IJASRM

International Journal of Advanced Scientific Research and Management, Volume 8 Issue 12, Dec 2023

www.ijasrm.com

ISSN 2455-6378

Ecotoxicity of beta-Cyfluthrin on Developmental Stages of Zebrafish (*Danio* sp.)

Sandhya Kadiru[1] and Roshan D'Souza[1,*]

[1] Department of Zoology, Sophia College for Women (Autonomous), Mumbai, Maharashtra, India – 400 026

*

Abstract

The extensive and indiscriminate use of insecticides gives rise to significant risks for both human health and the environment. Agricultural runoff into waterbodies like rivers and ponds results in unintended exposure of aquatic organisms to these insecticides. Beta-Cyfluthrin, a type II synthetic pyrethroid is widely used in India as an insecticide. In this study, we investigated the developmental toxicity of beta-Cyfluthrin at concentrations of 0.25, 0.5, 1.0, 2.0 and 4.0 µg/L on zebrafish (*Danio* sp.) embryos at 24, 48, 72, and 96 hours post-fertilization (hpf). The cumulative mortality increased in a time and dose-dependent manner and LC50 was 2.869 at 96 hpf. The hatch rate and hatchability were inversely proportional to concentration of beta-Cyfluthrin. Spontaneous trunk contractions (STCs) also decreased in embryos exposed to higher concentrations. Severe cardiac abnormalities like pericardial edema, failure of the heart to loop properly and increased heartbeat rate were observed in the treatment groups. Additionally, beta-Cyfluthrin led to yolk sac edema and spinal curvature in the treated embryos. This research underscores that exposure to beta-Cyfluthrin during the early life stages of zebrafish results in developmental defects and its unintended release in to aquatic ecosystems could potentially cause environmental harm.

Keywords: *Beta-Cyfluthrin, Developmental toxicity, Zebrafish embryo, Pyrethroid*

1. Introduction

Being a global agricultural powerhouse, pesticides are used on a widespread scale in India. The most common method of pesticide application is spraying in the form of aerosols. In India, the irrigation and drainage systems in agricultural fields are closely interlinked with natural aquatic bodies. This leads to unintended entry of pesticides into ponds, lakes and rivers. This can result in exposure of non-target species like aquatic invertebrates and vertebrates like fishes to the harmful effects of pesticides (Smith and Stratton, 1986; Wijngaarden *et al*., 2005).

Synthetic pyrethroids (SPs) are one of the most commonly used pesticides in modern agriculture. They are neurotoxicants and are highly toxic to aquatic invertebrates and fishes (Benli, 2005; Morgan *et al*., 2007; Gan *et al*., 2008; Sepici-Dinçel *et al*., 2009).

Beta-Cyfluthrin is a second generation type II synthetic pyrethroid. It is used to control pests like ants, silverfish, cockroaches, termites, weevils, fleas, mosquitoes and flies and is easily available in the markets (Extoxnet, 1996). It acts as a contact and oral toxin. It affects the nerve function and disrupts the sodium channels leading to depolarization and hyperexcitability. It also shows weak anti-estrogenic activity (Johnson and Finley, 1980; Tyler *et al*., 2000; Du *et al*., 2010). The LC50 values of beta-cyfluthrin toxicity for various fish species are 0.068 µg/L for rainbow trout (*Oncorhynchus mykiss*), 330.9 ng/L/96 hr for golden orfe (*Leuciscus idus*), and 28 ng/L/96 hr for bluegill sunfish (*Lepomis macrochirus*). In *Mysidopsis bahia* (Mysid shrimp), the LC50 was 0.0022 µg/L. Bioaccumulation in the muscles of Nile tilapia (*Tilapia nilotica*) was 0.009 mg/L after

https://doi.org/10.36282/IJASRM/8.12.2023.1919

five days, when exposed to a dose of 0.001 mg/L (Al-Makkawy and Madbouly, 1999; Tomlin, 2006). The half-life of beta-Cyfluthrin is approximately 12 days in water with sunlight and 193 days in the dark (Casjens, 2008).

Cypermethrin, a synthetic pyrethroid was detected at different sites in the surface waters of Thamirabarani, a perennial river in Tamil Nadu, India ranging from 0.006 to 0.034 mg/kg. (Arisekar *et al*., 2018). This raises the concern of potential movement of pyrethroids to aquatic water bodies and its impact on non-target organisms. Even though synthetic pyrethroids are highly toxic to invertebrates and fishes, relatively few studies have been conducted to assess the impact of beta-Cyfluthrin on aquatic organisms.

Zebrafish (*Danio* sp.) is a very useful model for ecotoxicity impact studies due to its transparent embryos, small size, and short generation time and well tested protocols. The objective of the present study was to investigate the impact of beta-Cyfluthrin on embryo and larval stages of zebrafish (*Danio* sp.). The results will provide better understanding of ecotoxicity of beta-Cyfluthrin and characterize accurately the environmental risk associated with pyrethroid runoff after its agricultural applications.

2. Materials and Methods

2.1 Chemicals

Beta-Cyfluthrin (Cyfluthrin [(R)-cyano-(4-fluoro-3-phenoxyphenyl) methyl] (1S)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate), 96.4%] was obtained from Sigma-Aldrich, USA). 1% stock solution was prepared in acetone and stored in darkness at 4^{o}C. The test solutions with concentrations of 0.25, 0.5, 1.0, 2.0 and 4.0 µg/L were prepared by diluting the stock in E3 medium (Cold Spring Harbor Protocols, 2011). The above concentrations were selected based on literature survey as well as pilot trials conducted. All the other chemicals were of AR grade and obtained from SD-Fine Chem, India.

2.2 Maintenance and acclimatization of zebrafish

Five pairs of mature breeding zebrafish were obtained from the local aquarium. They were housed in 10 L glass aquaria under semi-static conditions. The aquarium was supplied with activated charcoal filtered tap water, which was aerated and filtered constantly. The fishes were exposed to 14:10 light dark cycle. The temperature of the water was maintained at 28±10°C. The fishes were fed with live artemia *ad libitum*. The aquaria was cleaned daily by siphoning the waste and by changing 10% of the water. The fishes were acclimatized for two weeks before breeding.

2.3 Breeding and egg collection

The eggs were obtained by mass spawning. The breeding pairs were kept in a breeding tank with a mesh at the bottom to prevent cannibalization of the eggs by the adults. Spawning was triggered by exposing the fishes to 30 mins of light in the morning. The eggs were collected, debris removed and rinsed repeatedly in E3 medium till clean. Unfertilized eggs were removed by checking under a dissection microscope.

2.4. Experimental Setup and exposure to beta-Cyfluthrin

Throughout the experimental study the fishes were maintained in the Sophia College for Women, CPCSEA registered zebrafish maintenance facility, Registration No. 1936/Po/Re/S/17/CPCSEA as per the protocols recommended by the IAEC. Seven 24-well plates were set up with 2 ml of test solution in each well. The plates were as follows: one negative control, one solvent control (SC) (with 4.0 µg/L of acetone) and five experimental groups with different concentrations of beta-Cyfluthrin (0.25, 0.5, 1.0, 2.0 and 4.0 µg/L). Fertilized embryos at 3 hpf were added to the test plates – one egg in each well. The experimental groups had 20 embryos exposed to beta-Cyfluthrin with 4 internal control in each plate. The embryos were monitored once every 24 hours for a period of 4 days. The mortality in the negative control and solvent control plates were monitored at ≤ 90% according to FET guidelines (OECD, 2013). Any experimental plate with a mortality ≥ 10% in the internal control embryos were discarded. The entire experiment was done in triplicates. The eggs were incubated at 28 ± ºC with a light dark cycle of 14:10 hours. The E3 medium was changed every 24 hours. The endpoints such as mortality, hatching, hatch rate, sinus venosus-bulbous arteriosus (SV-BA) distance and heart rate were measured every 24 hours. Any

https://doi.org/10.36282/IJASRM/8.12.2023.1919

IJASRM

International Journal of Advanced Scientific Research and Management, Volume 8 Issue 12, Dec 2023

www.ijasrm.com

ISSN 2455-6378

malformations such as pericardial edema and yolk sac edema were observed. Any dead embryos were discarded every 24 hours. Lack of visible heartbeat was considered as an indicator of mortality. Observation of heart malformation was done from 48 hpf onwards. The toxicity assays were done by following the OECD FET guidelines. The identification of the embryo stages was done using Kimmel *et al*, 1995.

2.5. Statistical analysis

The data was presented as mean ± standard deviation (SD). Statistical differences between control and treatment groups were evaluated by one-way ANOVA, followed by Bonferroni and Holm pairwise comparison of means. The differences were considered statistically significant when **p<0.01. LC50 was calculated using Finney's Probit data analysis method.

3. Results and Discussion

3.1. Effect of Solvent

Acetone in E3 medium was used as solvent control. Statistical analysis showed that there was no significant difference between the negative control and solvent control for all tests in this study.

3.2. Effect on Survival and Hatching

The cumulative mortality was calculated for 24, 48, 72 and 96 hpf. The cumulative mortality increased in a time and dose dependent manner (Fig.1). Cumulative mortality at 0.25 and 0.5 µ/L were the similar to control, while the embryos exposed to 2.0 and 4.0 µg/L of beta-Cyfluthrin showed significant difference with respect to control from 24 hpf onwards. This is in agreement with earlier work done on other synthetic pyrethroids like deltamethrin, trans-permethrin, fenvalerate, cypermethrin and flucythrinate where the increasing concentration of pesticide lead to increased mortality in fishes, especially in the early life stages (Clark et al., 1989; Rahmi et al., 2005; Amin and Hashem, 2012).

3.3. Effect on hatch rate and hatchability

Hatching is a crucial event in the developmental history of fishes where a complex set of biochemical and physical pathways enables the weakening and

Table 1: LC50 of zebrafish embryos exposed to different concentrations of beta-Cyfluthrin (µg/L) at 24, 48, 72 and 96 hpf.

Exposure Time (hours)	LC50 (µg/L)
24	10.638
48	4.830
72	3.583
96	2.869

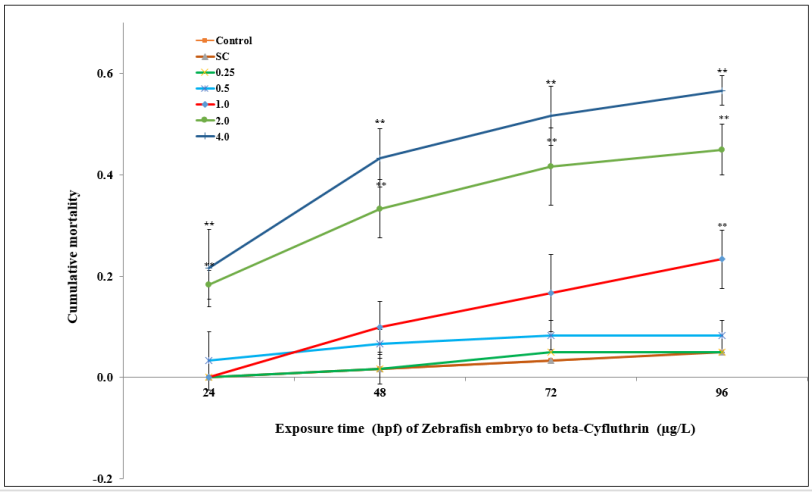

Fig. 1: Cumulative mortality of zebrafish embryos exposed to different concentrations of beta-Cyfluthrin (µg/L) at 24, 48, 72 and 96 hpf. Data are expressed as mean ± SD (n=3 replicates of 20 embryos in each concentration). Asterisks denote significant differences between control and treatments at different time periods. (p < 0.01), SC - Solvent Control**

https://doi.org/10.36282/IJASRM/8.12.2023.1919

IJASRM

International Journal of Advanced Scientific Research and Management, Volume 8 Issue 12, Dec 2023

www.ijasrm.com

ISSN 2455-6378

destruction of the chorion, allowing the embryo to hatch (De Gaspar et al., 1999). In this study, we observed the hatching of embryos at intervals of 5 hours starting from 50 hpf to 65 hpf. The embryos started hatching asynchronously from 48 hpf. All the embryos had hatched out by 65 hpf. The hatch rate was calculated by the following formula: Hatch rate = No of eggs hatched at time 'X'/ No of eggs alive at time 'X'.

It was observed that hatching was delayed with increase in exposure concentration. The hatch rate at 50 hpf was similar in both control and treated groups. However, by 55 hpf, the hatch rate embryos in concentrations 2.0 and 4.0 slowed down and showed significant difference with respect to control by 60 hpf (Fig.2).

Hatchability is the ratio of eggs hatched with respect to the total number of fertile eggs. The hatchability of zebrafish was not affected by the increase in beta-Cyfluthrin concentration except for concentrations of 2.0 and 4.0 µg/L, which showed significant decrease with respect to control (Fig.3). Earlier work done shows that exposure to deltamethrin resulted in decrease in hatch rate in common carp (*Cyprinus carpio* L.) embryos and larvae (Köprücü and Aydin, 2004). Zebrafish exposed to different pyrethroid insecticides like d-Tetramethrin, cyphenothrin, deltamethrin and l-cyhalothrin showed a similar decrease in hatchability (Sharma and Ansari, 2010; Yang et al., 2014; Mendis et al., 2018). However esfenvalerate accelerated hatching in zebrafish, while permethrin did not have any effect on hatchability (Zhang et al., 2017; Wang et al., 2020).

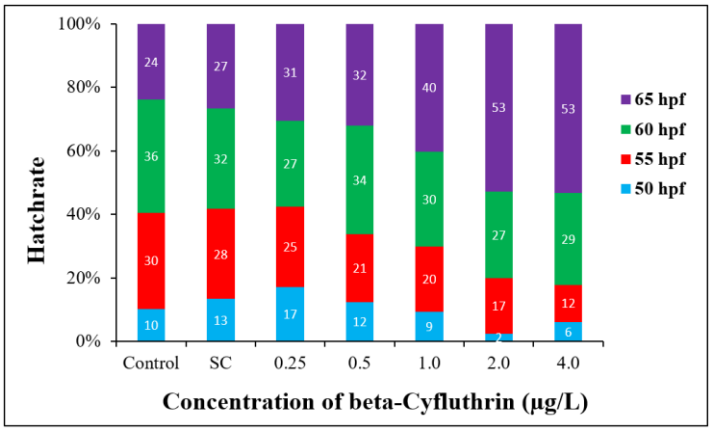

Fig. 2: Hatch rate of zebrafish embryos exposed to different concentrations of beta-Cyfluthrin (µg/L) from 50 to 65 hpf expressed in %. Data are expressed as mean ± SD (n=3 replicates of 20 embryos in each concentration), SC - Solvent Control

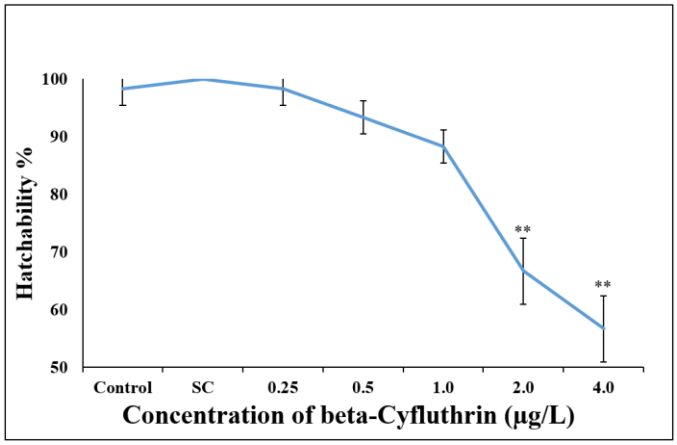

Fig. 3: Hatchability of zebrafish embryos exposed to different concentrations of beta-Cyfluthrin (µg/L) after 96 hpf. Data are expressed as mean ± SD (n=3 replicates of 20 embryos in each concentration). Asterisks denote significant differences between control and treatments at different time periods. (p < 0.01), SC - Solvent Control**

https://doi.org/10.36282/IJASRM/8.12.2023.1919

International Journal of Advanced Scientific Research and Management, Volume 8 Issue 12, Dec 2023

www.ijasrm.com

ISSN 2455-6378

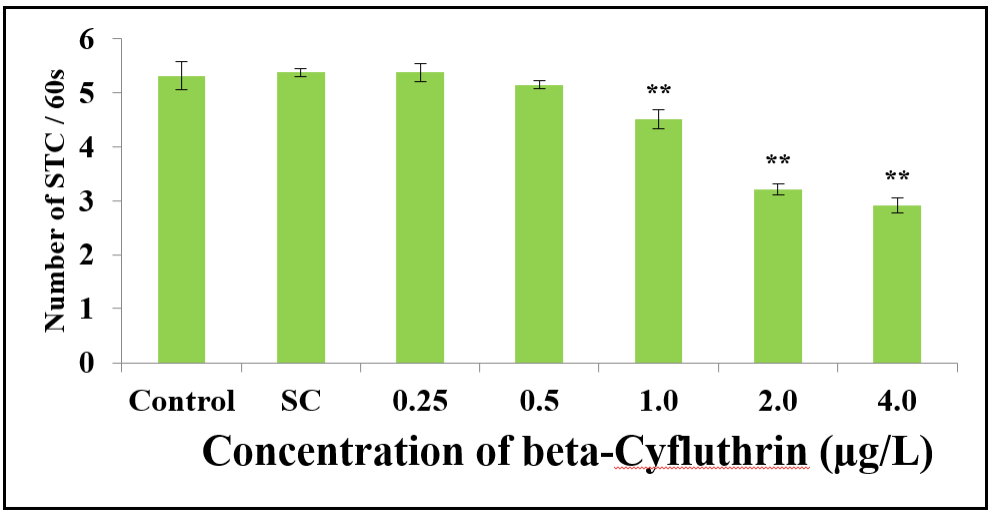

Fig. 4: Number of spontaneous contractions (STC) occurring in zebrafish embryos exposed to different concentrations of beta-Cyfluthrin (µg/L) at 24 hpf for 60 seconds. Data are expressed as mean ± SD (n=3 replicates of 20 embryos in each concentration). Asterisks denote significant differences between control and treatments (p<0.01). SC - Solvent Control**

3.4 Effect of beta-Cyfluthrin on spontaneous trunk contractions (STC)

In zebrafish, involuntary movements termed spontaneous contractions start at 17 hpf. Spontaneous trunk contractions are indicated by jerky movements by the embryo within the chorion and are due to development of neurons adjacent to the somites. During the course of normal development, the frequency of spontaneous contractions reach their peak at 19 hpf and gradually decrease (Saint-Amant and Drapeau., 1998; Fraysse *et al*., 2006; Jin *et al*., 2009).

In this study, we recorded the STC for a period of 60s at 24 hpf. It was observed that the frequency of STC was inversely proportional to the concentration of beta-Cyfluthrin. Embryos exposed to concentration of 1.0, 2.0 and 4.0 µg/L showed a concentration dependent decrease (Fig.4). Kimmel *et al*., 1974 postulates that STC's lead to hatching as it helps in rupturing the chorion. This decrease in STC's on exposure to beta-Cyfluthrin aligns with the decrease in hatch rate (Fig.2).

3.5 Effect on Cardiac Structure and Function

During the development of zebrafish, the heart is one of the first organs to form and start functioning. Hence the development of the heart is a useful endpoint in toxicity studies. Lack of heartbeat is one of the lethal endpoints according to Fish Embryo Acute Toxicity (FET) Test guidelines (Stainier *et al*., 1993; Lee *et al*., 1994; OECD, 2013).

The organogenesis of the heart begins with formation of a tube which later loops into an S-shaped structure made up of two chambers, the atrium (A) and the ventricle (V) (Antkiewicz *et al*., 2005; Ramasubramanian *et al*., 2008). These two chambers lie side by side and can be easily seen in the lateral view (Manner *et al*., 2010).

In the present study, we observed the cardiac morphology from 48 hpf to 96 hpf. It was observed that the embryos exposed to beta-Cyfluthrin showed failure in the looping process and as a result the ventricle was positioned in front of the atrium. The atrium and ventricle were narrower and string shaped compared to control (Fig. 8E).

Pericardial edema was observed in the treated embryos at 48, 72 and 96 hpf. The embryos exposed to concentrations 2.0 and 4.0 µg/L showed significant difference from 48 hpf onwards, while embryos in 1.0 µg/L developed edema from 72 hpf onwards (Fig. 5).

The distance between the junction of heart with inflow tract at sinus venosus (SV) and the junction of the heart with outflow tract at bulbous arteriosus (BA) can be measured as an index of the cardiac looping and is known as SV-BA distance (Fig. 8C) (Stainier *et al*., 1993; Lee *et al*., 1994). In the present study, the SV-BA distance was measured using oculometer under 100X magnification at 72 and 96 hpf. It was observed that there was no significant difference in SV-BA distance between control and experimental embryos at 72 hpf. However, embryos exposed to 2.0 and 4.0 µg/L showed significant increase in SV-BA (µm) at 96 hpf with respect to control. This indicates that the heart did not loop properly during development, resulting in increased SV-BA distance (Fig. 6).

https://doi.org/10.36282/IJASRM/8.12.2023.1919

International Journal of Advanced Scientific Research and Management, Volume 8 Issue 12, Dec 2023

www.ijasrm.com

ISSN 2455-6378

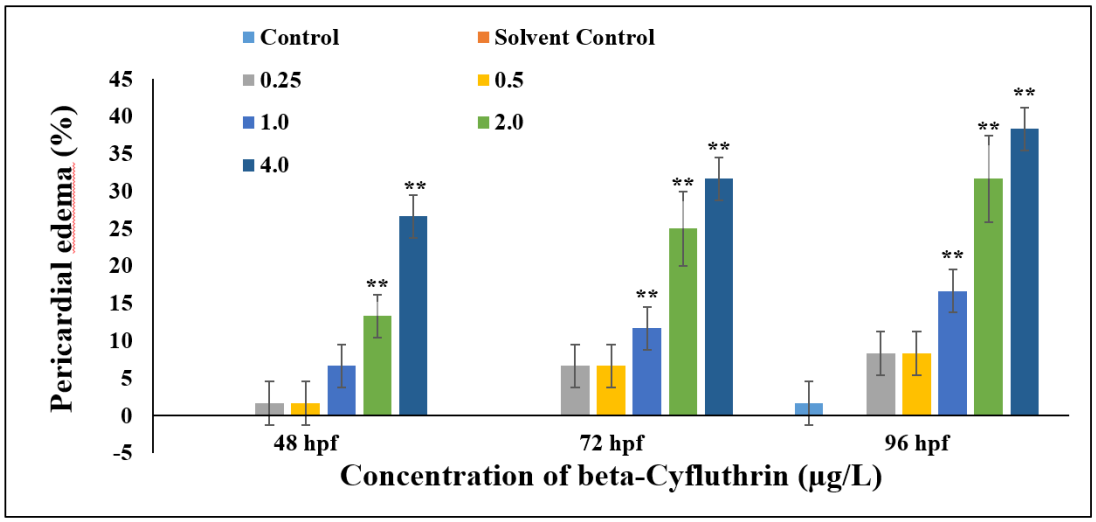

Fig. 5: Pericardial edema occurring in zebrafish embryos exposed to different concentrations of beta-Cyfluthrin (µg/L) at 48, 72 and 96 hpf. Data are expressed as mean ± SD (n=3 replicates of 20 embryos in each concentration). Asterisks denote significant differences between control and treatments at different time periods (p < 0.01).**

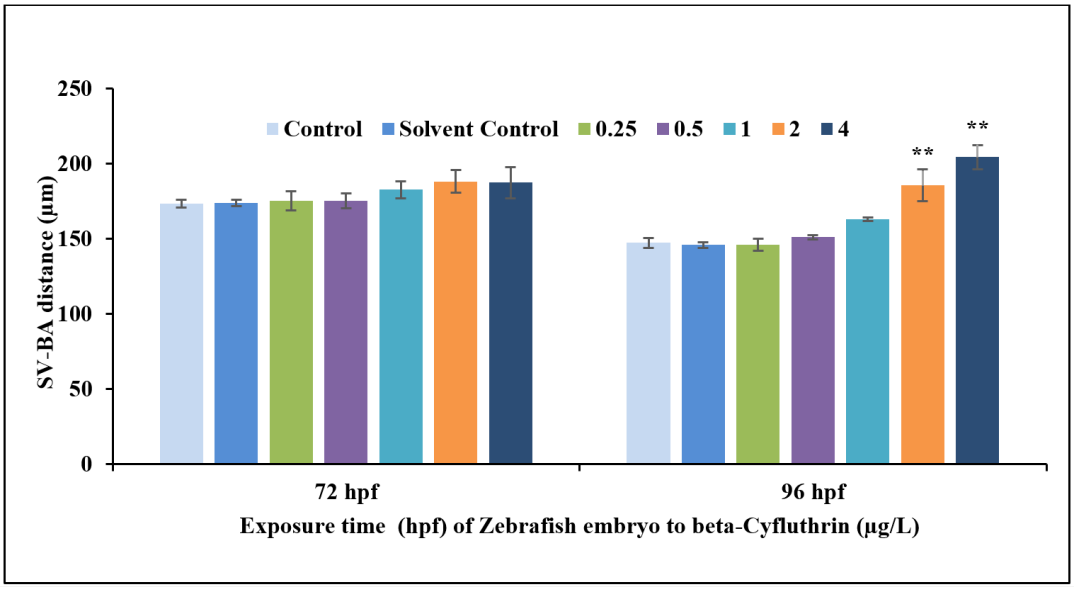

Fig. 6: SV-BA distance in zebrafish embryos exposed to different concentrations of beta-Cyfluthrin (µg/L) after 72 and 96 hpf. Data are expressed as mean ± SD (n=3 replicates of 20 embryos in each concentration). Asterisks denote significant differences between control and treatments at different time periods (p < 0.01).**

Comparison of the SV-BA distance between 72 and 96 hpf indicates that in untreated embryos, the SV-BA distance decreases from 72 to 96 hpf. This is due to the tube like heart folding into an S-shaped loop, thereby decreasing in length. However, Fig. 6 shows that embryos in concentrations 2.0 and 4.0 ug/L showed a significant increase in SV-BA distance from 72 hpf to 96 hpf, indicating failure of the looping of the cardiac tube.

The rate of heart beat is one of the important cardiac functions and is an important indicator of early life stage toxicity (Henry *et al.*, 1997). The embryos exposed to increasing concentration of beta-Cyfluthrin showed significant increase in the rate of heartbeat with respect to control in a time-dependent manner (Fig. 7). The increased rate of heartbeat could be due to pericardial edema and abnormal looping of the heart (Yamauchi *et al.*, 2005). A comparison of the heart rate with the incidence of pericardial edema shows that increasing levels of pericardial edema correlated to increasing heart rate (beats/min). The results suggest that beta-Cyfluthrin impairs both the

https://doi.org/10.36282/IJASRM/8.12.2023.1919

cardiac structure and function of zebrafish embryos.

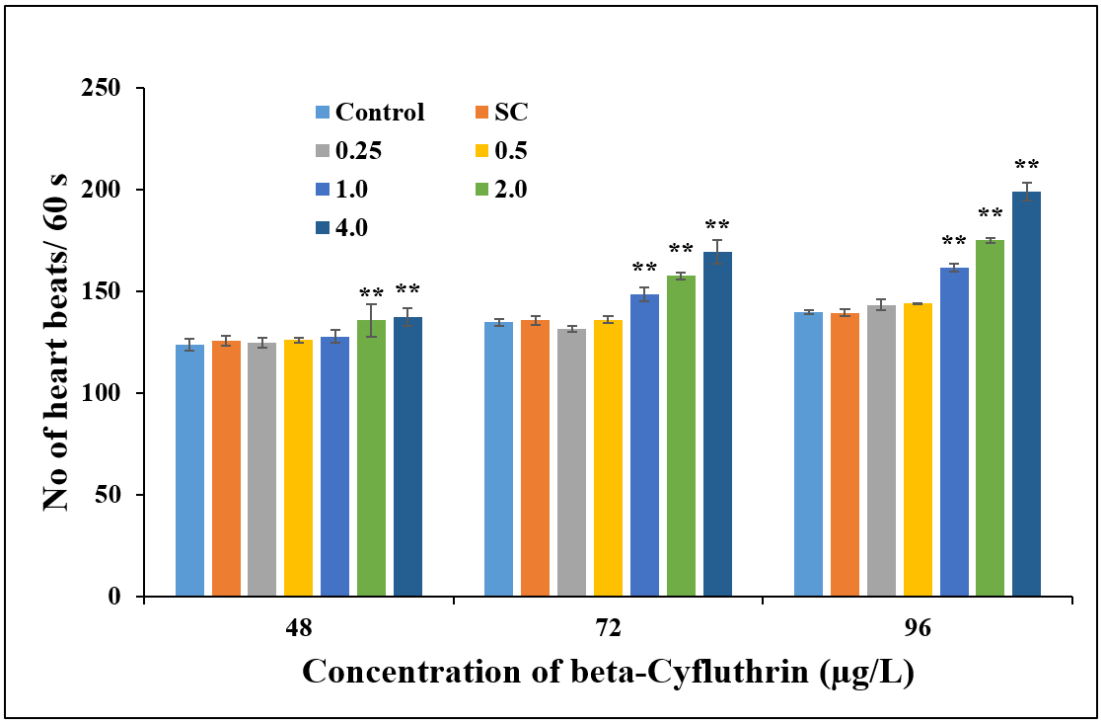

Fig. 7: Number of heart beats occurring in zebrafish embryos exposed to different concentrations of beta-Cyfluthrin (µg/L) at 48/72/96 hpf for 60 s. Data are expressed as mean ± SD (n=3 replicates of 20 embryos in each concentration). Asterisks denote significant differences between control and treatments at different time periods (p < 0.01).**

It should be noted that abnormalities caused by beta-Cyfluthrin did not result from an inability to form a functional heart in the initial stages of development. With the increase in time and concentration, the beta-Cyfluthrin exposed embryos developed cardiac abnormalities and deteriorated in function compared to the control.

Prior research have shown that synthetic pyrethroids like ectomethrin, tralomethrin and α-cypermethrin can cause cardiovascular defects like pericardial edema and increased heart rate (Othman and Mahmoud, 2019; Ghazouani *et al*., 2020; Guo *et al*., 2023).

3.6 Effect of beta-Cyfluthrin on the yolk sac

In zebrafish developmental toxicity studies, yolk sac edema is a frequently observed malformation. Many compounds including pesticides have been shown to cause yolk sac edema in zebrafish embryos (Cao *et al*., 2016; Oliveira *et al*., 2017). "Blue sac syndrome" which is characterized by pericardial and yolk sac edema is observed in many developmental toxicity screens in zebrafish (Hill *et al*., 2004). This suggests that the incidence of yolk sac edema is an important toxicological outcome for zebrafish developmental studies (Sant and Laragy, 2018).

Yolk sac edema was observed in the treated embryos at 48, 72 and 96 hpf. The embryos exposed to concentrations 2.0 and 4.0 µg/L showed significant difference from 48 hpf onwards, while embryos in 1.0 µg/L developed edema from 72 hpf onwards (Fig. 9). Earlier research show that exposure to deltamethrin, a widely used type II pyrethroid resulted in yolk sac edema in zebrafish (Kuder and Gundala, 2018).

.

https://doi.org/10.36282/IJASRM/8.12.2023.1919

International Journal of Advanced Scientific Research and Management, Volume 8 Issue 12, Dec 2023

www.ijasrm.com

ISSN 2455-6378

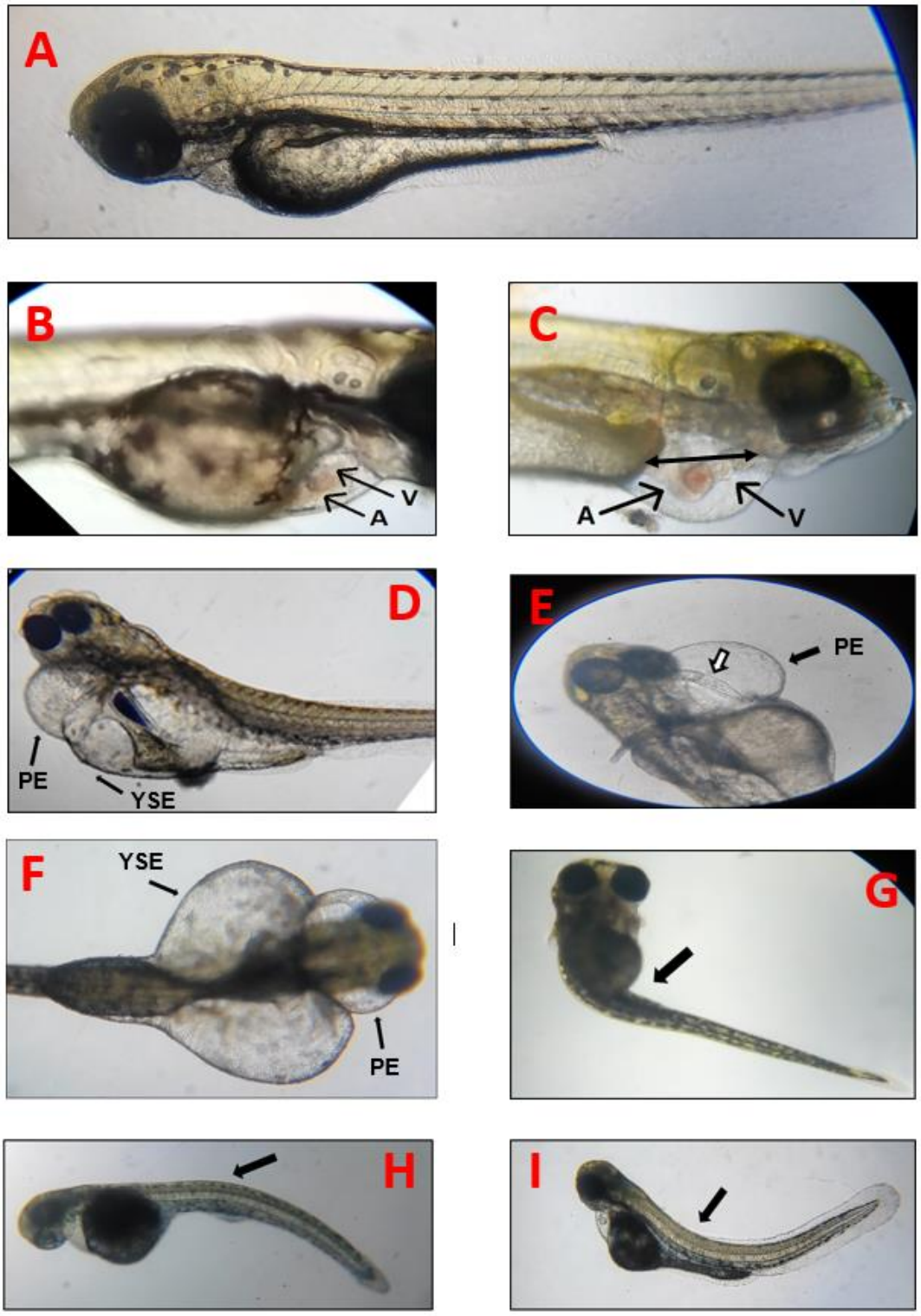

Fig. 8: Morphological effects of beta-Cyfluthrin on zebrafish developmental stages. A- Normal embryo at 48 hpf; B and C - embryo with pericardial edema (A-auricle, V ventricle) at 48 and 96 hpf respectively, ⇔ indicates the SV-BA distance; D – embryo with pericardial edema (PE) and yolk sac edema (YSE) at 72 hpf; E – embryo with pericardial edema (PE) at 72 hpf showing failure in looping of the heart tube, marked by ⇦ ; F- embryo with severe pericardial edema (PE) and yolk sac edema (YSE) (dorsal view) at 72 hpf; G - embryo with scoliosis at 48 hpf, markd by ← ; H - embryo with kyphosis at 48 hpf, marked by ← and I- embryo with lordosis at 48 hpf, marked by ←

https://doi.org/10.36282/IJASRM/8.12.2023.1919

International Journal of Advanced Scientific Research and Management, Volume 8 Issue 12, Dec 2023

www.ijasrm.com

ISSN 2455-6378

3.7 Effect of beta-Cyfluthrin on Spinal Curvature

Skeletal and muscle tissue are vulnerable to organic chemical exposure. Exposure of zebrafish embryos to various type I pyrethroids like permethrin, resmethrin, and bifenthrin and type II pyrethroids like deltamethrin, cypermethrin, and λ-cyhalothrin have resulted in curvature of the spine (DeMicco *et al.*, 2010). The current study shows that exposure to beta-Cyfluthrin resulted in defects in spinal curvature. There was significant difference in incidence of abnormal curvature of the spine in embryos exposed to 2.0 and 4.0 µg/L of beta-Cyfluthrin at 72 and 96 hpf compared to control (Fig. 10). Scoliosis (sideways curvature of the spine –Fig. 8G), kyphosis (inward curvature of the spine – Fig. 8H) and lordosis (outward curvature of the spine Fig. 8I) were observed in embryos exposed to 1.0, 2.0 and 4.0 µg/L of beta-Cyfluthrin

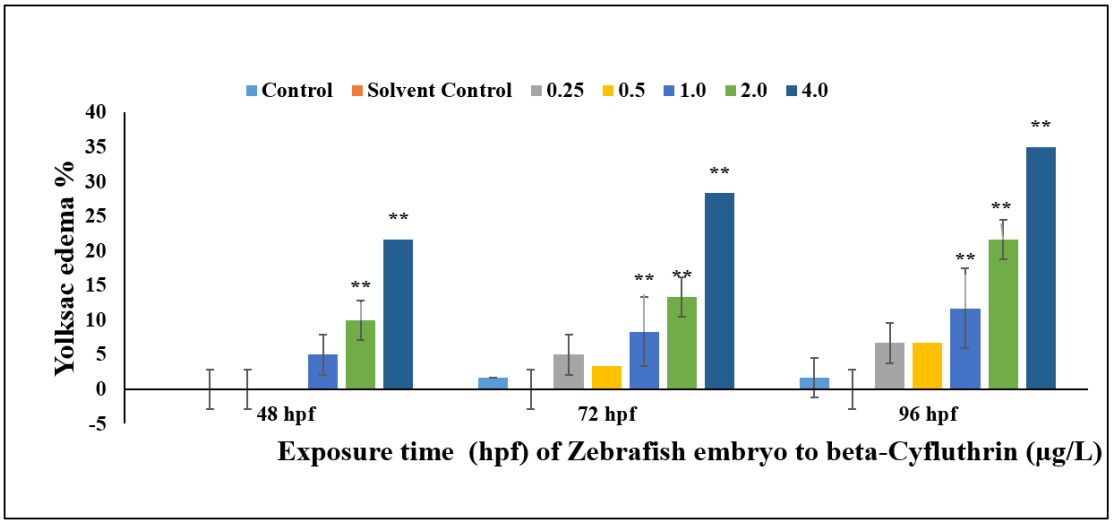

Fig. 9: Yolk sac edema occurring in zebrafish embryos exposed to different concentrations of beta-Cyfluthrin (µg/L) at 48, 72, and 96 hpf. Data are expressed as mean ± SD (n=3 replicates of 20 embryos in each concentration). Asterisks denote significant differences between control and treatments at different periods (p < 0.01)**

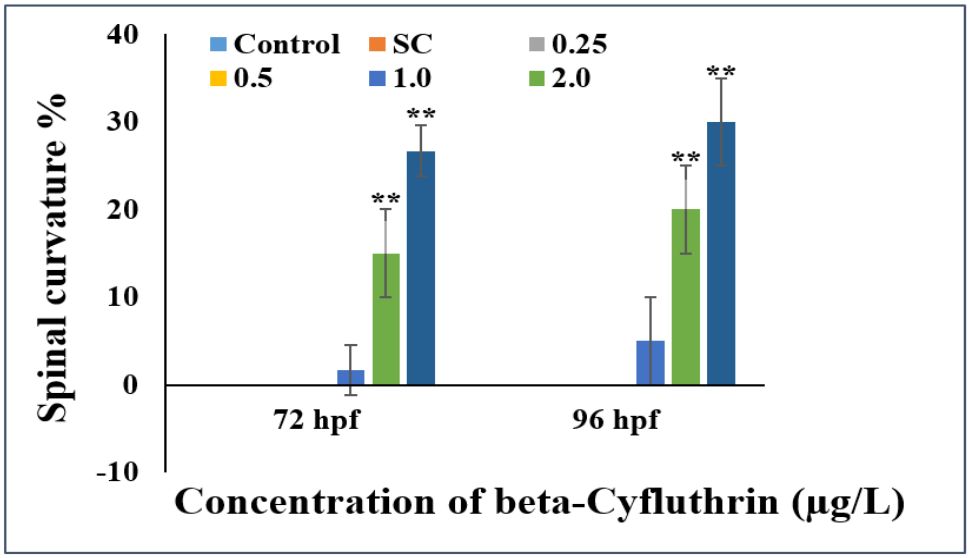

Fig. 10: Abnormal curvature of the spine occurring in zebrafish embryos exposed to different concentrations of beta-Cyfluthrin (µg/L) at 72 and 96 hpf. Data are expressed as mean ± SD (n=3 replicates of 20 embryos in each concentration). Asterisks denote significant differences between control and treatments at different time periods (p < 0.01).**

https://doi.org/10.36282/IJASRM/8.12.2023.1919

4. Conclusion

The results from the present study show that beta-Cyfluthrin can lead to developmental defects in zebrafish. The increase in mortality and abnormalities showed a concentration-dependent and time-dependent manner. As the toxicity of beta-Cyfluthrin to the developmental stages of zebrafish have not been studied extensively, this study forms the initial basis for further exploration of developmental and cardiac toxicity of beta-Cyfluthrin in zebrafish. Beta-Cyfluthrin is widely used in India as an insecticide and this results in significant potential risk to non-target species, especially aquatic life. It is crucial to develop methods of application of beta-Cyfluthrin to crops which will minimize the likelihood of it entering aquatic ecosystems.

References

[1] Al-Makkawy H K, and Magdy Diab Madbouly, Persistence and Accumulation of Some Organic Insecticides in Nile Water and Fish. Resources, conservation and recycling, 27 (1&2):105-115, (1999).

[2] Amin K A, Hashem K S, Deltamethrin-induced oxidative stress and biochemical changes in tissues and blood of catfish (*Clarias gariepinus*): Antioxidant Defense and Role of Alpha-tocopherol. BMC Vet Res, 8:45–52, (2012).

[3] Antkiewicz D S, Burns C G, Carney S A, Peterson R E and Heideman W, Heart Malformation is an Early Response to TCDD in Embryonic Zebrafish. Toxicological Sciences, 84(2) : 368-377, (2005).

[4] Arisekar U, Robinson J, Rajendran S and Geevaretnam J, Pesticides Contamination in the Thamirabarani, a Perennial River in Peninsular India: The First Report on Ecotoxicological and Human Health Risk Assessment. Chemosphere, 267: 129251, (2021).

[5] Benli A C K, Investigation of Acute Toxicity of Cyfluthrin on Tilapia Fry (*Oreochromis niloticus* L. 1758). Environmental Toxicology and Pharmacology, 20(2): 279-282, (2005).

[6] Cao F, Liu X, Wang C, Zheng M, Li X, & Qiu L, Acute and Short-term Developmental Toxicity of Cyhalofop-butyl to Zebrafish (*Danio rerio*). Environmental Science and Pollution Research, 23:10080-10089, (2016).

[7] Casjens H and Branch E M, Environmental Fate of Cyfluthrin. Department of Pesticide Regulation, (2008).

[8] Clark J R, Goodman L R, Borthwick P W, Patrick Jr J M, Cripe G M, Moody P M and Lores E M, Toxicity of Pyrethroids to Marine Invertebrates and Fish: A Literature Review and Test Results with Sediment-Sorbed Chemicals. Environmental Toxicology and Chemistry: An International Journal, 8(5): 393-401, (1989).

[9] Cold Spring Harbor Protocol, (2011). E3 medium (for zebrafish embryos). https://cshprotocols.cshlp.org/content/2011/10/pdb.rec66449, accessed on 19 Nov 2023.

[10] De Gaspar Ignacio, Blanquez M J, Fraile B, Paniagua R andArenas M I, The Hatching Gland Cells of Trout Embryos: Characterisation of N-and O-Linked Oligosaccharides. The Journal of Anatomy, 194(1): 109-118, (1999).

[11] DeMicco A, Cooper K R, Richardson J R and White L A, Developmental Neurotoxicity of Pyrethroid Insecticides in Zebrafish Embryos. Toxicological Sciences, 113(1): 177-186, (2010).

[12] Du G, Shen O, Sun H, Fei J, Lu C, Song L and Wang X, Assessing Hormone Receptor Activities of Pyrethroid Insecticides and Their Metabolites in Reporter Gene Assays. Toxicological Sciences, *116*(1): 58-66, (2010).

[13] Extension Toxicology Network (1996) EXTOXNET Pesticide Information Profiles, Cyfluthrin, Oregon State University: Corvallis, OR. http://extoxnet.orst.edu/pips/cyfluthr.htm, accessed Oct 2023.

[14] Fraysse B, Mons R and Garric J, Development of a Zebrafish 4-Day Embryo-Larval Bioassay to Assess Toxicity of Chemicals. Ecotoxicology and

https://doi.org/10.36282/IJASRM/8.12.2023.1919

Environmental Safety, *63*(2): 253-267, (2006).

[15] Gan J, Spurlock F, Hendley P and Weston D P, Synthetic Pyrethroids: Occurrence and Behavior in Aquatic Environments. American Chemical Society, (2008).

[16] Ghazouani L, Feriani A, Mufti A, Tir M, Baaziz I, Mansour H B and Mnafgui K, Toxic Effect of Alpha Cypermethrin, An Environmental Pollutant, On Myocardial Tissue in Male Wistar Rats. Environmental Science and Pollution Research, 27: 5709-5717, (2020).

[17] Guo S, Wu Y, Li W and Xiao P, Tralomethrin Causes Cardiovascular Toxicity in Zebrafish (*Danio rerio*) Embryos. Environmental Toxicology, 38 (11): 2761-2771, (2023).

[18] Henry T R, Spitsbergen J M, Hornung M W, Abnet C C and Peterson R E, Early Life Stage Toxicity of 2, 3, 7, 8-Tetrachlorodibenzo-P-Dioxin in Zebrafish (*Danio rerio*). Toxicology and Applied Pharmacology, *142*(1): 56-68, (1997).

[19] Hill A J, Bello S M, Prasch A L, Peterson R E and Heideman W, Water Permeability and TCDD-induced Edema in Zebrafish Early-life stages. Toxicological Sciences, *78*(1): 78-87, (2004).

[20] Jin M, Zhang X, Wang L, Huang C, Zhang Y and Zhao M, Developmental Toxicity of Bifenthrin in Embryo-larval Stages of Zebrafish. Aquatic Toxicology, *95*(4): 347-354, (2009).

[21] Johnson W W and Finley M T, Handbook of acute toxicity of chemicals to fish and aquatic invertebrates: Summaries of toxicity tests conducted at Columbia National Fisheries Research Laboratory. US Department of the Interior, Fish and Wildlife Service, 137:1965-78, (1980).

[22] Kimmel C B, Ballard W W, Kimmel S R, Ullmann B and Schilling T F, Stages of Embryonic Development of the Zebrafish. Developmental Dynamics, *203*(3): 253-310, (1995).

[23] Kimmel C B, Patterson J and Kimmel R O, The Development and Behavioral Characteristics of the Startle Response in the Zebrafish. Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology, *7*(1): 47-60, (1974).

[24] Köprücü K and Aydın R, The Toxic Effects of Pyrethroid Deltamethrin on the Common Carp (*Cyprinus carpio* L.) Embryos and Larvae. Pesticide Biochemistry and Physiology, *80*(1): 47-53, (2004).

[25] Kuder R S and Gundala H P, Developmental Toxicity of Deltamethrin and 3-Phenoxybenzoic Acid in Embryo–larval stages of Zebrafish (*Danio rerio*). Toxicology Mechanisms and Methods, *28*(6): 415-422, (2018).

[26] Lee R K, Stainier D Y, Weinstein B M and Fishman M C, Cardiovascular Development In The Zebrafish: Endocardial Progenitors Are Sequestered Within The Heart Field. Development, *120*(12): 3361-3366, (1994).

[27] Männer J, Wessel A and Yelbuz T M, How Does The Tubular Embryonic Heart Work? Looking For The Physical Mechanism Generating Unidirectional Blood Flow In The Valveless Embryonic Heart Tube. Developmental Dynamics, *239*(4): 1035-1046, (2010).

[28] Mendis J C, Tennakoon T K and Jayasinghe C D, Zebrafish Embryo Toxicity of a Binary Mixture of Pyrethroid Insecticides: D-Tetramethrin And Cyphenothrin. Journal of Toxicology, 1: 23-24, (2018).

[29] Morgan M K, Sheldon L S, Croghan C W, Jones P A, Chuang J C and Wilson N K, An Observational Study of 127 Preschool Children at Their Homes and Daycare Centers in Ohio: Environmental Pathways to Cis-And Trans-Permethrin Exposure. Environmental Research, 104(2): 266-274, (2007).

[30] OECD, Test No. 236: Fish Embryo Acute Toxicity (FET) Test. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, (2013).

[31] Oliveira J M, Galhano V, Henriques I, Soares A M and Loureiro S, Basagran® Induces Developmental Malformations and Changes the Bacterial Community of

https://doi.org/10.36282/IJASRM/8.12.2023.1919

Zebrafish Embryos. Environmental Pollution, 221: 52-63, (2017).

[32] Othman A I and Mahmoud H S, Chronic Toxicity of Ectomethrin: A Biochemical and Histological Study. Egyptian Journal of Histology, *42*(3); 526-533, (2019).

[33] Rahmi A, Kenan K, Mustafa D, Sibel S K, Murat P, Acute Toxicity of Synthetic Pyrethroid Cypermethrin on the Common Carp (*Cyprinus carpio*) Eembryo and Larvae. Aquacult Int, 13:205, (2005).

[34] Ramasubramanian A, Nerurkar N L, Achtien K H, Filas B A, Voronov D A and Taber L A, On Modeling Morphogenesis of the Looping Heart Following Mechanical Perturbations, 130(6): 06-18, (2008).

[35] Saint-Amant L and Drapeau P, Time Course of the Development of Motor Behaviors in the Zebrafish Embryo. Journal of Neurobiology, *37*(4): 622-632, (1998).

[36] Sant K E and Timme-Laragy A R, Zebrafish as a Model for Toxicological Perturbation of Yolk and Nutrition in the Early Embryo. Current Environmental Health Reports, 5: 125-133, (2018).

[37] Sepici-Dinçel A, Benli A Ç K, Selvi M, Sarıkaya R, Şahin D, Özkul I A and Erkoç F, Sublethal Cyfluthrin Toxicity to Carp (*Cyprinus Carpio* L.) Fingerlings: Biochemical, Hematological, Histopathological Alterations. Ecotoxicology and Environmental Safety, *72*(5): 1433-1439, (2009).

[38] Sharma D K and Ansari B A, Effect of The Synthetic Pyrethroid Deltamethrin and the Neem-Based Pesticide Achook on the Reproductive Ability of Zebrafish, *Danio Rerio* (Cyprinidae). Fisheries & Aquatic Life, *18*(3): 157-161, (2010).

[39] Smith T M and Stratton G W, Effects of Synthetic Pyrethroid Insecticides on Nontarget Organisms. Residue Reviews: Reviews of Environmental Contamination and Toxicology, 1: 93-120. (1986).

[40] Stainier D Y, Lee R K and Fishman M C, Cardiovascular Development in the Zebrafish: Myocardial Fate Map and Heart Tube Formation. Development, *119*(1): 31-40, (1993).

[41] Tomlin C D S, The Pesticides Manual: A World Compendium. British Crop Protection Council, 14*:*351. (2006).

[42] Tyler C R, Beresford N, Van Der Woning M, Sumpter J Pand Tchorpe K, Metabolism and Environmental Degradation Of Pyrethroid Insecticides Produce Compounds with Endocrine Activities. Environmental Toxicology and Chemistry: An International Journal, *19*(4): 801-809, (2000).

[43] Wang X H, Souders II C L, Xavier P, Li X Y, Yan B and Martyniuk C J, The Pyrethroid Esfenvalerate Induces Hypoactivity and Decreases Dopamine Transporter Expression in Embryonic/Larval Zebrafish (*Danio rerio*). Chemosphere, 243:125416, (2020).

[44] Wijngaarden R P V, Brock T C and Brink P J V D, Threshold Levels For Effects of Insecticides in Freshwater Ecosystems: A Review. Ecotoxicology, 14: 355-380, (2005).

[45] Yamauchi M, Kim E Y, Iwata H and Tanabe S, Molecular Characterization of the Aryl Hydrocarbon Receptors (AHR1 And AHR2) from Red Seabream (*Pagrus major*). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, *141*(2): 177-187, (2005).

[46] Yang Y, Ma H, Zhou J, Liu J and Liu W, Joint Toxicity of Permethrin and Cypermethrin at Sublethal Concentrations to the Embryo-Larval Zebrafish. Chemosphere, 96: 146-154, (2014).

[47] Zhang Q, Zhang Y, Du J and Zhao M, Environmentally Relevant Levels of λ-cyhalothrin, Fenvalerate, and Permethrin Cause Developmental Toxicity and Disrupt Endocrine System in Zebrafish (*Danio rerio*) Embryo. Chemosphere, 185: 1173-1180, (2017)

.

https://doi.org/10.36282/IJASRM/8.12.2023.1919