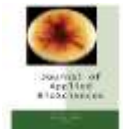

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# Effect of acute exposure to cotton insecticide PYRO FTE 472 EC (Chlorpyrifos 400 g/l and Cypermethrin 72 g/l) in African catfish *Clarias gariepinus* embryos

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## ABSTRACT
*Objective:* This study aims to determine the acute toxicity of binary insecticide Pyro FTE 472 EC (Chlorpyrifos 400 g/l; Cypermethrin 72 g/l) on the egg and embryo of African catfish *Clarias gariepinus*.
*Methodology and Results*: The fertilized eggs were exposed to six concentrations (T0: 0; T1: 20; T2: 70; T3: 120; T4: 170 and T5: 220 ppm) of Pyro in troughs placed in aquariums to determine the effects on survival, hatching, and development. The arithmetic method of Karber was used to calculate LC50 values. Results showed that eggs/embryos mortality significantly increased with increasing Pyro concentrations. The 24h-LC50 and 48h-LC50 values of Pyro for eggs/embryos were 102.26 and 90.40 ppm, respectively. Paradoxically, hatching success increased significantly from T1 to T3. Rates of tailless and short-tail embryos and immobility and agglutination of embryos increased with increasing Pyro concentrations.
*Conclusions and application of findings*: The present study revealed that Pyro FTE 472 EC, a binary pesticide used extensively in the fields against insect pests of cotton plants during the flooding period of reproduction of *C. gariepinus* could harm the development of this specie. A reduction in the quantity of this biocide in the cotton fields is essential in order not to reach the lethal doses for the eggs/embryos of *C. gariepinus*. This study, first report, indicates thus for biomonitoring the limit values of Pyro in aquatic biotopes to preserve the eggs/embryos of *C. gariepinus* in particular and of other fish in general.
**Keywords:** Pesticides, hatching, malformation, *Clarias gariepinus*, aquatic environment

## INTRODUCTION
In 2018, global fish production reached 179 million tonnes, with 156 million tonnes used for human consumption (FAO, 2020). This is equivalent to an estimated annual supply of 20.5 kg of fish per capita (FAO, 2020). Most of these fish and other aquatic organisms that humans consume live in rivers that are the final receptacles for pollutants of all kinds

(Agbohessi *et al*., 2015a, b and 2020). Among these pollutants, are agricultural pesticides of various kinds, used in the fields to control plant pests and increase crop production and productivity (Agbohessi *et al*., 2011 and 2012, Agbohessi, 2014; Agbohessi *et al.*, 2015b). These pests are becoming more and more numerous, especially with global warming which offers them new favourable conditions (GIEC, 2014; Agbodji *et al*., 2017). The proliferation of these pests and diseases leads to an increase in the doses of pesticides and other chemicals used in the fields with numerous consequences on the environment (Agbohessi *et al*., 2011). Global warming, synonymous with a temperature rise, also leads, especially in tropical countries where the heat is intense, to an acceleration of the biodegradation of these chemical pesticides into derivatives that are more toxic than the initial products (Agbohessi *et al*., 2012). In Benin (West Africa), among the agricultural pesticides used, there is Pyro FTE 472 EC, newly introduced in the technical cotton production route. It is a binary insecticide based on Chlorpyrifos (400 g/l) and Cypermethrin (72 g/l), used as acaricidal binary in the third window of phytosanitary treatment from the 77th day after the emergence of the young cotton plant to control the appearance of the second generation of *Helicoverpa armigera* (CRA-CF, 2019). Chlorpyrifos is a non-systemic acaricide of the Organophosphate family (Jarviren and Tanner, 1982). Its cholinesterase inhibitory effect is the most sensitive known in fish. It is believed to be very toxic to freshwater fish (Jarviren and Tanner, 1982). Cypermethrin is a synthetic non-systemic Pyrethroid (INERIS, 2016). It is also believed to be toxic to aquatic life. The main target of Cypermethrin is the neuromuscular system (RECA-Niger, 2013). Douny *et al*. (2021) recently determined in the water reservoirs of the cotton basin of northern Benin in the sediments the concentrations of 1.0 µg/kg of Chlorpyrifos and 0.8-1.8 µg/kg of Permethrin at Batran, and of 0.8-13.0 µg/kg of Permethrin at Sori. The same authors also reported the presence of Chlorpyrifos up to 1.9-3.3 µg/kg in Nile tilapia *Oreochromis niloticus* caught in Batran and the same insecticide in African catfish *Clarias gariepinus* in Gambanè with concentrations varying from 2.5 to 4.5 µg/kg. Studies have shown the harmful effects of Chlorpyrifos and Cypermethrin on fish, but to our knowledge, there is no published data on the impact of acute Pyro concentrations on the embryonic phase of this species. In fact, in the north of Benin, where nearly 70% of the national cotton production is concentrated, the period of intense use of pesticides in the fields coincides with the period of reproduction of several species of fish including *C. gariepinus* in the natural environment (Agbohessi *et al*., 2013; 2015a and 2020). It is therefore obvious that this delicate phase of fish life is exposed to high concentrations of these pollutants compared to the enormous quantities of pesticides used in the fields. The present experiment, therefore, aims to study, in laboratory conditions, the impact of acute Pyro concentrations on the embryonic phase of this species. The task is to determine the effect of acute concentrations of Pyro on the survival, hatching, and deformities of embryos.

## MATERIALS AND METHOD

**Pesticide collection:** The cotton insecticide Pyro FTE 472 EC commonly used by farmers in the cotton basin of Benin, was obtained from the "Société de Distribution des Intrants (Bénin)" for this study. Pyro FTE 472 EC is formed from Cypermethrin (72 g/l) and Chlorpyrifos (400 g/l). The chemical properties of these active ingredients are listed in table 1. Pyro is in liquid form. The test solutions are obtained by mixing Pyro directly with dechlorinated tap water, as is done in a farming environment. All working stock

solutions were made immediately prior to the tests. Water used in the preparation of test solutions was tested for quality (nitrate 19.13 ± 0.02 mg/l, nitrite 0.02 ± 0.01 mg/l, and total hardness 83.0 ± 0.2 mg/l).

**Table 1**- Properties of the active ingredients of Pyro FTE 472 EC

| Trade name | Formulation | Active ingredient and concentration | Water solubility (mg/l) | Log Kow | Vapour pressure (Pa) | DT50 in water (days) | References |
|---|---|---|---|---|---|---|---|
| **Pyro FTE** | 472 EC (Emulsifiable Concentrate) | Chlorpyrifos (400 g/l) (Organophosphate) | 2.0 at 25 °C | 3.31-5.27 | $1.87 \times 10^{-5}$ at 25 ˚C | 41 | Jarviren and Tanner (1982); Kidd and James (1991); Amara (2012) |
| | | Cypermethrin (72 g/l) (Pyrethroid) | 0.2 at pH=7 | 5.5 | $5.6.10^{-9}$ at 20˚C and pH=7 | 136 | INERIS (2016); EPA (2006) |

**Fish collection:** Adult of *C. gariepinus* were collected from at a local farm (Royal Fish Benin) in Porto-Novo (6° 29' 49.999" N 2° 36' 18" E), Benin. These broodstock were carefully transported in 50 l plastic bins at the Research Laboratory in Aquaculture and Aquatic Ecotoxicology (LaRAEAq), University of Parakou (9° 20' 60" N 2° 37' 0.001" E), Benin, where they were individually acclimated for 12 days in plastic tanks (1000 l), according to Organisation for Economic Co-operation and Development (OECD) guideline 203 (OECD, 1992a). They were fed twice a day with 2 % of their biomass with TOP FEEDS (6-mm pellets, 40 % crude protein; Grand fish feed, Egypt).

**Collection of gametes and artificial fertilization:** One male (422.2 g) and one female (413.5 g) both healthy and matures were choose for spawning by examining the gonads based on the external morphological features. The male that was retained is very mature and the female has a soft and developed abdomen, a red and protuberant genital papilla with emission of a few oocytes by abdominal pressure. Both male and female breedings were artificially induced by intramuscular injection of Ovaprim® brand synthetic hormone. The Ovaprim® was administered at a dose of 0.5 ml/kg body weight of fish for the female and 0.25 ml/kg body weight of fish for the male. Hormone- injected fish were then kept in a moderately aerated glass aquarium (45 x 35 x 30 cm) containing dechlorinated tap water (50 l). About 24 h after hormone administration, eggs were stripped into plastic tray and testes were collected from male and cut into small pieces by using a scalpel for milt collection. Milt and eggs were stirred thoroughly into a plastic tray by using a clean and soft poultry feather for fertilization. After 2 min of gentle stirring, the eggs were washed with tap water to remove excess milt.

**Experimental design and handling:** The test design incorporated 18 aquaria (five tested concentrations and a zero-concentration used as control in triplicate). Each aquarium (5 l) was equipped with an air diffuser, which ensured full oxygenation of the water. Approximately 200 mg fertilized eggs (≈100 eggs) were incubated in a trough placed in each aquarium filled with 4 l test solution. The eggs were completely submerged and spread out so they did not touch each other. Exposure to Pyro was made under static conditions to avoid disturbing them during incubation in accordance with OECD guidelines 203 and 210 (OECD, 1992a, b) with some minor adaptations. During the test, the photoperiod was maintained at 12 h light to dark. The acute

toxicity procedure was preceded by 48 h range-finding tests to determine the concentrations at which the pesticides were lethal to eggs (data not shown). This preliminary test, which included the period from egg fertilization to egg hatching, was performed at nominal concentrations of 0; 30.0; 60.0; 90.0; 120.0 and 150.0 ppm of Pyro. The nominal concentrations in the final test were: 0.0; 20.0; 70.0; 120.0; 170.0 and 220.0 ppm named respectively T0, T1, T2, T3, T4 and T5. Control and treatments were run simultaneously. During exposure, water-quality parameters were measured daily in all aquaria using standard methods (temperature 27.0 ± 0.0 °C, pH 7.1 ± 0.2, dissolved oxygen 5.6 ± 0.1 mg/l). First, 30 minutes after the beginning of the incubation of the fertilized eggs, the unfertilized eggs found in each trough and which are recognizable by their whitish colour, were removed. Next, the number of fertilized eggs in each trough was counted. At 4 h intervals, the proportions of hatched eggs, dead eggs/embryos, and eggs/embryos with abnormalities (e.g. dead embryo within the egg, immobile free embryos, tailless or short-tailed vesiculated embryos, agglutination of free vesiculated embryos) were recorded. From the 12$^{th}$ hour, the aquaria were observed every hour to record the time of the first hatching by a trough. The hatching rate was calculated as the percentage of fertilized eggs from which the embryo hatched. Unhatched eggs that had not decayed were observed under a microscope to determine the percentage of dead embryos in the eggs. At hatching, embryos with tail deformities were classified as tailless or short-tailed embryos. Nominal concentrations were not confirmed by chemical analyses. Precise measurement of the actual concentrations was considered to be of minor importance in these increasing concentrations. In addition, the half-lives of Chlorpyrifos (41 days, (Javiren and Tanner, 1982)) and Cypermethrin (136.0 days, (INERIS, 2016)) in neutral and acidic environments were greater than the duration of our experiments (48 h). Furthermore, the active components were not very volatile (vapour pressures: Chlorpyrifos 3.35 mPA at 25 °C (Agritox, 2015); Cypermethrin 5.6 x $10^{-9}$ PA à 20 °C (INERIS, 2016)). A significant quantity of these compounds should therefore not be lost by volatilization during exposure.

**Data analysis:** The experimental unit is the incubation trough. The 12, 24, 36 and 48 h-LC50 for the eggs/embryos were determined by the arithmetic method of Karber (Dede and Kaglo, 2001) according to the formula :

**LC50 = LC100 - (Σ (mean mortality of two successive concentrations x differences between the two successive concentrations) / number of embryos per treatment)**.

Abbott's formula **(%Corrected = (1 - ((Number of survivors for treatment) / (Number of survivors for control)) × 100)** was used to correct the mortalities (Abbott, 1925).

The other results are expressed as the mean ± standard deviation. The incidence rates of hatching rates, dead eggs/embryos, dead embryos in the egg, nonmotile embryos, tailless or short-tailed vesiculated embryos, agglutination of free embryos, were analysed by one-way analysis of variance analysis (ANOVA I) . Means were compared with control values by Dunnett's test with $p < 0.05$ being considered statistically significant.

## RESULTS

**Egg/Embryo Mortality:** Figure 1 shows the effect of the acute concentrations of Pyro on the survival of eggs/embryos. After the correction by Abbott's formula to extract the naturally dead eggs/embryos, it is observed that the mortalities of the exposed

eggs/embryos increase with the increase of the pollutant in the environment, whatever the period. From the first hours of incubation, all the embryos (100%) died in the highest T5 concentration, then after 36 h in T4. Figure 2 presents the evolution of the median lethal concentrations every 12 h during the test. 12 h-LC50 > 24 h-LC50 > 36 h-LC50, 36 h-LC50 = 48 h-LC50.

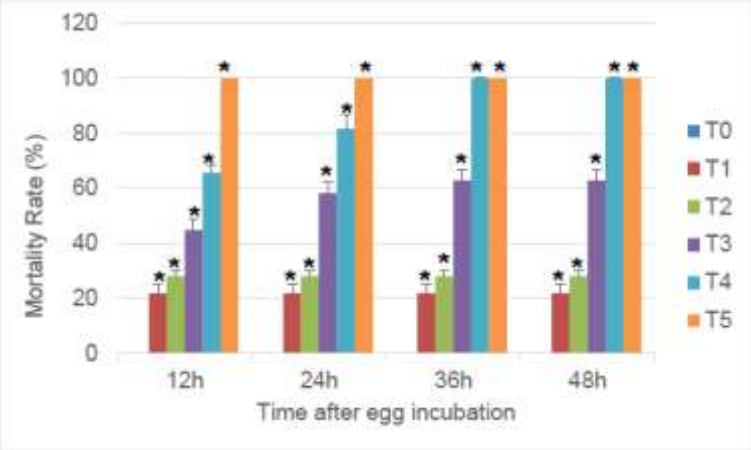


**Figure 1:** Effect of increasing the acute concentrations of Pyro FTE 472 EC on the survival of eggs/embryos of *C. gariepinus*. Values are mean ± SD (n = 3). Asterisk significantly different from the control treatments ($p<0.05$, Dunnett's test).

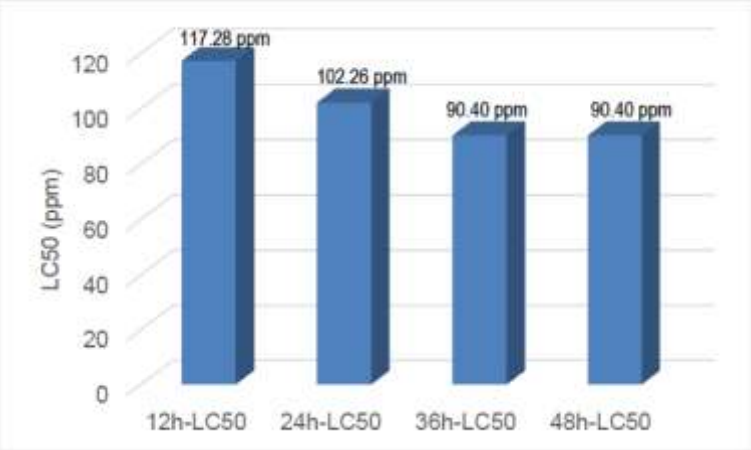


**Figure 2**: Evolution of the LC50, the median lethal concentration of Pyro FTE 475 EC during the 48h exposure of *C. gariepinus*

**Hatching rate:** The effect of increasing concentrations of Pyro on hatching rates is shown in figure 3. The rate in the control group was 45.4 ± 2.8 % and was significantly lower than in T1, T2 and T3 treatments of Pyro ($p<0.05$). The hatching rate increased with increasing concentrations of Pyro. Moreover, all embryos died without hatching at the highest concentrations tested; i.e., T4 and T5. Note that the first hatchings were observed in the treatments (T1, T2, and T3) 21 h after the beginning of the incubation. At the control, the first hatching was only observed after 24 h.

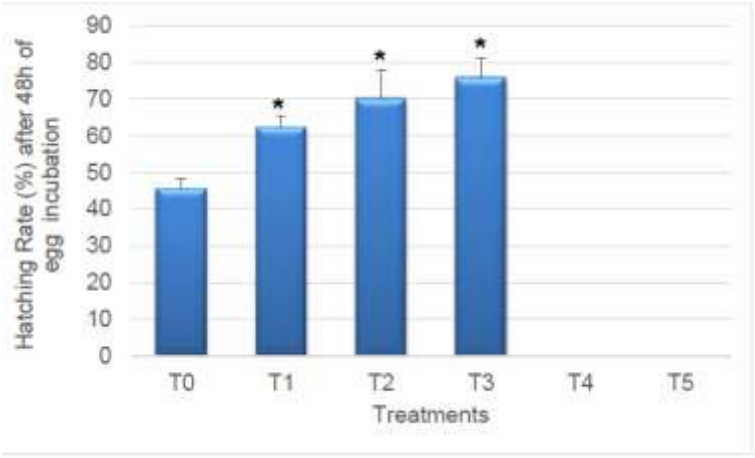


**Figure 3:** Effect of increasing the acute concentrations of Pyro FTE 472 EC on the hatching rate of eggs of *C. gariepinus*. Values are mean ± SD (n = 3). Asterisk significantly different from the control treatments ($p<0.05$, Dunnett's test).

**Deformity and behavioural abnormalities rates** Apart from short-tailed vesiculated embryos observed at a very low rate in the non-exposed eggs, it was noted in the eggs exposed to Pyro after hatching the rate of the tailless and short-tailed vesiculated embryos, which increases with the increase of the toxicant in the environment (Table 2). Dead embryos in the egg are also observed only in exposed eggs and increase with increasing concentrations of the pollutant in the environment, with levels of 100% in T4 and T5. The nonmotility and the agglutination of free embryos are the behavioural abnormalities observed only in exposed eggs, with an increase in rates when the pesticide increases in the environment.

**Table 2**: Rates of morphological and behavioural abnormalities caused by Pyro FTE 472EC in *C. gariepinus*

| | **T0** | **T1** | **T2** | **T3** | **T4** | **T5** |
|---|---|---|---|---|---|---|
| Deformity (%) | | | | | | |
| Dead embryos in the egg | 0 a | 09.45 ± 2.22 b | 16.33 ± 3.21 b | 23.11±1.64 b | 100 b | 100 b |
| Short-tailed vesiculated embryos | 03.08 ± 0.01 a | 12.22 ± 5.10 b | 13.74 ± 3.11 b | 15.88 ± 2.90 b | 0 a | 0 a |
| tailless vesiculated embryos | 0 a | 06.93±3.12 b | 09.33±2.63 b | 14.86±5.63 b | 0 a | 0 a |
| Behavioural abnormalities (%) | | | | | | |
| Immobility | 0 a | 0 a | 5.2 ± 4.42 b | 07.55 ± 1.74 b | 0 a | 0 a |
| Agglutination of free embryos | 0 a | 10.33 ± 2.54 b | 07.64±3.21 b | 12.75 ± 4.55 b | 0 a | 0 a |

Mean ± SD (n = 3)
Letter b significantly different from the corresponding control treatment (Dunnett's test, $p<0.05$)

## DISCUSSION

The study consists of determining the effect of acute concentrations of Pyro on the survival, hatching and deformations of embryos of *C. gariepinus*. It has been reported by several workers that chorion of fish provides no protection to the developing embryo exposed to various pesticides (Ansari and Ahmad, 2010). According to Helmstetter and Alden (1995), the chorion of fertilized eggs, when incubated in water, is permeable to lipophilic molecules with high n-octanol-water partition coefficients (Log kow). Pollutants with high Log kow more readily penetrate the chorion than those with low Log kow (Agbohessi *et al*.,

2013). In the present study, the two compounds constituting Pyro FTE 472 EC are all lipophilic (Log kow > 3). Thus, from the incubation of the fertilized eggs in the test solutions, these molecules quickly penetrated inside them by the chorion, which has a phospholipid nature. The more the test solution is concentrated in these molecules, the more there is an entry of these molecules by the chorion, and the extent of the toxic effect of this pollutant will be a function of the quantity of this one inside the eggs (Tyor and Harkrishan, 2016; Agbohessi *et al*., 2020). This explains why from the first hours of exposure, it was observed 100% egg/embryo mortality in the two highest concentrations of Pyro. This is also, what justifies that the mortalities of eggs/embryos increase as the concentration of the pollutant increases in the environment. Results of this kind had been proven by Prastika *et al*. (2021) in the exposure of fertilized eggs of Silver rasbora Rasbora *argyrotaenia* to Organophosphates. Rahman *et al*. (2020) revealed same results in the exposure of Zebrafish *Danio rerio* fertilized eggs to Sumithion. Tyor and Harkrishan (2016) obtained similar finding when exposed fertilized eggs of Common carp *Cyprinus carpio* to Imidacloprid. Agbohessi *et al.* (2013) obtained same results in the exposure of fertilized eggs of *C. gariepinus* to acute concentrations of Endosulfan and Tihan 175 O-TEQ. Malone and Blayloc (1970) reported that at concentration of 5-10 ppm almost all insecticides cause significant mortality of embryos. The LC50 in this study decreased as the duration of exposure progressed up to 36 h before levelling off. This means that as exposure progressed, the sensitivity of the embryos increased to Pyro. Indeed, when the fertilized eggs are brought into contact with solutions contaminated with pollutants, it takes time for the molecules to cross the chorion to find the embryos before acting. As these toxic molecules progress towards the embryos, there is an increase in their toxicity. This is what explains this decrease in the LC50 during exposure. Tyor and Harkrishan (2016) obtained similar results when exposed fertilized eggs of *C. carpio* to Imidacloprid. Similar finding were reported by Agbohessi *et al*. (2013) who showed that Flubendiamide (Log kow = 4.14) a fat-soluble molecule becomes more toxic to embryos as it enters the chorion. The 48h-LC50 obtained in the present study is 90.40 ppm. This value is very high compared to 78 ppm revealed for Imidacloprid on *C. carpio* (Tyor and Harkrishan, 2016), 5.47 ppb found for Chlorpyrifos on Banded gourami *Trichogaster fasciata* (Sumon *et al*., 2017), 1.34 ppb obtained for the same Chlorpyrifos on Gangetic mystus *Mystus cavasius* (Ali *et al*., 2018), 4.642 ppm recorded for Buprofezin on *C. gariepinus* (Marimuthu *et al*., 2013) and 0.999 ppm calculated for Diazinon on *C. carpio* (Aydin and Koprucu, 2005). In the control group, the hatching rate was 45.4 ± 2.8 %, a value very lower than those recorded by Kucharczyk *et al*. (2019) (87.9 - 97.1 %) but similar to 11.8 - 66.2% found in natural substrates by Macharia *et al*. (2005). The hatching rates of 62.2 to 75.9% obtained in the Pyro treatments from T1 to T3 in the present study are significantly higher than the rates of 0.12 to 68.9% in eggs/embryos of *C. gariepinus* subjected to Buprofezin (Marimuthu *et al*., 2013) and to the values of 3.3% recorded in eggs/embryos of the same species exposed to Atrazine (Opute and Oboh, 2020). During the normal hatching process of fish embryos, the chorion is digested by the hatching enzyme, which is a proteolytic enzyme secreted from hatching gland cells of the embryo (Marimuthu *et al*., 2013). Pollutant exposure might delay, prevent, or stimulate hatching by acting on secretion of hatching enzyme. The embryo itself by its movements inside the chorion can favour hatching (Agbohessi *et al*., 2013). In the present study, it was noted in the intermediate treatments (T1, T2, and T3) where the embryos did not all die, an increase in the hatching rate with the

increase in the pesticide concentrations in the environment, therefore stimulation of the hatching by Pyro in *C. gariepinus*. All the active molecules contained in Pyro being fat-soluble, their entry through the chorion into the egg would surely have favoured the synthesis of hatching enzymes or provided energy for the majority of embryos in the egg. This explains the appearance of the first hatchings in treatments T1, T2 and T3 compared to T0. The hatching stimulation recorded in this study is consistent with the results of Ahmad *et al*. (2020) in *D. rerio* subjected to Methonyl, glyphosate, Paraquat and Amitrol. Acceleration of hatching was also revealed by Fiorino *et al*. (2018) in exposing fertilized eggs of *D. rerio* to glyphosate and by Prastika *et al*. (2021) in *R. argyrotaenia* contaminated with organophosphates. Contrary to this observation, several authors have instead noted an inhibition of the hatching success of fish eggs exposed to pollutants. We can cite for example: Tyor and Harkrishan (2016) who showed a decreased of hatching success in *C. carpio* eggs subjected to Imidacloprid, De la Paz *et al*. (2017) who found that Triazole fungicides inhibit *D. rerio* hatching, Rahman *et al*. (2020) who reported that Sumithion caused a delay of hatching of *D. rerio* and Opute and Oboh (2020) who recorded a hatching success reduced in *C. gariepinus* contaminated to Atrazine. Several studies have shown that Chlorpyrifos, one of the Pyro molecules, has negative effects on the hatchability of different fishes. Thus, Sumon *et al*. (2017) revealed that in *T. fasciata*, Chlorpyrifos hinders the hatching of larvae. Sreedevi *et al*. (2014) reported an almost similar finding on the reduced hatching success of *D. rerio* embryos due to Chlorpyrifos toxicity. Reduced hatching success was also observed in Eastern rainbowfish *Melanotaenia splendida* exposed to Chlorpyrifos (Humphrey and Klumpp, 2003). Cypermethrin, the second molecule of Pyro has also been shown to delay hatching in several fish species such as *C. carpio* (Aydin *et al*., 2005) and *M. cavasius* (Ali *et al*., 2018). Thus, the effect of Pyro on hatching observed in the present study is probably the combined effect of the two molecules. For the two highest concentrations tested (T4 and T5), we noted 100% embryo mortality in the egg and much less in T1, T2 and T3. Indeed, the strong penetration of Pyro molecules into the eggs would probably have led to energy depletion to levels insufficient to support escape from the eggshell (Varo *et al.,* 2006). Tyor and Harkrishan (2016) observed the death of embryos in eggs of *C. carpio* subjected to Imidacloprid. Rahman *et al*. (2020) revealed high levels of unhatching eggs in *D. rerio* contaminated with Sumithion. Agbohessi *et al.* (2013) also reported dead embryos in eggs of *C. gariepinus* exposed to Tihan. In this study, it was observed nonmotile newly hatched embryos at T2 and T3. The immobility of free embryos precedes their death and surely results from an energy deficiency or an oxygen deficit created by the toxicant. Beyger *et al*. (2012) observed nonmotile larvae of Florida flagfish *Jordanella floridae* after exposure to 10 ppb Endosulfan for 96 h. Tailless or short-tailed vesiculated embryos were rated highly in treatments T1, T2, and T3 where the embryos were alive. Effects of Triclopyr and Paraquat on the tail shape of *D. rerio* larvae were reported by Ahmad *et al.* (2020). Curved or short-tailed larvae were also found in *D. rerio* embryos subjected to Chlorpyrifos (Kienle *et al*., 2009) and in *C. gariepinus* exposed to Flubendiamide (Agbohessi *et al*., 2013). The agglutinations of free embryos observed in Pyro treatments are probably due to the chemical stress-induced by Pyro on free embryos, which find comfort when they are next to each other.

## CONCLUSION AND APPLICATION OF RESULTS

The present study revealed that Pyro FTE 472 EC, a binary pesticide used extensively in the fields against insect pests of plants during the flooding period of reproduction of *C. gariepinus*, significantly affects the survival of eggs/embryos and induces malformations. This insecticide with embryotoxic effect on *C. gariepinus* must be used sparingly to avoid any threat to the survival of this species. However, further studies should be performed to better elucidate the mechanism by which this binary stimulates hatching in *C. gariepinus*. Other experiments can be carried out on other species such as *O. niloticus*, which is also present in ecosystems that receive these agricultural pesticides, to confirm the stimulatory effect on the larvae hatching of Pyro.

### Conflict of interest

The authors declare no competing interests.

**Authors' contribution**: (A.P.) conducted the toxicity tests and performed the statistical analysis, discussion and drafted the manuscript; (P.E.O.R.) participated in statistical analysis, discussion and drafted the manuscript; (H.M.A.B.) discussion and drafted the manuscript; (I.T.I) drafted the manuscript. All authors read and approved the final manuscript.

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## REFERENCES

Abbott WS, 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18:265-267.

Agbodji LK, Negloh K, Labare K, 2017. Un nouvel insecte majeur du Sorgho, *Poophilus costalis* au Togo: Dégâts et méthodes de lutte. Journal Recherche Scientifique de l'Université de Lomé, 19(2): 189 – 198.

Agbohessi TP, Imorou Toko I, Yabi A.J, Dassoundo-Assogba JFC, Kestemont P, 2011. Caractérisation des pesticides chimiques utilisés en production cotonnière et impact sur les indicateurs économiques dans la Commune de Banikoara au nord du Bénin. International Journal of Biological and Chemical Sciences, 5:1828–1841.

Agbohessi TP, Toko Imorou I, Kestemont P, 2012. Etat des lieux de la contamination des écosystèmes aquatiques par les pesticides organochlorés dans le bassin cotonnier béninois. Cahier d'Agriculture, 21: 46–56.

Agbohessi PT, Imorou Toko I, Houndji A, Gillardin V, Mandiki SNM, Kestemont P, 2013. Acute toxicity of agricultural pesticides to embryo-larval and juvenile African catfish *Clarias gariepinus*. Archives of Environmental Contamination and Toxicology, 64:692–700.

Agbohessi PT, 2014. Impact des pesticides agricoles sur le développement et la régulation du système reproducteur, le statut hépatique et la croissance des poissons dans le bassin cotonnier béninois. Thèse de Doctorat en Sciences, Université de Namur, Belgique, pp 307.

Agbohessi PT, Imorou Toko I, Ouédraogo A, Jauniaux T, Mandiki SNM, Kestemont P, 2015. Assessment of the health status of wild fish inhabiting a cotton basin heavily impacted by pesticides in

Benin (West Africa). Science of The Total Environment, 506-507: 567–584.

Agbohessi TP, Imorou Toko I, Atchou V, Tonato R, Mandiki SNM, Kestemont P, 2015. Pesticide used in cotton production affect reproductive development, endocrine regulation, liver status and offspring fitness in African Catfish *Clarias gariepinus* (Burchell, 1822). Comparative Biochemistry and Physiology Part C, 167: 157 – 172.

Agbohessi TP, Atchou V, Imorou Toko I, 2020. Effets chroniques du Tihan 175 O-TEQ et de l'endosulfan sur la phase embryo-larvaire de *Clarias gariepinus* (Burchell, 1822). Revue Afrique science, 17(6): 282 – 296.

Agritox. Base de données sur les substances actives phytopharmaceutiques, Chlorpyriphos-éthyl, 2015. http://www.agritox.anses.fr/php/sa.php?sa. (Accessed September 2021)

Ahmad F, Ali S, Richardson MK, 2020. Effect of pesticides and metals on zebrafish embryo development and larval locomotor activity. bioRxiv preprint doi: https://doi.org/10.1101/2020.10.05.326066

Ali MH, Sumon KA, Sultana M, Rashid H, 2018. Toxicity of cypermethrin on the embryo and larvae of Gangetic mystus, *Mystus cavasius*. Environmental Science and Pollution Research, 25: 3193 – 3199.

Ansari BA, and Ahmad MK, 2010. Toxicity of pyrethroid Lambacyhalothrin and Neemgold to the embryo of Zebrafish, *Danio rerio* (Cyprinidae). Journal of Applied Biosciences, 36:97-100.

Aydin R, and Koprucu K, 2005. The toxicity of diazinon on the common carp (*Cyprinus carpio* L.) embryos and larvae. Pesticide Biochemistry and Physiology, 82: 220- 225.

Aydin R, Koprucu K, Dorucu M, Koprucu SS, Pala M, 2005. Acute toxicity of synthetic pyrethroid cypermethrin on the common carp (*Cyprinus carpio* L.) embryos and larvae. Aquaculture International, 13:451–458

Beyger L, Orrego R, Guchardi J, Holdway D, 2012. The acute and chronic effects of endosulfan pulse-exposure on *Jordanella florida* over one complete life-cycle. Ecotoxicology and Environmental Safety, 76:71–78.

CRA-CF (Centre de Recherche Agricole Coton et Fibres), 2019. Point de la recherche cotonnière en 2018. CRA-CF/INRAB/MAEP/Bénin. 216p. Dépôt légal N°11783 du 18/11/2019, Bibliothèque Nationale du Bénin, 4ième trimestre. ISBN: 978-99982-53-51-3.

De la Paz J.F, Beiza N, Paredes-Zúñiga S, Hoare MS, Allende ML, 2017. Triazole Fungicides Inhibit Zebrafish Hatching by Blocking the Secretory Function of Hatching Gland Cells. International Journal of Molecular Sciences, 18: 710

Dede EB, Kaglo ED, 2001. Aqua-toxicological Effects of Water Soluble Fractions (WSF) of diesel fuel on *O. niloticus* fingerlings. Journal of Applied Sciences and Environmental Management, 5: 93-96.

Douny C, Zoumenou YMBG, Aïna M, Imorou Toko I, Igout A, Guedegba L, Chabi SK, Kestemont P, Scippo ML, 2021. Contamination of water, sediment and fish with residues of pesticides used in cotton production in Northern Benin. Archives of Environmental Contamination and Toxicology, 81: 367- 385.

FAO (Food and Agriculture Organisation), 2020. La situation mondiale des pêches et de l'aquaculture: La durabilité en action, Résumé. Rome, 28p. https://www.fao.org/3/ca9231fr/CA9231FR.pdf (Accessed on 12/26/2021)

Fiorino E, Sehonova P, Plhalova L, Blahová J, Svobodova Z, Faggiol C, 2018. Effects of glyphosate on early life stages: comparison between *Cyprinus carpio* and *Danio rerio*. Environmental Science and Pollution Research, 25: 8542–8549

GIEC, Changements climatiques. 2014. *Rapport de synthèse. Contribution des Groupes de travail I, II et III au cinquième Rapport d'évaluation du Groupe d'experts intergouvernemental sur l'évolution du climat*. Genève, Suisse, 161 p.

Helmstetter MF, and Alden RW III, 1995. Passive trans-chorionic transport of toxicants in topically treated Japanese medaka (*Oryzia latipes*) eggs. Aquatic Toxicology, 32:1–13

Humphrey C, and Klumpp DW, 2003. Toxicity of chlorpyrifos to the early life history stages of eastern rainbowfish *Melantaenia splendida splendida* (Peters 1866) in tropical. Environmental Toxicology, 18: 418–427.

INERIS (Institut National de l'Environnement Industriel et des Risques), 2016. Données technico-économiques sur les substances chimiques en France: Cyperméthrine, DRC-18-157877-10983A, pp48 (http://www.ineris.fr/substances/fr/)

Jarvinen AW, and Tanner DK, 1982. Toxicity of selected controlled release and corresponding un formulated technical grade pesticides to Fathead minnow *Pimephales promelas*. Environmental Pollution, 27:179–195.

Kienle C, Kohler H, Gerhardt A, 2009. Behavioural and developmental toxicity of chlorpyrifos and nickel chloride to zebra fish (*Danio rerio*) embryos and larvae. Ecotoxicology and Environmental Safety, 72: 1740–1747

Kucharczyk D, Kucharczyk DJ, Nowosad J, Omirzhanova N, 2019. Optimization of artificial insemination outcomes of African catfish (*Clarias gariepinus*) with differing hatchery conditions. Animal Reproduction Science, 211: 106222

Malone R, and Blayloc G, 1970. Toxicity of insecticide formulation on Carp embryos reared in vitro. The Journal of Wildlife Management, 34:462-463.

Macharia SK, Ngugi CC, Rasowo J, 2005. Comparative Study of Hatching Rates of African Catfish (*Clarias gariepinus* Burchell 1822) Eggs on Different Substrates. NAGA, WorldFish Center Quarterly, 28(3):23-26.

Marimuthu K, Muthu N, Xavier R, Arockiaraj J, Rahman MA, Subramaniam S, 2013. Toxicity of buprofezin on the survival of embryo and larvae of African catfish, *Clarias gariepinus* (Bloch). PLoS One 8, e75545.

OECD (Organisation for Economic Co-operation and Development), 1992. Guideline for testing of chemicals: fish acute toxicity test. 203, Paris, France

OECD, 1992. Guideline for testing of chemicals: fish early-life stage toxicity test. 201, Paris, France

Opute PA, and Oboh IP, 2020. Effects of Sub-Lethal Atrazine Concentrations on Embryogenesis, Larval Survival and Growth of African Catfish, *Clarias gariepinus* (Burchell, 1822). Aquaculture Studies, 21(1): 01-09.

Prastika AB, Sulmartiwi L, Lutfiyah L, 2021. Effect of sublethal dose organophosphate pesticides on embryogenesis and hatching rate of silver rasbora eggs (*Rasbora argyrotaenia*). *IOP* Conf. Series: Earth

and Environmental Science, 679: 012065.

Rahman MS, Islam SMM, Haque A, Shahjahan Md, 2020. Toxicity of the organophosphate insecticide sumithion to embryo and larvae of zebrafish. Toxicology Reports, 7: 317–323

RECA-NIGER (Réseau National des Chambres d'Agriculture du Niger), 2013. Fiche conseil pour la matière active: Cyperméthrine (insecticide), Famille: pyréthrinoïdes, RECA - Programme de Productivité Agricole en Afrique de l'Ouest – PPAAO Niger, pp 5.

Sreedevi B, Suvarchala G, Philip GH, 2014. Morphological and physiological abnormalities during development in zebrafish due to chlorpyrifos. Indian Journal of Scientific Research, 5(2): 1–8.

Sumon KA, Saha S, Van den Brink PJ, Peeters ETHM, Bosma RH, Rashid H, 2017. Acute toxicity of chlorpyrifos to embryo and larvae of banded gourami *Trichogaster fasciata*. Journal of Environmental Science and Health, Part B, 52: 92–98

Tyor AK, and Harkrishan, 2016. Effects of imidacloprid on viability and hatchability of embryos of the common carp (*Cyprinus carpio L*.). International Journal of Fisheries and Aquatic Studies, 4(4): 385-389.

Varo I, Amat F, Navarro JC, Barreda M, Pilarch E, Sarrerro R, 2006. Assessment of the efficacy of Artemia sp. (Crustacea) cyst chorion as barrier to chlorpyrifos (organophosphorus pesticide) exposure. Effect on hatching and survival. Science of the Total Environment, 366:148–153.