Toxicology International, *32(1) : 91-99; 2025. DOI: 10.18311/ti/2025/v32i1/45020*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Contents available at: www.informaticsjournals.com/index.php/toxi



The Role of *Phyllanthus niruri* in Ameliorating the Toxic Effects of Imidacloprid Insecticide in Zebra Fish Embryos

K. Gowsalya[1*], Asmi Khopkar[2], K. Elumalai[1], M. Elumalai[1] and Saramma Mini Jacob[2]

[1]Department of Zoology, Government Arts College for Men, Nandanam, Chennai – 600035, Tamil Nadu, India; gowsalyascholar2021@gmail.com

[2]Research and Development Wing, Sree Balaji Medical College and Hospital, Chromepet, Chennai – 600044, Tamil Nadu, India

Abstract

Imidacloprid (IMCP) is a commonly used pesticide for agricultural treatments worldwide. It percolates through the soil into water bodies and threatens the aquatic ecosystem. One of the biggest obstacles is eradicating the accumulated pesticides in edible freshwater fishes. Many medicinal plants are being investigated in helping to mitigate the toxic effects on fish. The objective of the present study was to evaluate the *in vivo* toxicity of zebrafish (*Danio rerio)* embryos exposed to sub-lethal concentration of IMCP and to mitigate the effect of IMCP with *P. niruri* aqueous Leaf Extract (PNLE). The male and female zebra fishes were allowed to breed and the embryos were collected. The fertilised embryos were divided into four groups (10 embryos each) – Group I (Control), Group II (IMCP), Group III (PNLE) and Group IV (IMCP and PNLE). The developmental stages of the embryos (24, 48, 72 and 96 h) in the control and treated groups were viewed under a light microscope and photographed. When compared with the control group, Group II showed major deformities, Group III results showed normal embryonic development as in Group I and Group IV showed normal embryonic development. Thus, in conclusion, the cytotoxic effects of imidacloprid in zebrafish embryos were mitigated by *P. niruri* aqueous extract.

Major Findings: This study shows that *Phyllanthus niruri* aqueous leaf extract (PNLE) mitigates Imidacloprid (IMCP) toxicity in zebrafish embryos. IMCP exposure causes developmental deformities, while PNLE treatment restores normal development. PNLE may serve as a natural detoxifier in aquatic ecosystems, benefiting environmental conservation and food safety.

Keywords: Deformed Embryos, Developmental Stages, Imidacloprid, *P. niruri*, Sub-Lethal Concentration, Zebra Fish

1. Introduction

Zebrafish (*Danio rerio*) is a benthopelagic freshwater fish belonging to the family minnow (Cyprinidae), Order (Cypriniformes), Class (Actinopterygii) and Phylum (Chordata). It is utilized for *in vitro, in vivo* and developmental toxicity research as an embryonic and larval model[1]. Zebrafish is a top model organism, and because of its inherent benefit, low maintenance costs, ease of availability, and generation of many transparent embryos, it is now widely employed in many scientific fields[2]. Zebrafish embryos provide excellent experimental models since they are less painful and fragile than adult fish[3]. Their applications in biology research span a vast array of subjects, such as chemical toxicity, behaviour,

**Author for correspondence*

Article Received on: 18.07.2024 Revised on: 21.11.2024 Accepted on: 06.12.2024

drug development, and mimicking human diseases through the use of forward and reverse genetic approaches in conjunction with large-scale, high-throughput screening[4]. Since zebrafish and humans have identical xenobiotic metabolisms[5], researchers can conclude and get a deeper understanding of how potentially dangerous substances may affect human health. Furthermore, zebrafish can offer crucial information about how pesticides affect aquatic ecosystems and human health, which can help create a safer and more sustainable environment. This is because they are exceptionally sensitive to the toxicity of pesticides[6]. As a result, the zebrafish embryo toxicity test was developed as a reliable model for toxicity studies, and test guidelines were established utilizing embryos as model organisms[7,8]. Normal embryonic stages in zebrafish and development events in each stage[9] are depicted in Figure 1 and Table 1, respectively.

Imidacloprid (IMCP) is a neonicotinoid and resembles nicotine's toxic nature towards insects. It appears as a colourless crystals and has a chemical formula of $C_9H_{10}ClN_5O_2$. Its solubility in water is 0.51 g/L (at 20°C), with its melting point ranging from 409.5 to 416.9 K. Due to its affordability, ease of application, and effectiveness, this pesticide is one of the most often used agricultural treatments worldwide. In the absence of light, it degrades at a very slow pace and remains in the soil for a very long time. This pesticide percolates through soil into water bodies leading to an upsurge in pH and toxicity of soil and water bodies. Imidacloprid endangers the aquatic environment and disrupts the aquatic system[10]. It is also classified as moderately hazardous to humans (Class-II WHO; toxicity category-II EPA) based on animal studies[11]. In aquatic ecotoxicology, zebrafish has emerged as a key indicator organism capable of accurately identifying the toxicity of minute environmental contaminants[12].

Phyllanthus niruri commonly known as "*Keezhanelli*" in the local language in Tamil Nadu, (India) or Stonebreaker is a tropical plant that has been used in traditional herbal medicine for centuries. The plant

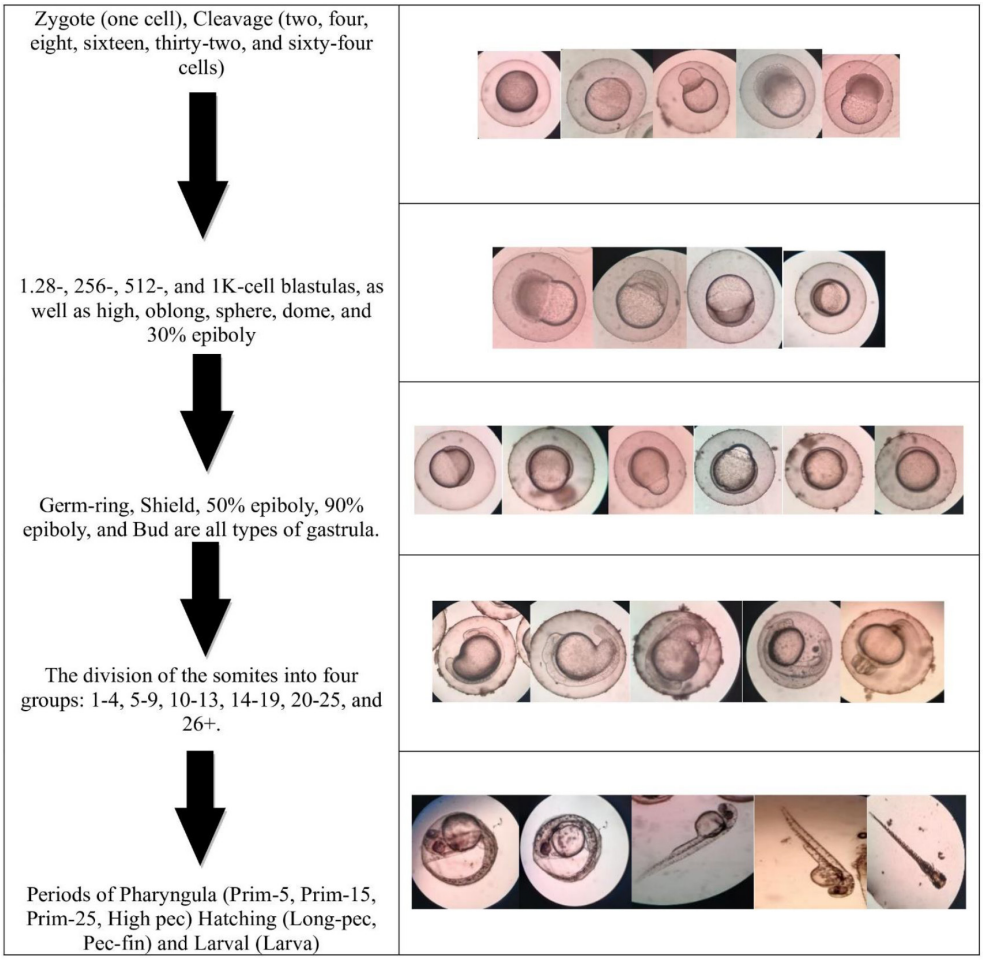


Figure 1. Zebrafish embryonic stages.

belongs to the Phyllanthaceae family and is native to tropical regions of the Americas, including the Amazon rainforest. The plant is a small, annual herb that grows to a height of about 30-40 cm. It has small green leaves and bears tiny, inconspicuous flowers. The entire plant, including the leaves, stems, and roots, is utilized for its medicinal properties. It contains various bioactive compounds, including lignans, alkaloids, flavonoids, and tannins. These compounds are believed to contribute to the plant's therapeutic effects. Several scientific studies have explored the potential of *P. niruri* in the treatment of kidney stones and liver disorders[13-15].

2. Objective of the Study

The objective of this study was to assess the *in vivo* toxicity of zebrafish embryos subjected to sub-lethal concentration of IMCP during the course of 24, 48, 72, and 96 h and analyzed the developmental stages of zebrafish embryos (Zygote, Cleavage, Blastula, Gastrula, Segmentation, Pharyngula, Hatchlings, Larval stage) and deformities in pesticide-treated embryos. In addition, the impact of *P. niruri* aqueous extract on the effects of IMCP-intoxicated embryos was studied.

3. Material and Methods

3.1 Fish Care and Breeding

Adult male and female zebrafishes (Figure 2) were purchased from the local fish market (Priya Dharshini Aquarium), Kolathur, Chennai and maintained at the Research and Development wing of the Sree Balaji Medical College and Hospital, Chennai. The female fishes and the male fishes were sexually differentiated by

Table 1. Events of embryonic stages in zebrafish

Stages of Development	Developmental events of each stage	Time period (min/h)
Fertilization	Formation of Zygote	External fertilization
Zygote	Cytoplasm Accumulated at the animal pole of the embryonic membrane(one-cell-stage)	45 min after fertilization
Cleavage	Two-cell stage median vertical division; four-cell stage vertical division; discoid partial cleavage Vertical and parallel to the first division's plane (eight-cell stage) and the second division's plane (sixteen-cell stage)	4 cell stage - 1 h 8 cell stage - 1.25 h 16 cell stage - 1.5 h
Blastulation	The formation of the interface between the blastoderm and yolk, the onset of the blastula stage, the late cleavage period, and the 256 blastomeres in the blast disc	Blastula- 2.25 to 5.25 h
Gastrulation	50% of the epiboly gets thin, the blastoderm-periblast interface becomes curved, and 75% of the epiboly ends and the blastoderm is almost closed.	Gastrula- 5.25 to 10 h
Segmentation	Initial development of somites Developed somites; an undifferentiated mesodermal component of the early trunk; a segmented tail; muscular twitches; an expanded tail; and the creation of otoliths	Segmentation- 10 to 24 h
Pharyngula period	The characteristics of the prototypic stage are as follows: spontaneous movement, tail detachment from the yolk, early pigmentation, reduced spontaneous movement, retinal pigmentation, tail degeneration, aortic arch circulation, tail pigmentation, string circulation, single aortic arch pair, early motility, initiation control over heart rate Organs tend to differentiate and develop (Long Pec, Pec fin)	Pharyngula- 24 to 48 h
Hatching Period	Regular heartbeat, yolk extension begins to taper, segmented blood vessels are visible, foregut development initiated, Sacculus gets thickened with two chambers (Prim 5, Prim 15, Prim 25, High pec) Extension of the tail and the organ development persists and step into the next phase of fingerlings Feeding behaviour tends to develop	Hatching- 48 to 72 h

their morphology and maintained in separate tanks for a period of one week before breeding. The tank and room temperature were maintained between 24°C-36°C with lighting conditions 14:10 h (Light: dark). Filters were used to maintain the water (RO water) quality and the tanks were cleaned regularly. The maintenance of water quality parameters is given in Table 2. The fish were fed twice daily with dry or live food; mostly with blood worm-infused flakes which were purchased commercially from the market (65% protein, 5% fat and 30% fibre). The breeding tank was set up with pebbles and a mesh plate under which the laid eggs got settled. Male and female zebra fishes were bred in the breeding tank, and the eggs laid post-breeding were used for this study. The unfertile and fungal-infected embryos were discarded and the fertilized healthy embryos were taken for experiments[16].

3.2 Preparation of Embryonic Medium

The Stock embryonic medium composed of 3.65 g sodium chloride (NaCl), 0.16 g potassium chloride (KCl), 0.55 g calcium chloride ($CaCl_2$), 1.01 g magnesium chloride ($MgCl_2$) and 100 µl of 1% of methylene blue[17]. The prepared stock was autoclaved for 30 min at 121°C and stored in a glass jar with a lid. The working standard was prepared by adding 20 ml of stock medium to 230 ml of autoclaved distilled water.

3.3 Wide Range and Narrow Range Test

The Stock Imidacloprid solution 17.8% SL was prepared at the concentration of 2 µl/ml (2 µl IMCP in 1 ml distilled water). From the IMCP stock, five different concentrations were prepared and tested for a wide range of concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 0.8, 0.9, 1.0) 0.1 mg/L concentrations resulted with 90% mortality thus, beyond 1.0 mg/L narrow range concentrations (0.02, 0.04, 0.06, 0.08, and 0.10 mg/L) were prepared.

Table 2. Water quality parameters for zebra fish maintenance

Water parameters	Range of maintenance	Method/ Instrument
pH	6.8 to 8.5	Digital pH meter
Temperature	24°C-36°C	Digital temperature meter
Total Dissolved Solids	0.13 mg/l Devis	Digital TDS meter
Dissolved oxygen	6 to 8 mg/l	Digital DO meter
Hardness	50 to 100 mg/l	EDTA titration method
Alkalinity	50 to 70 ppm	Digital titrator
Conductivity	200 to 2500 micro siemens	Probe and meter

3.4 Sub-Lethal Concentration and LC_{50} Calculation

The exposed sub-lethal concentration of IMCP against the embryo was ½ value of LC_{50} 96 hr. The LC_{50} and LC_{90} values for 24, 48, 72 and 96 h exposure of IMCP to zebrafish embryos were analyzed with SPSS Software (Version 20.0).

3.5 Preparation of *P. niruri* Leaf Extract (PNLE)

P. niruri leaves were collected from various locations and washed thoroughly with running tap water and shade-dried at laboratory condition. The plant was identified and confirmed by the Department of Botany at the Government Arts College, Nandanam, Chennai. The dried leaves were then powdered with mortar and pestle. 500 mg of leaf powder was soaked in 10 ml of distilled water in a test tube and heated at 100°C for 2 hrs. After successful incubation at 37°C for 24 h, the aqueous extract of leaves was centrifuged at 2000 rpm for 10 min at room temperature and 200 µl of the supernatant was utilized for the experiment[18].

3.6 Sample Exposure and Documentation

The experiment was conducted in triplicate to ensure reproducibility and reliability. Fertilized embryos were



Figure 2. Female and male zebrafish.

divided into four groups, each containing 10 embryos: Group I served as the control, Group II was exposed to 200 µl of Imidacloprid (IMCP), Group III was treated with 400 µl of *P. niruri* Leaf Extract (PNLE), and Group IV received 200 µl of IMCP along with 400 µl of PNLE to assess amelioration effects. The dosages were determined and fixed in a ratio of 1:2 (IMCP: PNLE) after performing wide-range and narrow-range tests to optimize effective concentrations. The embryos were subjected to treatment and subsequently observed for developmental changes and deformities over 24, 48, 72, and 96 h. Observations were made using a light microscope, and microphotographs were taken to document the developmental stages and any deformities using a Vivo T1 smartphone equipped with a 50 MP built-in camera and an external projecting lens to enhance magnification. The images were captured

Table 3. Acute embryotoxicity of IMCP 17.8% on *D. rerio* embryos

Exposure periods (Hrs)	Acute toxicity testing- Embryotoxicity (ppm)	
	LC_{50} (LCL - UCL)	LC_{90} (LCL - UCL)
24	0.057 (0.052-0.062)	0.109 (0.100-0.121)
48	0.047 (0.035-0.056)	0.087 (0.075-0.112)
72	0.040 (0.035-0.044)	0.077 (0.072-0.084)
96	0.035 (0.028-0.039)	0.074 (0.071-0.080)

LC_{50} – Lethal Concentration 50; LC_{90} – Lethal Concentration 90; LCL- Lower Confidence Limit; UCL- Upper Confidence Limit; Hrs - hours; ppm- parts per million.

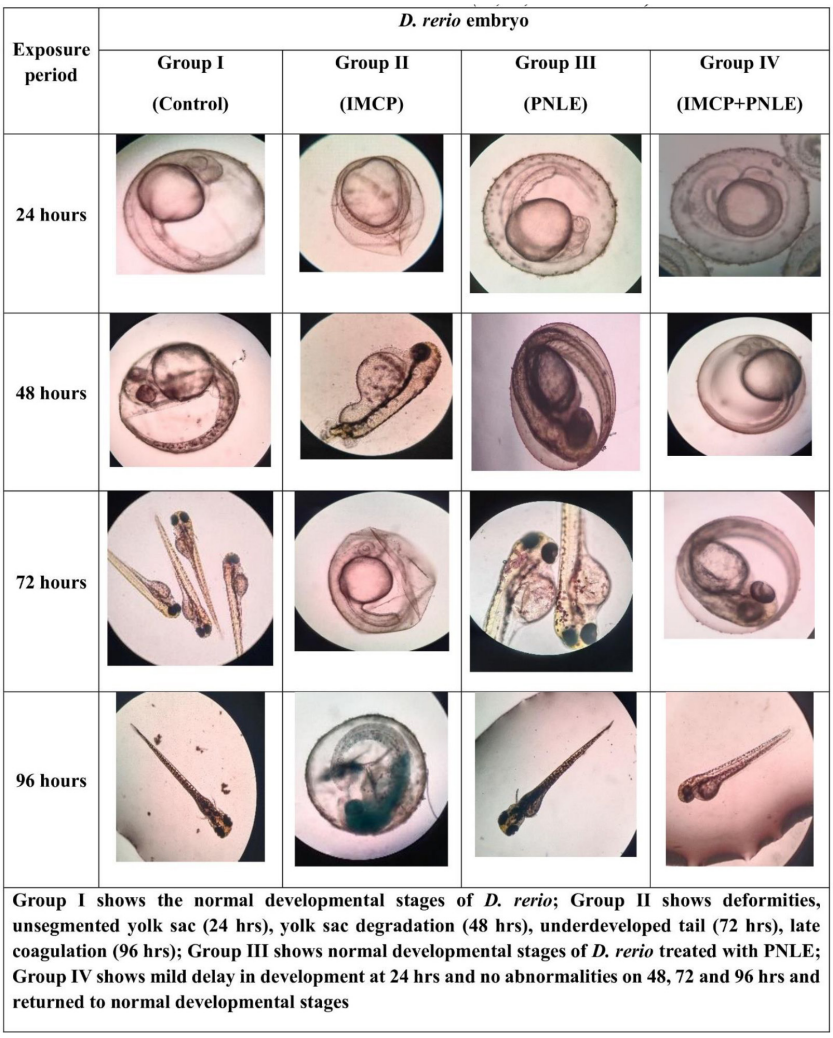


Figure 3. Morphological changes in *D. rerio* embryo to Control, IMCP treated, PNLE treated and IMCP with PNLE treated (24, 48, 72 and 96 h).

systematically for all groups at each time point to ensure consistent documentation. The control group served as a baseline for normal development, while comparisons were made with treated groups to evaluate the impact of IMCP and the potential protective role of PNLE.

4. Results

In *D. rerio* embryos, LC_{50} on exposure with IMCP was found to be 0.057, 0.047, 0.040 and 0.035 mg/L for 24, 48, 72 and 96 h respectively and LC_{90} was found to be 0.109, 0.087, 0.077 and 0.074mg/L for 24, 48, 72 and 96 h respectively (Table 3). Hence the sub-lethal concentration of IMCP was 0.0175 mg/L. The embryo developmental stages (24, 48, 72 and 96 h) of control and treated Groups (I, II, III, and IV) were observed under a microscope and the microphotographs are shown in Figure 3. Fungal-infected embryos were photographed and discarded. Various abnormalities observed in the study when treated with IMCP were, egg coagulation, premature hatchling, decapitated tail, maimed body with a single eye, cardiac edema and absence of heart development, severed and degraded yolk sac, dead embryo with slender body, collapsed body structure, ruptured dead embryo, stunted growth, flexure in the tail and dispersed body structure (Figure 4). The embryos treated with PNLE showed

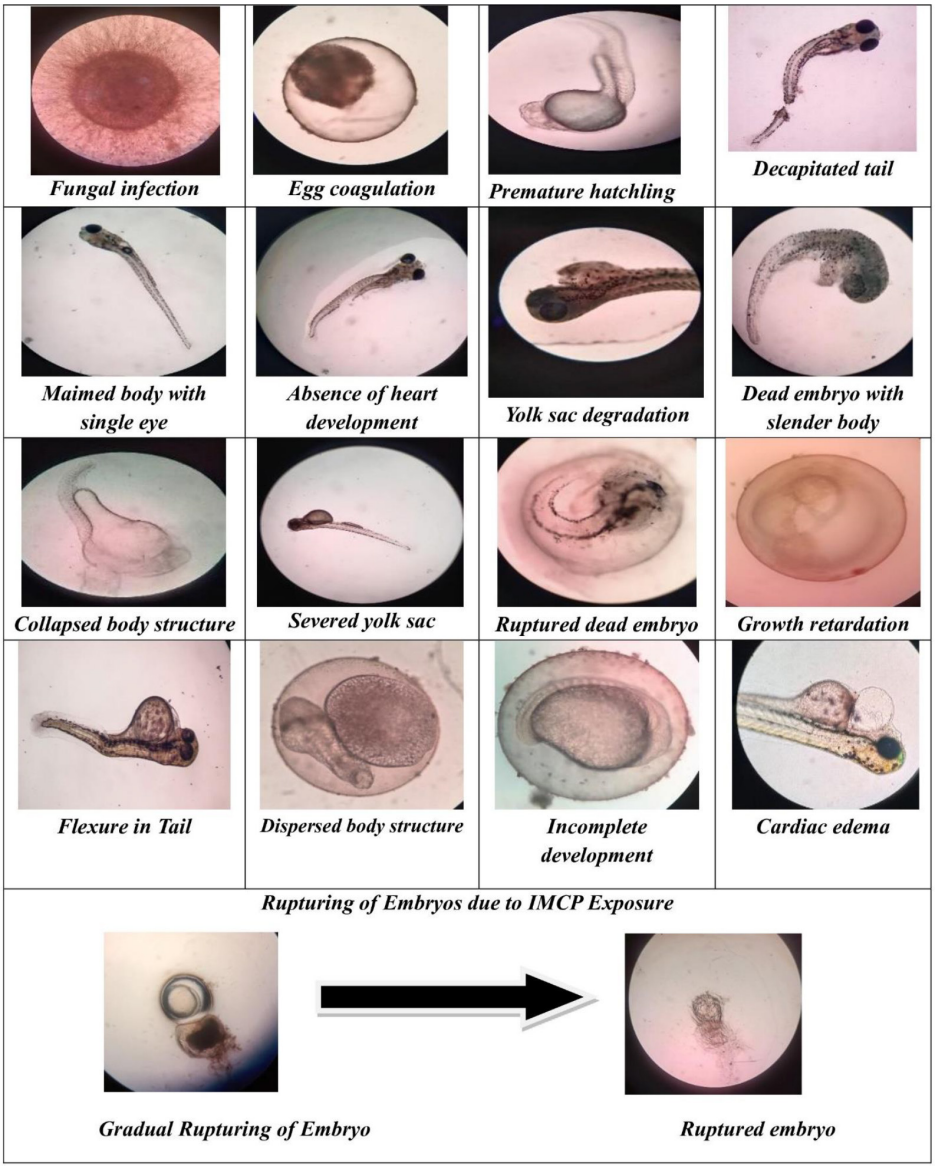


Figure 4. Various abnormalities resulted from exposure to IMCP (Group II).

normal development. Nevertheless, there was a slight delay in the embryos' development at 24 h when they were exposed to the combination group (IMCP+PNLE), but normal development was observed at 48, 72, and 96 h. No abnormalities were seen (Figure 3).

5. Discussion

Many species have been employed as proxy indicators of pollution exposure over time to determine the hazards to human health. zebrafish have been evolving significantly in recent years and are now widely used as models in many fields, including toxicity studies[19]. To assess the harmful effects of IMCP on zebrafish embryos and larvae, several endpoints have been examined. Although there is an adequate quantity of research to evaluate the level of toxicity of various pesticides on the morphology of fish embryos, there is still a knowledge gap about how to mitigate the effects of pesticides on embryo morphology. Thus, our results provide insight into reducing the impact of IMCP using *P. niruri* aqueous leaf extract.

In our study, the acute toxicity of IMCP in the developmental stages of zebrafish was found to be 0.057, 0.047, 0.040 and 0.035 mg/L for 24, 48, 72 and 96 h respectively. A study from Uttar Pradesh, India reported the LC_{50} of IMCP for zebrafish as 0.423, 0.352, 0.297 and 0.270 ml/l after 24, 48, 72 and 96 h, respectively[20]. Consequently, this indicates that IMCP is more toxic in comparison to our findings. However, a study from Gujarat reported acute toxicity of IMCP in adult zebrafish at 27.5 mg/L concentration of imidacloprid for 24 h[21]. A study from Bangladesh found LC_{50} of IMCP for the embryos of freshwater common carp was 12, 708.8, 611 and 274.9 µg/L and for the larvae of common carp was 93719, 21691, 2352.7, and 1292.6 µg/L for 24, 48, 72 and 96 hrs respectively. Therefore, the LC_{50} value for Imidacloprid varied greatly in zebrafish when compared to our study and also for other freshwater fishes. The major deformities were noted at the sub-lethal doses of IMCP (300 and 1000 µg/L)[22]. Whereas in another freshwater fish *Labeo rohita* (HAMILTON), LC_{50} values obtained were 14,260 mg/L, 13,710 mg/L and 11,900 mg/L for 24, 48 and 96 h[23].

In this study, *D. rerio* embryos exposed to IMCP (Group II) showed progressive developmental delays, morphological deformities and abnormalities. Toxic effects of IMCP such as egg coagulation, lack of larvae hatchings and pericardial edema were observed in zebrafish embryos in a study from Brazil[24]. A study from Turkey reported damage and degeneration of reproductive organs in male and female adult zebrafishes when exposed to IMCP[10], Research suggests that exposure to IMCP leads to damage of cerebral neurons through oxidative stress, cell death and inflammation[12]. Other investigators have assessed IMCP toxicity in several other fishes. Morphological abnormalities similar to our results were reported in common carp on exposure to IMCP[25]. Kai *et al.* reported that IMCP was highly toxic to medaka fish embryos which resulted in loss of blood circulation, loss of symmetry and displaced eyeballs which were also recorded in the present study[26]. Embryonic toxicities noted in catfish *C. gariepinus* were a reduction in heartbeat rate and rate of hatching, and malformations such as changes in the epiboly, deformed head, pericardial edema, rupturing of the yolk sac and lordosis[27]. Imidacloprid has been associated with potential health risks to humans, particularly through exposure via contaminated food and water. Studies suggest that acute imidacloprid poisoning can cause central nervous system depression, atrial fibrillation, hypokalemia and respiratory arrest[11].

In the present study, *P. niruri* potentially mitigated the effect of IMCP toxicity on the embryo morphology of zebrafish. *P. niruri* is a plant with medicinal properties that has been used widely for centuries in Ayurveda, other systems of Indian Medicine (Siddha and Naturopathy) and worldwide alternate systems of medicine. Its phytochemical properties have been studied extensively for the presence of terpenoids, steroids, saponins, flavonoids, anthocyanin, lignans, tannins, polyphenols and coumarins[18]. These compounds exhibit excellent anti-oxidant activity and it is also well known for their therapeutic effects and hence taken into account for the amelioration of IMCP toxicity. The morphological deformities on exposure to IMCP (Group II) were not observed when IMCP was administered with PNLE (Group IV). Almost identical results were observed by Khandia *et al.*[18], where *P. niruri* helped to reduce biochemical parameters such as the liver enzymes, amylase, triglyceride and cholesterol levels in IMCP-treated chick embryos. Another study from Nigeria looked at the effects of *P. niruri* when administered to Wistar rats exposed to Chlorpyrifos, an organophosphate insecticide[28]. They concluded that *P. niruri* was able to mitigate the sensory-motor and cognitive impairment which was induced by sub-acute Chlorpyrifos exposure. The ability of *P. niruri* may be attributed to polyphenol

compounds especially flavonoids that are present which help by scavenging the free radicals thus preventing cell death. Studies have shown that other plants mitigate the toxicity of insecticides. One study observed that submerged plant *E. canadensis* reduced the toxicity of carbamate insecticides[29]. More research studies can highlight the plant sources that can minimize the toxicity of common pesticides.

6. Conclusion

In conclusion, our study suggests that the agricultural insecticide – Imidacloprid is toxic to zebrafish embryos. It led to multiple developmental deformities and death. *P. niruri* leaves helped to mitigate adverse effects on IMCP-intoxicated embryos. The study suggests that *P. niruri* can mitigate the toxic effects of IMCP-induced developmental abnormalities, further studies could help to investigate the potential of *P. niruri* in ameliorating the effects of insecticide poisoning.

7. References

1. Chahardehi AM, Arsad H, Lim V. Zebra fish as a successful animal model for screening toxicity of medicinal plants. Plants. 2020; 9(10):1345. https://doi.org/10.3390/plants9101345 PMid:33053800 PMCid:PMC7601530
2. Mu X, Shen G, Huang Y, Luo J, Zhu L, Qi S, *et al*. The enantioselective toxicity and oxidative stress of beta-cypermethrin on zebrafish. Environ Pollut. 2017; 229:312-320. https://doi.org/10.1016/j.envpol.2017.05.088 PMid:28601763
3. Kluver N, Konig M, Ortmann J, Massei R, Paschke A, Kühne R, *et al*. Fish embryo toxicity test: Identification of compounds with weak toxicity and analysis of behavioral effects to improve prediction of acute toxicity for neuro toxic compounds. Environ Sci Technol. 2015; 49(11):7002-7011. https://doi.org/10.1021/acs.est.5b01910 PMid:25939044
4. Ahmad F, Ali S, Richardson MK. Effect of pesticides and metals on Zebrafish embryo development and larval locomotor activity. BioRxiv. 2020. https://doi.org/10.1101/2020.10.05.326066
5. Anselmo Cd, Sardela VF, de Sousa VP, Pereira HMG. Zebrafish (Danio rerio): A valuable tool for predicting the metabolism of xenobiotics in humans? Comp Biochem Physiol C Toxicol Pharmacol. 2018; 212, 34-46. https://doi.org/10.1016/j.cbpc.2018.06.005 PMid:29969680
6. Bertoni Í, Sales BCP, Viriato C, Peixoto PVL, Pereira LC. Embryo toxicity induced by triclopyr in Zebrafish (*Danio rerio*) early life stage. Toxics. 2024; 12(4): 255. https://doi.org/10.3390/toxics12040255 PMid:38668478 PMCid:PMC11054795
7. Glaberman S, Padilla S, Barron MG. Evaluating the zebrafish embryo toxicity test for pesticide hazard screening. Environ Toxicol Chem. 2017; 36(5):1221-1226. https://doi.org/10.1002/etc.3641 PMid:27699829 PMCid:PMC7737843
8. OECD. Test No. 203: Fish, acute toxicity test, OECD guidelines for the testing of chemicals, Section 2, OECD Publishing; 2019. https://doi.org/10.1787/9789264069961-en
9. Nagel R. DarT: The embryo test with the zebrafish *Danio rerio* – A general model in ecotoxicology and toxicology. ALTEX. 2002; 19 Suppl 1:38-48.
10. Akbulut, C. Acute exposure to the neonicotinoid insecticide imidacloprid of zebrafish (*Danio rerio*) Gonads: A histopathological approach. Ann Limnol. 2021; 57:23. https://doi.org/10.1051/limn/2021021
11. Mundhe SA, Birajdar SV, Chavan SS, Pawar NR. Imidacloprid poisoning: An emerging cause of potentially fatal poisoning. Indian J Crit Care Med. 2017; 21(11):786-788. https://doi.org/10.4103/ijccm.IJCCM_152_17 PMid:29279642 PMCid:PMC5699009
12. Chung K-T, Chen L-W. Tseng H-W. Wu C.-H. Neonicotinoid imidacloprid affects the social behavior of adult zebrafish by damaging telencephalon neurons through oxidation stress, inflammation, and apoptosis. Life. 2023; 13:1418. https://doi.org/10.3390/life13061418 PMid:37374200 PMCid:PMC10304127
13. Pucci ND, Marchini GS, Mazzucchi E, Reis ST, Srougi M, Evazian D, *et al*. Effect of Phyllanthus niruri on metabolic parameters of patients with kidney stone: A perspective for disease prevention. Int Braz J Urol. 2018; 44(4):758-764. https://doi.org/10.1590/s1677-5538.ibju.2017.0521 PMid:29617079 PMCid:PMC6092661
14. Sowjanya K, Girish C, Bammigatti C, Lakshmi NCP. Efficacy of *Phyllanthus niruri* on improving liver functions in patients with alcoholic hepatitis: A double-blind randomized controlled trial. Indian J Pharmacol. 2021; 53(6):448-456. https://doi.org/10.4103/ijp.IJP_540_20 PMid:34975132 PMCid:PMC8764976
15. Varsha KP. Various health benefits and phyto chemical constituents of *Phyllantus niruri*. J Pharm Innov. 2022; SP-11(6):1886-1895.
16. Ali S, van Mil HG, Richardson MK. Large-scale assessment of the zebrafish embryo as a possible predictive model in toxicity testing. PLoS One. 2011; 6(6):e21076. https://doi.org/10.1371/journal.pone.0021076 PMid:21738604 PMCid:PMC3125172
17. Williams SY, Renquist BJ. High throughput *Danio rerio* energy expenditure assay. J Vis Exp. 2016; (107):e53297. https://doi.org/10.3791/53297
18. Khandia R, Pathe CS, Vishwakarma P, Dhama K, Munjal A. Evaluation of the ameliorative effects of *Phyllanthus niruri*

on the deleterious insecticide imidacloprid in the vital organs of chicken embryos. J Ayurveda Integr Med. 2020; 11(4):495-501. https://doi.org/10.1016/j.jaim.2019.03.003 PMid:31757597 PMCid:PMC7772494

19. Curcio V, Macirella R, Sesti S, Ahmed AIM, Talarico F, Tagarelli A, *et al*. Morphological and functional alterations induced by two ecologically relevant concentrations of lead on *Danio rerio* gills. Int J Mol Sci. 2022; 23(16):9165. https://doi.org/10.3390/ijms23169165 PMid:36012426 PMCid:PMC9409012
20. Yadav V, Ahmad S, ZahraK. Imidacloprid toxicity and its attenuation by aqueous extract of *Moringa oleifera* leaf in zebra fish, *danio rerio*. Int J Curr Pharm Res. 2020; 12(2):32-38. https://doi.org/10.22159/ijcpr.2020v12i2.37483
21. Shukla S, Jhamtani RC, Dahiya MS, Agarwal R. Oxidative injury caused by individual and combined exposure of neonicotinoid, organophosphate and herbicide in zebrafish. Toxicol Rep. 2017; 4:240-244. https://doi.org/10.1016/j.toxrep.2017.05.002 PMid:28959645 PMCid:PMC5615116
22. Islam MA, Hossen MS, Sumon KA, Rahman MM. Acute toxicity of IMCP on the developmental stages of common carp Cyprinus carpio. J Toxicol Environ Health Sci. 2029; 11: 244-251. https://doi.org/10.1007/s13530-019-0410-8
23. Reddy AP, Veeraiah K, Rao TS, Vivek Ch. The effect of confidor on histology of the gill, liver and kidney of fish *Labeo rohita* (Hamilton). Int J Bioassays. 2015; 4(02):3682-3685.
24. Santiago MR, Salvo LM, Badaro-Pedroso C, Costa EMF. Single and mixed exposure to distinct groups of pesticides suggests endocrine disrupting properties of imidacloprid in zebrafish embryos. J Environ Sci Health B. 2023; 58(3):217-228. https://doi.org/10.1080/03601234.2023.2184158 PMid:36861322
25. Bhardwaj JK, Kamboj H, Tyor AK. The toxicity of IMCP on early embryonic stages and growth rate of hatchlings of common carp, *Cyprinus carpio*. Toxicology International. 22: 29(1): 105-115. https://doi.org/10.18311/ti/2022/v29i1/28317
26. Kai H, Mita A, Yamaguchi M. Effects of neonicotinoid pesticide metabolic compounds on medaka (*Oryzias latipes*) embryo development. Biology (Basel). 2023; 12(12):1460. https://doi.org/10.3390/biology12121460 PMid:38132286 PMCid:PMC10740882
27. Erhunmwunse NO, Tongo I, Omigie K. Embryonic toxicity of Imidacloprid: Impact on hatchability, survivability, swimming speed and cardiac function of catfish, *Clarias gariepinus*. Ecotoxicol. 2023; 32(1):127-134. https://doi.org/10.1007/s10646-023-02625-y PMid:36652124
28. Ambali SF, Makinde AO, Shittu M, Adeniyi SA, Mowuogwu FO. Alleviating effect of *Phyllanthus niruri* on sensorimotor and cognitive changes induced by *Subacute chlorpyrifos* exposure in Wistar rats. Am J Med Sci. 2012; 2(3):50-58. https://doi.org/10.5923/j.ajmms.20120203.05
29. Brogan WR 3rd, Relyea RA. Multiple mitigation mechanisms: Effects of submerged plants on the toxicity of nine insecticides to aquatic animals. Environ Pollut. 2017; 220(Pt A):688-695. https://doi.org/10.1016/j.envpol.2016.10.030 PMid:27823867