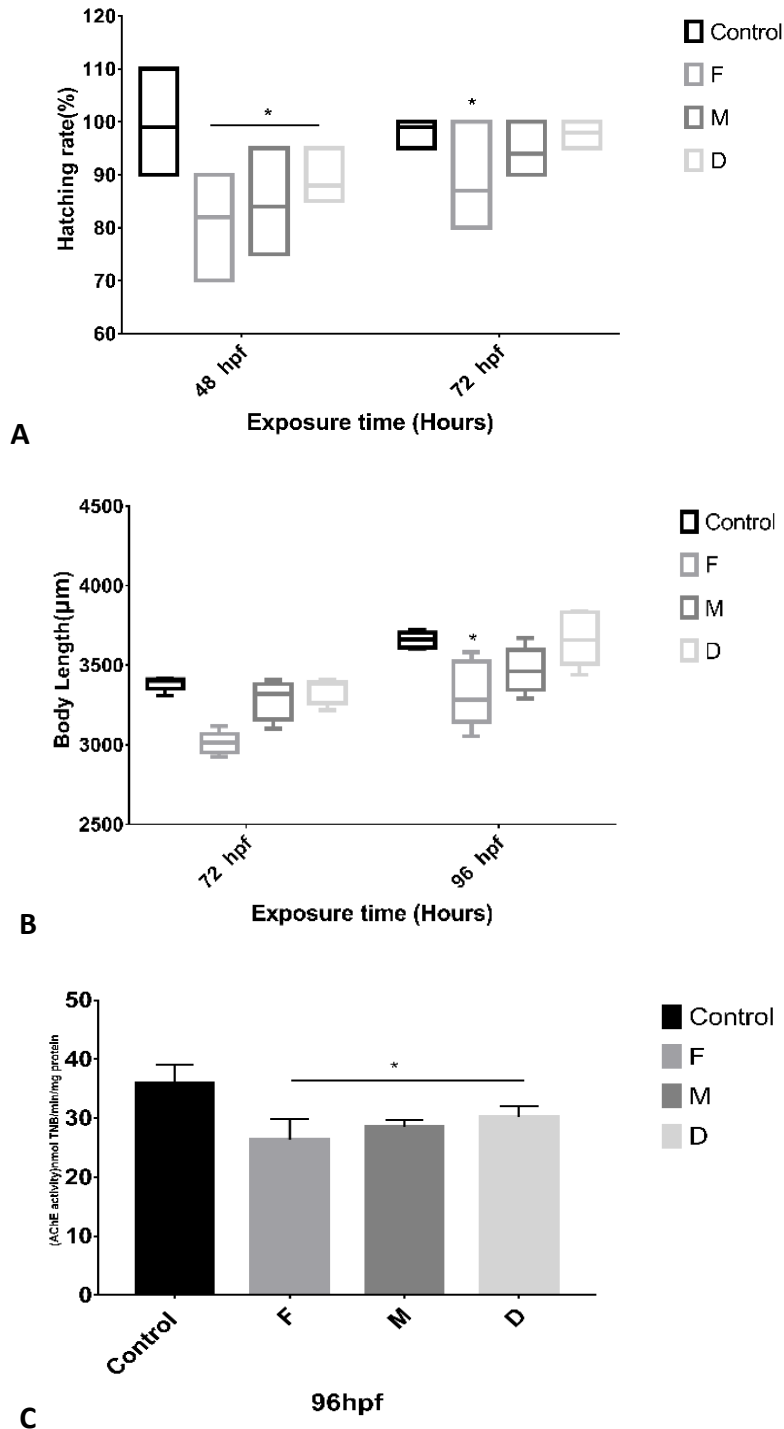


Figure 5. Represents the a) Hatching rate of zebrafish embryos at different time points (48 and 72 hpf) b) Body length (72 and 96 hpf) c) AChE activity at 96 hpf exposed to Fenproprathrin (0.016 mg/L), Meothrin (0.152 mg/L) and Danitol (0.369 mg/L). One-way ANOVA for AChE and Two-way ANOVA for rest followed Sidak's post analysis was used at $P<0.05$ (*).

Discussion

The present study reveals that the 96h LC₅₀ value of FEN (99.9%) is 0.156 mg/L which resembles the previous finding of embryotoxicity value (0.16 mg/L) by Wang et al. (2020), but lower compared to that of Fenpropathrin's adult zebrafish lethal toxicity value (0.0056 mg/L) in the same study. Reduction in toxicological properties were reported by 610.89% and 748.71% in Meothrin and Danitol respectively when compared to that of the analytical grade. Lower toxicity of Meothrin and Danitol can mainly be attributed to the specificity of formulation i.e. Controlled Release System (CRS) by microencapsulation. Controlled release system formulations are not usually much effective in target organisms, but this mechanism also notably reduces negative impacts on non-targeted organisms by lowering exposure concentrations (Stevenovic et al., 2017). The higher mortality rate that was observed during the first 24 hpf was later decreased till it attained 96 hpf in all the test chemicals. This might be due to two important considerations a) during the early stages embryos have lower detoxifying potentials and their plasma membrane is susceptible to alterations b) inhibiting activities of the toxins towards specific pathways, rather than arising from a general dysfunction in embryogenesis (Sarmah et al., 2020). Moreover, early life stages are usually the most sensitive in fish development, but in contrast, some findings suggest that chorion might reduce the penetration of toxicants into embryos (Pamanji et al., 2015 and Stevenovic et al., 2017).

One of the lethal endpoints is coagulation, which results from the denaturation of the yolk protein (ADFG, 2013). Some chemicals can induce an inflammatory response leading to the production of protease, which destroys the structural conformation of the protein. This is due to the inability of nutrients to pass through the yolk syncytial layer which leads to tissue damage and ultimately leads to the death of the embryo (FyIn, 2003). Modifications in lipid synthesis and metabolism may also affect the yolk sac, any defect in the yolk sac may prevent nutrient supply during embryogenesis, which could be the cause of abnormal development (Goetz & Dix, 2009).

An increase in pericardial edema observed during this study could be linked to the decrease in heart rate associated with slow blood flow in exposed embryos (Ranjani et al., 2020). Moreover, Retinoic acid deficiency is also known to cause pericardial edema (Hou, 2008). The RBC accumulation (hyperemia) in treated embryos is most likely due to heart muscle weakness, edema formation, and a decrease in heart rate (Maharajan et al., 2017). The impairment caused by Fenpropathrin and its formulation in the developing heart of zebrafish is an important indication of cardiac toxicity.

During embryogenesis, the notochord is a transient structure that serves to support the vertebrae and spine (Zeng et al., 2018). Thus, the malformed axial spinal

curvature observed in the present study could be attributed to notochord abnormalities caused by Fenpropathrin and its formulations in zebrafish embryos (Kudar & Gundala, 2018). Tail deformation, notochord bending, and malformed curvature were observed in embryos/larvae exposed to test chemicals. The notochord sheath is made up of collagen, and a defect in collagen synthesis could be the cause of bent notochords (Kuder et al. 2018). The observed bent notochord and tail deformation might result in abnormal swimming. Ren et al. (2016) discovered that muscle AChE inhibition is a factor disorder in the movement of zebrafish.

The present study shows high TI in technical grade Fenpropathrin whereas the TI value decreases with the decrease in percentage (%) of the active ingredient in both commercial formulations. When a substance has a TI lower than 1, it may cause the death of the fish with little malformation being observed (Ma et al., 2019).

The heart size of all the embryos exposed to effective concentrations of FEN and its commercial formulations elongated significantly. Similar kinds of observations were reported in zebrafish embryos exposed to PPF and monocrotophos by Maharajan et al., (2018) and Pamanji et al., (2015) respectively. The elongation of the heart might be due to the loss of attachment between the heart and the common cardinal vein which migrate dorsally and hence mechanical stretching of the heart muscle occurs (Maharajan et al., 2018).

The present study displayed a significant reduction in the heartbeat of zebrafish embryos exposed to effective concentrations of FEN and its two formulations. Yamauchi et al. (2006) reported that malformation in the pericardium was one of the main causes of heart and blood circulation abnormalities in fish. Lin et al. (2007) described the reduction in the heart rate which might also be attributed to the inhibition of AChE activity and edema formation.

The influence of xenobiotics pollutants on the movement of zebrafish embryos is most likely due to disruption to the nervous system (Frayse et al., 2006). In the present study, the reduction in spontaneous movement is indicative of the embryo's CNS (Central nervous system) may have an injury or delayed developmental progress. Another reason might also be due to the uncontrolled action potential of motor neurons and joint development of muscular and motor neuron systems which further evoke frequent spontaneous movements in the embryos (Xia et al., 2021; Kimmel et al., 1995). Ma et al., (2019) observed a significant reduction in the number of spontaneous tail movements of zebrafish involving neonicotinoid pesticides. As Spontaneous movement is one of the first behavioral mechanisms in zebrafish to be developed, it is not surprising that it was the most sensitive and point tested (Selderslaghs et al., 2010).

Touch response in zebrafish embryos gets initiated as early as 24 hpf, a touch would make the

dechorionated embryos coil partially, with a brief swimming episode which has been reported in other studies as well (Brustein et al., 2003). In general, Rohon-Beard sensory neurons in the spinal cord are promptly activated to meet tail touch and perception of head simulation, which is being adjusted by trigeminal neurons (Kimmel et al. 1990). In addition, to this, an intact hindbrain has also been noted as an essential development for the touch response in zebrafish (Saint-Amant et al., 1998).

The present study suggests that the impact of the head touch response was observed more pronounced when compared to that of the tail touch response. Ma et al., (2019), observed similar results in zebrafish embryos exposed to acetamidiprid where the head touch response was more sensitive than the tail touch. This might be due to the fact that tail touch would only activate a small number of Mauthna cells, whereas head touch would activate more reticulospinal neurons which induce a significant response (Foreman et al., 1993).

The hatching rate has been frequently used for assessing embryo developmental toxicity. A decrease in the hatching rate induced by FEN and its formulations might be due to the adverse effects on neurotransmitters and the weakening of spontaneous muscle movements resulting in delayed hatching (Pandey et al., 2014). The impaired growth observed in the present study might be due to the impact of FEN and its formulations on the disruption of amino acid/ glucose metabolism. The delayed hatching also leads to the shortened body lengths of the embryos. A similar trend can be seen in a study conducted by Yu et al., (2022) in zebrafish embryos treated with FEN.

AChE is a key enzyme in the breakdown of the neurotransmitter acetylcholine into choline and acetate (Viera et al., 2018). During the present study, the acute and effective dose of Fenpropathrin and its formulation caused significant AChE inhibition activity. Similar kinds of findings have also been reported by Banaee et al. (2012), where exposure to Fenpropathrin reduces acetylcholine esterase in freshwater fish *Alburnus mossulensis*. Xing et al. (2013) also reported AChE inhibition causes neurotoxic alterations in carps when exposed to atrazine and chlorpyrifos. The result of AChE activity in the present investigation may be due to concentration and time-based variability. However, the relationship between AChE and the compounds is still very unclear.

Conclusion

In conclusion, our study clearly indicated that Meothrin and Danitol are less toxic than Fenpropathrin. However, all of them cause marked effects in the embryogenesis of Zebrafish. Moreover, due to the control release mechanism, the intensity of toxicity of the commercial formulations decreases than its technical grade. The present study also demonstrated the intensity of malformations, teratogenic potential,

behavioral abnormality, cardiotoxicity, and neurotoxicity which enlightens different toxicity aspects of Fenpropathrin, Meothrin, and Danitol that are essential for aquatic toxicological assessment. Moreover, this study is also the first kind of developmental toxicity of Meothrin and Danitol in aquatic organisms, a much-needed safety assessment database. Further emphasis should be given to the in-depth study of different pathways and molecular interactions of aquatic organisms when they come in contact with these chemicals.

Ethical Statement

Before commencement of the experiment, the collected zebrafishes were maintained following the guidelines of the Departmental Animal Ethics Rule, Department of Aquatic Environment Management, College of Fisheries, Assam Agricultural University. Zebrafish embryos are defined as in vitro tests so any experiment with them is considered to be under animal welfare (EU,2010).

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Author Contribution

Raktim Sarmah, Hemanta Pokhrel, Rajdeep Dutta conceptualize and design the study. Raktim Sarmah, Dipanka Nath, Ruhul Ameen acquired and analyzed the data. Raktim Sarmah, Rajdeep Dutta and Sarada Kanta Bhagabati interpreted the data and Drafted the Manuscript.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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