



Embryonic toxicity of Imidacloprid: Impact on hatchability, survivability, swimming speed and cardiac function of catfish, *Clarias gariepinus*

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Abstract

Imidacloprid is a systemic insecticide that belongs to the neonicotinoid class of chemicals that act on the central nervous system of insects. Imidacloprid is used to control sucking insects, chewing insects such as termites, soil insects, and fleas on pets, as well as to treat structures, crops, soil, and seeds. As a result of these factors, this pesticide may end up in the aquatic environment via municipal discharges and runoff. Although the presence of imidacloprid in aquatic environments has been underreported as widespread, the toxic effects of this pesticide may have serious implications on aquatic organisms, particularly at environmentally relevant concentrations and demand more attention. Given this knowledge, the goal of this study was to investigate the effects of imidacloprid on *Clarias gariepinus* embryonic development. *Clarias gariepinus* embryos (3 h post-fertilization) were exposed to environmentally relevant concentrations of imidacloprid (10, 30, 100, and 500 µg/L) until 48 h post-fertilization using a modified fish embryo acute toxicity test (OECD TG 236). A stereomicroscope was used to assess hatchability, deformity, heart rate, and swimming speed as endpoints. According to our results of the developmental acute toxicity test, imidacloprid significantly reduced the hatching rate and heartbeats of *C. gariepinus* embryos. It also influenced the swimming kinematics of exposed embryos and caused teratogenic effects such as yolk sac rupture, pericardial oedema, lordosis, an abnormally shaped head, and altered epiboly. Our results allow us to conclude that imidacloprid is a toxic pesticide in the early life stages of *C. gariepinus* due to its high teratogenic potential.

Keywords Fish embryos · Hatchability · *Imidacloprid insecticide* · *Swimming speed*.

Introduction

The application and usage of pesticides tend to end up in lakes, rivers, oceans, and other aquatic environments (Kumar et al. 2009; Inyang et al. 2016; Seiyaboh and Izah 2017; Ojesanmi et al. 2017) and may pose a widespread threat to aquatic life (Ogamba et al. 2015). Neonicotinoids

have become a popular class of insecticides in recent years due to their favourable safety profiles and the constraints placed on other insecticides, and their use for resistance management (Simon-Delso et al. 2015). Their effectiveness against a variety of insects, as well as their diversity in application, high potency at low dosages, low volatility, and high water solubility, make them one of the most important pesticide classes (Vieira et al. 2018).

Imidacloprid ((*E*)-1- (6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin- 2-ylideneamine) is a synthetic insecticide of the neonicotinoid class of pesticides. It is a neuroactive insecticide that disrupts the nicotinic acetylcholine receptors (nAChRs) in the central nervous system of insects by completely and irreversibly blocking the post-synaptic nAChRs consequently resulting in the impairment of normal nerve impulses (Lewis et al. 2016). Imidacloprid has a lower affinity to the receptors in vertebrates than in insects and is very effective in small concentrations hence is considered a safer option (Overmyer et al. 2005). The insecticide market is dominated by imidacloprid

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products which are registered for use in over 120 countries and on over 140 different crops (Jeschke and Nauen 2008). As a result of its high solubility in water, it affects non-target aquatic organisms (Sumon et al. 2018) owing to spray drift and runoff (Shan et al. 2020).

Spray drift, leaching, or run-off in agricultural settings can expose non-target aquatic organisms to imidacloprid and photolysis may exacerbate imidacloprid dissipation when it enters aquatic environments (CCME 2007, Siregar et al. 2021). Imidacloprid has been found in surface waters near agricultural areas as high as 200 µg/L in the Netherlands (van Dijk 2010), and 15 µg/L in Sweden (Kreuger et al. 2010) and 3.29 µg/L in the United States (Keith and Kean 2012).

Several studies have revealed that imidacloprid alters the physiological processes in fish, notably in the embryo, such as decreased viability and hatching success (Tyor and Harkrishan, 2016). Imidacloprid has also been linked to oxidative stress, DNA damage (Vieira et al., 2018), lower hatching rate of fertilized eggs (Islam et al., 2019), and histological abnormalities in tissues of *Labeo rohita* (Qadir and Iqbal 2016).

One way to learn about the harmful effects of pollutants in aquatic environments is to conduct a toxicity test with a fish model (Van der Oost et al. (2003)). Fish early developmental studies have been favoured over short-term adult toxicity assessments due to their lower cost (Villeneuve et al. 2014). Despite being an important study candidate, there have been few ecotoxicological documentations on the impacts of imidacloprid on the African catfish embryos. *C. gariepinus* (African catfish) is common in tropical freshwater habitats and popular among the locals for food (Ogueji et al. 2018). Therefore this study was conducted to determine the potential acute effects of imidacloprid on the developmental, hatchability, cardiovascular activity, and swimming capability of *Clarias gariepinus* embryos/larvae.

Materials and Methods

Fish handling and maintenance

Adult catfish of both sexes (male and female) were obtained from the breeding facilities at the Faculty of Agriculture (University of Benin; Nigeria) and transported to the Laboratory for Ecotoxicology and Environmental Forensics, University of Benin. Fish maintenance, breeding conditions and egg production was conducted based on the procedure of Erhunmwunse et al. 2021 and following the OECD TG 236 procedure (OECD, 2013). Twenty freshly laid eggs (< 2 hpf) were transferred to each well in 50-well Petri dishes filled with

the respective test solutions of 5mls and deionized water as the control in a static renewal assay changed every 24 h (Nagel 2002). The experimental water quality parameters were obtained in the manner described by American Public Health Association APHA (2005), with average values ranging from 25.4 to 27.0 °C (temperature), 5.7 to 6.4 (pH), 6.10 to 6.51 mg/L (dissolved oxygen), 11 to 19 S/cm (electrical conductivity), 10 mg/L (total hardness), 9 to 59 mg/L (total suspended solid), and 0–0.06 mg/L. (salinity).

Chemical

Imidacloprid (CAS Number 138261-41-3) was obtained from a local shop in Benin City. Imidacloprid stock solutions were prepared once at 100 mM in deionized water and used in the acute toxicity testing, hatching, heartbeat and swimming assays.

Procedure

The acute toxicity test in catfish embryo was conducted in five concentrations (0.0, 10.0, 30.0, 100.0, and 500.0 µg/L) and three replicates of imidacloprid. These concentrations were chosen based on data from previous environmental concentrations reported by van Dijk 2010, Kreuger et al. 2010) and Keith and Kean 2012. Hatching results were monitored at 24, 36 and 48 h exposure while heartbeat, swimming, and teratogenic effects were all observed and recorded at 48 h. Eggs were presumed dead when no heartbeat was observed (while the larvae were considered dead when they showed no form of movement even when prodded with a glass rod (Nguyen and Janssen 2001). The rupture of the egg's membrane was considered as hatching and percentage hatchability was calculated based on how many eggs hatched between 24 hpf and 48 hpf. The changes in appearance and development of the larvae were observed using an inverted microscope and an image analyzer (Amscope software) at 24 and 48 h. Embryos from each group were randomly selected for swimming movement and heartbeat measurement. Embryos with obvious defects were excluded for swimming speed and heartbeat measurement only. The rate of the heartbeat and the swimming speed of the embryo were measured using the methods employed by Erhunmwunse et al. 2021. At 24 and 48 hpf, the swimming movement patterns and speed of the larvae were determined by first making videos with a digital camera (AMSCOPE microscope). The video tracks (Fig. 1) were obtained by processing the video files with the Kinovea (0.8.15) program. The ratio of the distance moved to the swimming time was used to calculate the fish's swimming speed in one minute (60 s).

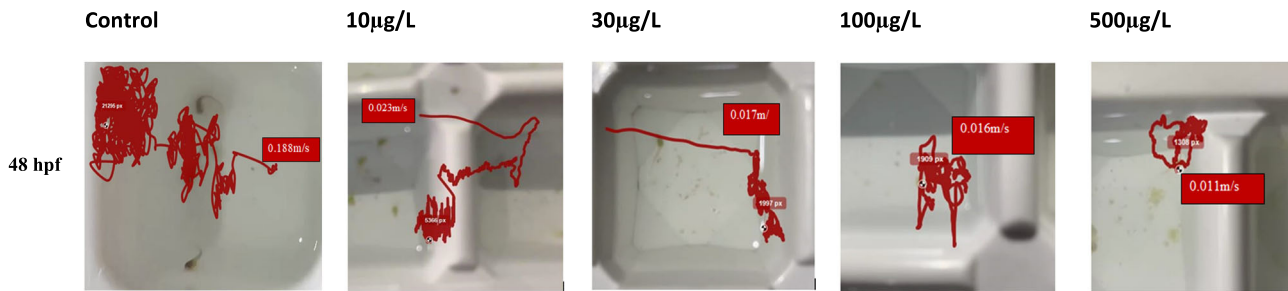


Fig. 1 Catfish larvae video tracks showing the swimming speed of larvae in different concentrations of imidacloprid at 48 hpf for 60 s. The red and black rectangle indicates the endpoints of each video track while the red lines show the movement tracks ($n = 20$)

Statistical Analysis

Graphic and statistical analyses were done with GraphPad Prism 8 software. Heart rates and swimming performance data were expressed as mean \pm SD. Kaplan–Meier survival curve was used to determine the % survivability of catfish embryo exposed to imidacloprid. One-way analysis of variance (ANOVA) was employed to determine significant differences by testing the variable at $p < 0.05$ level of significance.

Results

Morphological and developmental changes in fish embryo

Several embryo developmental changes were observed in the catfish embryos/larvae exposed to imidacloprid (Fig. 2) and the developmental defects in the embryos/larvae were observed to be concentration and time-dependent (Fig. 2). Larvae exposed to imidacloprid showed more severe indications of poisoning; particularly at 500 $\mu\text{g/L}$. Teratogenic effects were seen as early as 24 h after fertilization. The larvae abnormalities were primarily yolk retention ($> 95\%$), aberrant spinal curvature (50%), a poorly developed eyeball (10%), and poorly formed barbels (15%) at imidacloprid concentrations of 10 $\mu\text{g/L}$ (Fig. 2B3–2B4). The tail was twisted (20%), there was yolk sac oedema (35%), and there was no formed eyeball (15%) at imidacloprid concentrations of 30 $\mu\text{g/L}$ (Fig. 2C5–2C6). The head of the larvae exposed to imidacloprid concentrations of 100 $\mu\text{g/L}$ (Fig. 2D7–2D8) had an abnormal increase (30%), and the yolk sac was distorted ($> 60\%$), the eyeball was underdeveloped ($> 15\%$), there was ruptured yolk retention with the presence of pericardial oedema ($> 40\%$). For embryos exposed to imidacloprid concentrations of 500 $\mu\text{g/L}$, the yolk sac was maintained, no barbels formed (40%), there was lordosis (60%), and the body and eyeball were poorly developed (35%) (Fig. 2E9–2E10).

As the concentration and duration increased, new abnormalities were detected, which were added to the accumulation of most of the deformities already reported at lower concentrations and time.

Hatchability of fish embryo

In the control group, catfish embryos began hatching at 24 hpf and finished at 36 hpf, while other concentrations showed varying hatching times (24–48hpf). In general, the hatching rates of the control embryos were higher than those of the imidacloprid-exposed embryos (Fig. 3). The percent hatchability from different concentrations of 10, 30, 100, and 500 $\mu\text{g/L}$ were 95, 65, 50, and 35%, respectively (Fig. 3), with the hatchability ($p < 0.01$) of 30 $\mu\text{g/L}$ and ($p < 0.001$) 100, and 500 $\mu\text{g/L}$ being significantly different from the control.

Swimming speed/distance covered by exposed embryo

At 48 hpf, behavioural endpoints (swimming speed and distance covered) were examined to detect changes in behaviour and possibly neurological impairments in the catfish embryo/larvae caused by exposure to the neonicotinoid insecticide. The swimming speed of the larvae in the control group was substantially faster than that of the exposed groups. The swimming speed (0.011 m/s) of the embryos exposed to 500 $\mu\text{g/L}$ imidacloprid was substantially lower ($p < 0.05$) than the control group (0.188 m/s) at 48 hpf. The distance covered by the embryos showed a decreasing order of all concentrations from the control in this order control $> 10 > 30 > 100 > 500$ $\mu\text{g/L}$.

Cardiovascular activities of fish embryo

The rate of the heartbeat was assessed at 48 hpf. The heartbeat rate showed a concentration-dependent decrease peaking at the highest concentration (500 $\mu\text{g/L}$). This increase was significantly different from the control ($p < 0.001$) (Fig. 4).

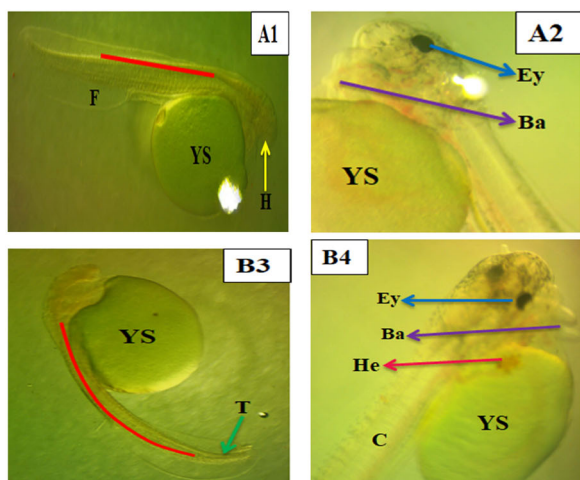


Fig. 2 Developmental abnormalities in *C. gariepinus* embryo/larvae exposed to imidacloprid at 24 and 48 hpf. Ey Eye (Blue arrow), Ba Barbel (Purple arrow), He Hemorrhage (Pink arrow), H Head (Yellow arrow), Td Tail Distortion (Green arrow), Eb Epiboly (White arrow), Bs Bent Spine (Redline), Hr Heart (Orange arrow), C Cord, S Somite, YS Yolk Sac, YSE Yolk Sac Edema, YSD Yolk Sac Distortion, YSR Yolk Sac Rupture, Pe Pericardial Edema (Peach arrow), I Iordosis (Curving body trunk). Scale bar = 200 pixels) (1 = 24 h, 2 = 48 h, A (Control), B (10 µg/L), C (30 µg/L), D (100 µg/L), E (500 µg/L)

Survivability

Death in the larvae was assumed when movement ceased and embryo/larvae lay suspended, and confirmation was

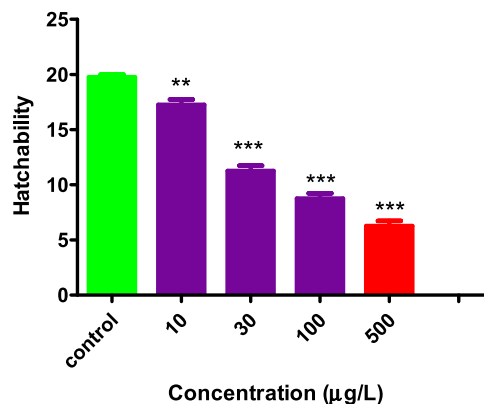


Fig. 3 Imidacloprid impact on the hatchability of exposed *C. gariepinus* embryos at 48 hpf

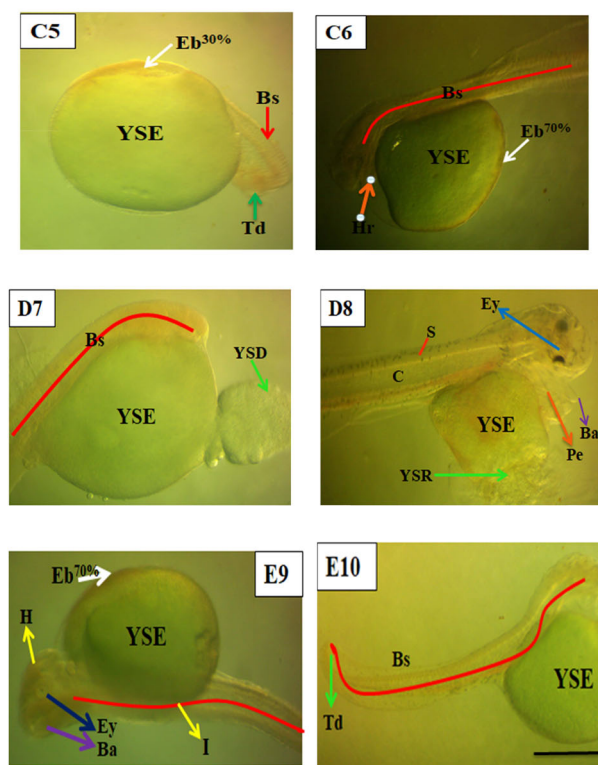


Fig. 4 Rate of the heartbeat of *C. gariepinus* embryos at 48 hpf. Bar graph indicate mean ($n = 20$). *** p -values of significant changes over controls ($p < 0.001$)

established when no breathing was noted at 24 and 48 hpf. Mortality was not recorded in the control group throughout the experimental time, however, there was mean mortality of 2.50 ± 0.28 in 10 µg/L, 4.75 ± 0.47 in 30 µg/L, 5.00 ± 0.41 in 100 µg/L and 7.50 ± 0.28 in 500 µg/L of imidacloprid at 24 h. In 48 h, mortality at 10 µg/L was 4.70 ± 0.25 , 12.00 ± 0.91 in 30 µg/L, in 100 µg/L the mean mortality was 14.50 ± 0.95 and 16.75 ± 0.48 in 500 µg/L imidacloprid. In the first 24 h, the survival rate decreased with increasing imidacloprid concentrations, but after 48 h, the survival rate dropped dramatically in the larvae exposed to 30–500 µg/L of imidacloprid Fig. 5.

Discussion

Pesticides in the aquatic environment have recently become a source of widespread concern due to their continuous discharge and possible harm (Al-Ahmadi 2019). This study looked at the effects of imidacloprid on the hatchability, developmental, cardiovascular, and swimming performance of African catfish embryos/larvae (*Clarias gariepinus*).

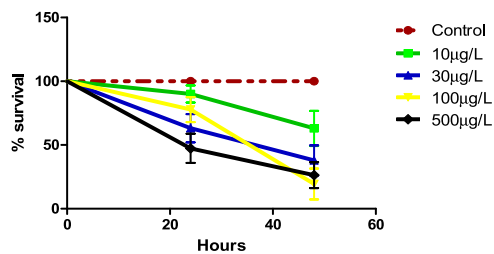


Fig. 5 Kaplan–Meier survival curve analysis of catfish embryo exposed to imidacloprid ($n = 20$ per group) (log-rank test, $p < 0.001$). (Chi-squared test, mean \pm SE * $p < 0.05$, ** $p < 0.01$)

Morphological and developmental changes in fish embryo

The developmental effect seen in this study revealed that imidacloprid can cause significant embryonic teratogenesis in *Clarias gariepinus*. The effects of highest dominance from exposure to imidacloprid in the developing embryos were yolk retention, spinal malformation, poorly developed eyeball, poorly formed barbels, and pericardial oedema, and these effects became more obvious as the imidacloprid concentration increased, implying that the pesticide was teratogenic to the fish. Similar results were reported in zebrafish embryos exposed to imidacloprid ($\geq 20 \mu\text{g/L}$), with similar developmental defects such as lordosis, haemorrhage, yolk oedema, and tail malformations being observed in a concentration-dependent manner as evident in our study in concentrations $\geq 30 \mu\text{g/L}$ (Vignet et al. 2019). Malformations such as yolk-sac oedema, deformed body structure, malformed head shape, lordosis, and pericardial oedema have also been reported in common carp embryos/larvae exposed to imidacloprid (Islam et al. 2019).

Similar deformities have been observed in zebrafish embryos and larvae exposed to acetamiprid (Ma et al. 2019). Such defects include a bent spine, an uninflated swim bladder, pericardial edema, and yolk sac edema, with the bent spine being the most common. In our study, the observed spinal teratogenesis could have been caused by a build-up of imidacloprid, which can diminish neuromuscular coordination in the African catfish larvae, resulting in lower collagen levels in the spinal column (Ekrem et al. 2012). However, more research is needed to fully comprehend the processes of notochordal abnormalities in African catfish larvae produced by imidacloprid poisoning.

Hatchability of fish embryo

Fish demonstrate considerable susceptibility to pollutants and toxins in their early life stages and reproductive ability (Zhang et al. 2008). The lower rate of hatching can be linked to structural and functional problems that occurred during the embryo's development, which is an exceptionally essential

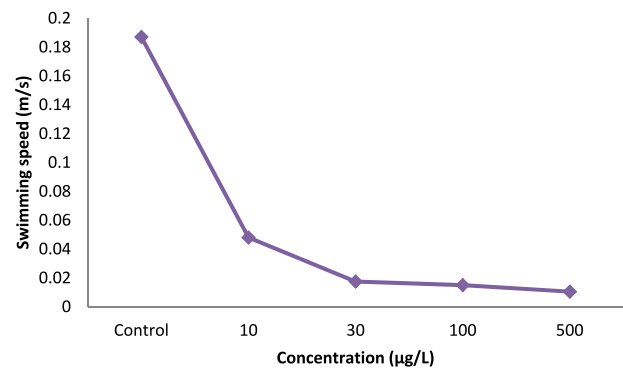


Fig. 6 Mean swimming speed of imidacloprid exposed embryo *C. gariepinus* larvae at 48 hpf. Line graph indicate mean \pm SE ($n = 20$)

time of embryogenesis in fish (Liu et al. 2016, Li et al. 2018). Our findings show that imidacloprid is extremely harmful to catfish embryos and impacts the hatching rate. With increasing imidacloprid concentrations, the hatching rate decreased considerably. Similarly, imidacloprid was found to lower the hatching rate of common carp (*Cyprinus carpio*) embryos in a concentration-dependent manner (Tyor and Harkrishan 2016; Islam et al. 2019). Other pesticides like Diazinon (Aydin and Koprucu 2005), cypermethrin (Aydin et al. 2005; Richterva et al. 2015), deltamethrin (Koprucu and Aydin 2004), and cyhalothrin (Koprucu and Aydin 2004) have all been found to have similar effects on common carp embryos (Richterva et al. 2014).

Swimming speed/distance covered of exposed embryo

Organism behaviour is a reaction that reflects the effects of environmental stimuli on organisms at all trophic levels and can represent physiological processes as a consequence of those stimuli (Saaristo et al. 2018). As a result, behavioural toxicity indicators are ideal for evaluating the effects of aquatic pollutants on distinct fish populations (Scott and Sloman 2004). In measuring behavioural responses of catfish exposed to toxicants, the speed at which fish swim is an extremely important and simple method. (Xia et al. 2018). There is currently no information on the behaviour of catfish larvae exposed to imidacloprid. The speed and total swimming distance in a given period were used to evaluate swimming behaviour in this study. With increasing concentrations of imidacloprid in the exposed larvae, speed and total swimming distance decreased at 48 hpf (Figs. 1 and 6).

The video tracking data (Fig. 1) demonstrated that larvae exposed to the highest concentration (500 µg/L) moved 17 times (0.011 m/s) slower in 60 s at 48 hpf. These findings are comparable to those of Siregar et al. (2021), who found that after 72 h of exposure to imidacloprid, the freshwater shrimp *Neocaridina denticulata*' swimming speed and distance were reduced. Similarly, acetamiprid inhibited

sudden movement patterns and caused body shortening and other malformations in exposed zebrafish embryos, thereby reducing swimming ability (Ma et al. 2019). Spine malformations and alteration in the swimming bladder in embryos exposed to neurotoxicants have been previously noted to impair swimming function in larval fish (Yan et al. 2016; Ma et al. 2019).

The disruptive impact of imidacloprid on the activity of some enzymes and the alteration in the swimming bladder may be to blame for the decrease in swimming speed and distance covered by the embryo/larvae in our experiment as some larvae had difficulty with buoyancy (Ma et al. 2019). The reduced distance travelled by catfish larvae is a behavioural parameter that indicates the toxic effects of imidacloprid. Our research found that the distance travelled by the larvae decreased as the concentration of imidacloprid increased, as evidenced by the shorter tracks (red lines) of catfish embryos exposed to the highest concentration compared to the control. Hussain et al. (2020) discovered a reduction in the distance travelled by zebrafish larvae exposed to Fipronil. Wu et al. 2015 discovered a similar pattern in *Danio rerio* exposed to Fipronil. Jin et al. (2015) discovered that increasing chlorpyrifos concentrations affected the swimming speed of zebrafish embryos after 96 h. The distance travelled and swimming speed of mosquitofish exposed to chlorpyrifos for 96 h was significantly reduced compared to controls, according to Kavitha and Rao (2008).

The ecological significance of this type of behaviour is that such animals are at the mercy of predators in times of danger (Bownik and Szabelak 2021).

Cardiovascular activities of fish embryo

Heart rate is an essential parameter in many small fish model applications and a suitable metric for environmental studies evaluating adverse developmental and cardiotoxic impacts of pollutants (Gierten et al. 2020). The development of the heart is a very sensitive process and during embryonic development, exposure to environmentally toxic molecules can affect it (Sarmah and Marrs 2013). From our results, at 48 hpf, the heart rates of the catfish larvae exposed to imidacloprid decreased as the concentration of imidacloprid increased. This is consistent with the findings of Ma et al. (2019), who found that exposing zebrafish embryos to acetamiprid, a neonicotinoid insecticide, caused a decrease in heartbeat rate as the concentration increased. Siregar et al. (2021) found that imidacloprid significantly reduced the heartbeat rates of *N. Denticulata*. The reduced heart rates may have been caused by the exposure to imidacloprid which stimulated the acetylcholine receptor continuously and acted as a nAChR agonist (Watson et al. 2014; Matsuda et al. 2001). Among the investigated indices

(endpoints) in the exposed larvae, oedema, spinal malformation, induced yolk deformation, lordosis (developmental abnormalities) proved to be more sensitive tools than heartbeats and hatching in diagnosing the exposure of catfish embryos to imidacloprid.

Conclusion

This study aimed to determine the impact of imidacloprid on the survival, hatching, heartbeat and swimming performance of Catfish embryo, *Clarias gariepinus*. Our data clearly show that exposure to environmental concentrations of imidacloprid induced changes in hatchability, survivability, cardiac function and swimming behaviour. The discovered abnormalities in swimming behaviour and cardiac function may jeopardize the embryo's physiology and development, contributing to the high mortality rate. Given the growing concern about pesticide use, it is critical to gain a better understanding of the toxicity as well as the mechanisms causing these effects.

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Author contributions CRediT authorship contribution statement ENO: Conceptualization, Methodology, Software, Data curation, Writing - original draft, Software, Validation, Writing - reviewing & editing, Supervision. IT: Conceptualization, Methodology, Software, Data curation, Writing - original draft, Supervision, Software, Validation, Writing - review & editing. OK: Conceptualization, Software.

Compliance with ethical standards

Conflict of interest These authors declare no competing interests.

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