EFFECTS OF ACUTE EXPOSURE TO COTTON INSECTICIDE THALIS 112 EC (EMAMECTIN BENZOATE 48 G.L-1 AND ACETAMIPRID 64 G.L-1) IN AFRICAN CATFISH CLARIAS GARIEPINUS EMBRYOS

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ABSTRACT

Thalis 112 EC, a binary insecticide based on Emamectin benzoate (48 g.L-1) and Acetamiprid (64 g.L-1), is widely used in agriculture in Benin, to control cotton pests including Helicoverpa armigera. In order to assess the impact of acute concentrations of this binary on the development of eggs/embryos of the African catfish Clarias gariepinus, an experiment was conducted in the laboratory. The fertilized eggs of C. gariepinus (n ≈ 100) were exposed to six concentrations of Thalis (T0: 0.0; T1: 10.0; T2: 60.0; T3: 110.0; T4: 160.0 and T5: 210.0 ppm), each in three replicates. The arithmetic method of Karber was used to calculate LC50 values. The 24h-LC50 and 48h-LC50 values of Thalis for eggs/embryos were 124.09 and 117.58 ppm, respectively. High Thalis concentrations significantly increased eggs/ embryos mortality and decreased hatching success ($p<0.05$, Dunnett's test). Rates of various physical deformities such as short-tail and lordosis, and the abnormalities such as black pigmentation on yolk sac, intense lethargy, etc., also climbed with increasing Thalis concentrations ($p<0.05$, Dunnett's test). The findings from the current study showed that Thalis exerts adverse effects on embryo development of C. gariepinus. They constitute an alert on the toxic effect of chemical pesticides used in Benin on the first developmental stages of fish inhabiting aquatic ecosystems.

Keywords: Aquatic environment, fish, hatching, malformation, pesticides.

RESUME

TOXICITÉ AIGUË DE L'INSECTICIDE THALIS 112 EC (EMAMECTINE BENZOATE 48 G.L-1 ET ACÉTAMIPRIDE 64 G.L-1) CHEZ LES EMBRYONS DU POISSON-CHAT AFRICAIN CLARIAS GARIEPINUS

Thalis 112 EC, un insecticide à base d'Emamectine benzoate (48 g.L-1) et d'Acétamipride (64 g.L-1), est largement utilisé en cotonculture au Bénin, pour lutter contre les ravageurs dont Helicoverpa armigera. Dans le but d'évaluer l'impact des concentrations aigues de ce binaire sur le développement des oeufs/embryons du poisson-chat africain Clarias gariepinus une expérience a été menée en laboratoire. Les œufs fécondés de C. gariepinus (n ≈ 100) ont été exposés à six concentrations de Thalis (T0 : 0,0 ; T1 : 10,0 ; T2 : 60,0 ; T3 : 110,0 ; T4 : 160,0 et T5 : 210,0 ppm), chacune en trois répétitions.

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La méthode arithmétique de Karber a été utilisée pour calculer les LC50. Les 24h-LC50 et 48h-LC50 de Thalis pour les œufs/embryons étaient de 124,09 et 117,58 ppm, respectivement. L'augmentation des concentrations du polluant a augmenté de manière significative la mortalité des œufs/embryons et a diminué le succès de l'éclosion (p<0,05; test de Dunnett). Les taux de diverses déformations physiques telles que la queue courte et la lordose, et les anomalies telles que la pigmentation noire sur le sac vitellin, la léthargie intense, etc., ont augmenté avec l'augmentation de la concentration de Thalis (p <0,05, test de Dunnett). Les résultats de la présente étude indiquent que Thalis exerce une toxicité développementale sur les embryons de C. gariepinus. Ces résultats constituent une alerte sur l'effet toxique des pesticides chimiques utilisés au Bénin sur les premières phases de développement des poissons dans les écosystèmes aquatiques.

Mots clés: Milieu aquatique, poisson, éclosion, malformation, pesticides.

INTRODUCTION

To fight effectively against crop pests, especially those of cotton, several pesticide formulations are used in Benin and west Africa countries such as Mali, Burkina Faso, Côte d'Ivoire, etc. (CRA-CF, 2019; Guedegba *et al.*, 2019). There are several formulations among these are, Thalis 112 EC (Emamectin benzoate 48 g.L-1, Acetamiprid 64 g.L-1), Vizir C 92 EC (Cypermethrin 72 g.L-1, Abamectin 20 g.L-1), Pyrinex Quick 212 EC (Deltamethrin 12 g.L-1, Chlorpyrifos 200 g.L-1) and Pyro FTE 472 EC (Cypermethrin 72 g.L-1, Chlorpyrifos 400 g.L-1) (CRA-CF, 2019). These binary insecticides have been introduced into the technical itinerary of cotton in Benin since the 2017-2018 agricultural campaign. Thalis is an aphicidal binary, used in the first and second windows to fight against stinging-sucking insects and the first generation carpophagous moths Helicoverpa armigera (CRA-CF, 2019). Based on the volume of this pesticide observed among cotton growers, this insecticide seems to be the most used in the fields. Several studies conducted revealed that in the Benin cotton basin, the doses of pesticides recommended for the treatment of crops are not necessarily those practiced by farmers (Agbohessi *et al.*, 2011; Douny *et al.,* 2021). These farmers increase the recommended amounts of pesticides at their will (Agbohessi *et al.*, 2011). Several studies have also shown the contamination of aquatic ecosystems in the cotton basin of northern Benin by agricultural pesticides (Agbohessi, 2014; Agbohessi *et al.*, 2015b; Douny *et al*., 2021). The most recent of these studies is on water reservoirs and indicates the concentrations of 1.0 µg/kg of Chlorpyrifos and 0.8-1.8 µg.kg-1 of Permethrin at Batran reservoir, and of 0.8-13.0 µg.kg-1 of Permethrin at Sori reservoir (Douny *et al.*, 2021) in the sediments. The same authors also reported the presence of Chlorpyrifos up to 1.9-3.3 µg.kg-1 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-1. In Niger, studies revealed in the water of the Tabalak River Dicofol ranging to 808 µg.L-1 and DDT ranging to 2 µg.L-1 (Youchaou Tawayé *et al.*, 2021). DDT contents of 1306 µg.kg-1 in the sediments of the Ebrié lagoon in Cote d'Ivoire were found by Marchand and Martin (1985), while Mawoussi (2008) obtained a rate of 164.31 µg.kg-1 of Endosulfan in the sediments of the Agbansiandi River in Togo. These chemical pesticides present in these different compartments of aquatic biotopes have acute and chronic effects on the different development phases of aquatic species such as fish (Agbohessi *et al.* 2015a et b).

Emamectin benzoate is of the Avermectin family (Agritox, 2014). C. gariepinus juveniles were highly sensitive to Ivermectin, with an LC50 of 15 µg.L-1 under static conditions (Ogueji *et al.*, 2019). Acetamiprid is a molecule of the first generation of the Neonicotinoid family (Annabi *et al.*, 2019). Its 96-h LC50 was 182.9 ppm for O. niloticus (Guedegba *et al.*, 2019) and 265.7 ppm for C. gariepinus juveniles (Houndji *et al.*, 2020). Studies have also shown the harmful effects of Emamectin benzoate and Acetamiprid on fish, but to the best of our knowledge, there is no published data on the impact of acute Thalis concentrations on the embryonic phase of C. gariepinus. While in the north part of Benin, where nearly 70% of the national cotton production is concentrated, the period of intense use of pesticides in the fields matches with the period of reproduction of several species of fish including C. gariepinus in the natural environment (Agbohessi *et al.*, 2013; Agbohessi *et al*., 2015a; Agbohessi et al., 2020). It is 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 aims to study, in laboratory conditions, the impact of acute Thalis concentrations on the embryonic phase of this species. This involves evaluating the effect of acute concentrations of Thalis on the survival, hatching and malformations of embryos.

MATERIALS AND METHODS

The experiment was conducted in August - September 2020, at the Research Laboratory in Aquaculture and Aquatic Ecotoxicology (LaRAEAq), University of Parakou (9° 20' 60" N 2° 37' 0.001" E), in Benin, following the 203 and 210 guidelines of the Organisation for Economic Co-operation and Development (OECD) (OECD, 1992a and b) with some minor adaptations.

PESTICIDE COLLECTION

The insecticide Thalis 112 EC formed of Emamectin benzoate (48 g.L-1) and Acetamiprid (64 g.L-1) and commonly used by farmers in the cotton basin of Benin, was purchased from the local market of the «Société de Distribution des Intrants (Bénin)». The chemical properties of these active ingredients are listed in Table 1. Thalis is in liquid form. The test solutions are obtained by mixing Thalis directly with dechlorinated tap water, as it is done in a farming environment. All working stock solutions were made immediately before the tests. Water used in the preparation of test solutions was tested for quality (nitrate 20.07 ± 0.03 mg.L-1, nitrite 0.03 ± 0.01 mg/L-1, and total hardness 81.0 ± 0.1 mg.L-1).

Table 1: Properties of the active ingredients of Thalis 112 EC.

Propriétés des principes actifs de Thalis 112 EC..

Trade name	Formulation	Active ingredient and concentration	Nater solubility (mg/l) at 25 °C	Log Kow at 20 °C	Vapor pressure	DT50 in water (days)	References
Thalis	112 EC (Emulsifiable Concentrate)	Emamectin benzoate (48 g/l) (Avermectin)	24	5	4 µPa at 21 °C	7	Agritox (2014) ANSES (2012)
		Actamiprid (64 g/L) (Neonicotinoid)	4.25×10^{2}	0.8	1 x 10-8 mmHg	420 at 25 °C	Annabi *et al.* (2019)

FISH COLLECTION

Adults of C. gariepinus were collected from closed circuit at a local farm (Royal Fish Benin) in Porto-Novo (6° 29' 49.999" N 2° 36' 18" E), Benin. These broodstock were thoroughly transferred to the LaRAEAq, University of Parakou, Benin, where they were individually acclimated for 12 days in plastic tanks (1000 L). They were fed twice daily 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 (637.2 g) and one female (428.4 g) both healthy and ready for breeding were chosen. Gonads were examined based on external morphological features. The study retained a mature male, while the female had a soft and developed abdomen, a red and protuberant genital papilla with emission of a few oocytes by abdominal pressure. Both male and female broods were artificially induced by intramuscular

injection of Ovaprim. The Ovaprim was administered at a dose of 0.5 mL.kg-1 body weight of fish for the female and 0.25 mL.kg-1 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 a plastic tray, and testes were collected from the 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 of fertilized 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 that, they did not touch each other. Exposure to Thalis was made under static conditions to avoid disturbing them during incubation (OECD, 1992 a and b). 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 concentration at which the pesticide was 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; 20.0; 60.0; 100.0; 140.0 and 180.0 ppm of Thalis. The nominal concentrations in the final test were: 0.0; 10.0; 60.0; 110.0; 160.0 and 210.0 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 26.5 ± 0.1 R"C, pH 7.1 ± 0.1, dissolved oxygen 5.6 ± 0.1 mg.L-1).

First, 30 min after the beginning of the incubation of the fertilized eggs, the unfertilized eggs found in each trough which are recognizable by their whitish colour, were removed. Next, the number of fertilized eggs in each trough was counted and recorded. At 4 hour intervals, the proportions of hatched eggs, dead eggs/embryos, and eggs/embryos with abnormalities (e.g. dead embryo within the egg, lordosis, short-tailed vesiculated embryos, black pigmentation on yolk sac, etc) were recorded. From the 12 hpf (hours post-fertilization), 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 whose yolk vesicles contain black spots were classified as black pigmentation on yolk sac embryos. Embryos that have deformities in the spine were classified as embryos with lordosis.

CALCULATION OF LC50

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 - (Ó (mean mortality of two successive concentrations x differences between the two successive concentrations) / number of embryos per treatment). But before Abbott's formula (% Corrected = (1 - ((Number of survivors for treatment) / (Number of survivors for control)) × 100) was used to correct the mortalities (Abbott, 1925).

STATISTICAL ANALYSIS

The experimental unit is the incubation trough. Results are expressed as the mean ± standard deviation. The incidence rates of hatching rates, dead eggs/embryos, dead embryos in the egg, embryos with lordosis, short-tailed vesiculated embryos, black pigmentation on yolk sac embryos, were analyzed by one-way analysis of variance (ANOVA I). Means were compared with control values by Dunnett's test with $p < 0.05$ being considered statistically significant.

RESULTS

EGG/EMBRYO MORTALITY

Most of the eggs/embryos exposed to Thalis died between 24 and 36 hpf, but at 24 hpf all the eggs/embryos (100%) were already dead in the T5 treatment, the highest concentration of Thalis (Table 2). At 48 hpf, all the free embryos of C. gariepinus were already dead regardless of the treatment. After correction by Abbott's formula, it is noted that 24 h-LC50 =124.09 ppm and 36 h-LC50 = 48 h-LC50 =117.58 ppm (Figure 1).

Table 2: Number of dead eggs/embryos or free-living embryos of *Clarias gariepinus* exposed to Thalis 112 EC at different times.

Nombre d'œufs/d'embryons morts ou d'embryons libres de Clarias gariepinus *exposés à Thalis 112 EC à différents moments.*

Treatments (ppm)			Number of incubated eggs	Number of eggs/embryos dead at 12 hpf	Number of eggs/embryos dead at 24 hpf	Number of eggs/embryos dead at 36 hpf	Number of eggs/embryos dead at 48 hpf	Free embryos mortality after hatching at 48 hpf (%)
0	TO	T01	134	09	09	09	09	
		T02	139	08	09	09	09	07.02 ± 1.52
		T03	134	12	12	12	12	
10.0	T1	T11	150	10	11	34	34	
		T12	158	12	13	39	39	100*
		T13	134	13	14	50	50	
600	T2	T21	136	11	13	40	40	
		T22	117	10	10	30	30	100*
		T23	136	13	16	49	49	
110.0	T3	T31	130	08	12	39	39	
		T32	103	11	12	38	38	100*
		T33	101	11	12	38	38	
160.0	T4	T41	127	13	16	71	71	
		T42	145	11	11	55	55	100*
		T43	128	18	21	79	79	
210.0	T5	T51	109	90	109	109	109	
		T52	131	70	131	131	131	100*
		T53	122	47	122	122	122	

hpf= hours post-fertilization ; * Significantly different from the corresponding control treatment (Dunnett's test, p<0.05).
*hpf= heures post-fertilisation ; * Significativement different du traitement témoin (Test de Dunnett, p<0,05).*

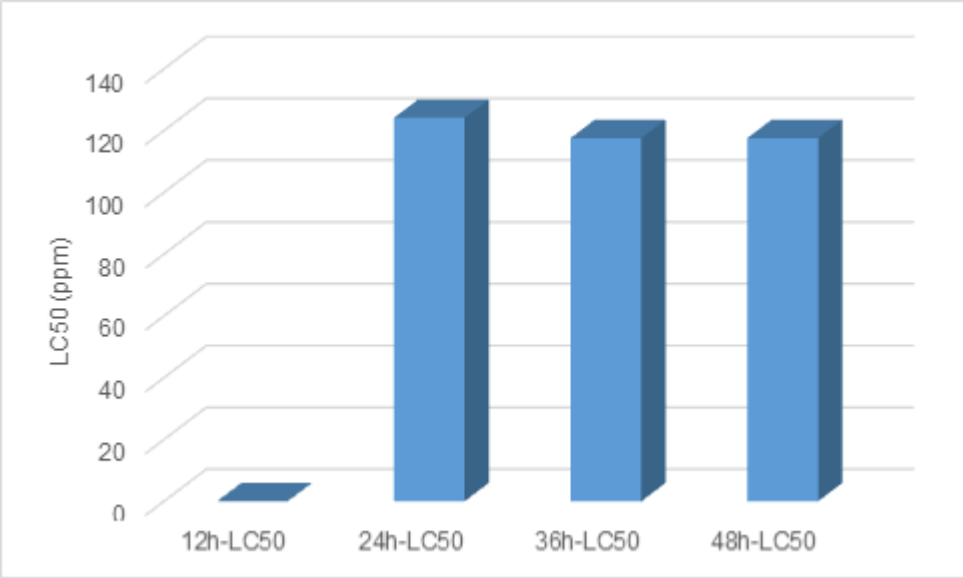

Figure 1: Evolution of the LC50, the median lethal concentration of Thalis 112 EC during the 48 h exposure of Clarias gariepinus.

Evolution de la CL50, la concentration létale médiane de Thalis 112 EC au cours de l'exposition de 48 h de Clarias gariepinus.

HATCHING RATE

At 12 hpf no hatching had been noted in any treatment including the control (Table 3). The first hatching appeared in treatment T4 followed by T3, then T2, T1, and T0. The maximum hatching of C. gariepinus eggs occurred between 24 and 36 hpf, whatever the treatment. The hatching rate in the control is 82.96% and the more the Thalis concentration increases, the more the hatching rate of C. gariepinus eggs decreases (Figure 2).

Table 3: Number of hatching eggs/embryos of Clarias gariepinus exposed to Thalis 112 EC at different times.

Nombre d'œufs éclos/embryons de Clarias gariepinus exposés à Thalis 112 EC à différents moments

Treatments (ppm)			Number of incubated eggs	Number of eggs hatching at 12 hpf	Number of eggs hatching at 24 hpf	Number of eggs hatching at 36 hpf	Number of eggs hatching at 48 hpf	Meantime of onset of the first hatching (hpf)
0	TO	T01	134	0	05	125	125	
		T02	139	0	06	130	130	23.67 ± 0.57
		T03	134	0	05	122	122	
10.0	T1	T11	150	0	02	139	139	
		T12	158	0	08	145	145	23.67± 0.57
		T13	134	0	25	118	118	
600	T2	T21	136	0	10	123	123	
		T22	117	0	13	101	101	23.33 ± 0.57
		T23	136	0	22	123	123	
110.0	T3	T31	130	0	98	117	117	
		T32	103	0	17	90	90	22.33 ± 0.57
		T33	101	0	09	89	89	
160.0	T4	T41	127	0	101	101	101	
		T42	145	0	121	124	124	21.0 ± 0.0
		T43	128	0	99	100	100	
210.0	T5	T51	109	0	0	0	0	
		T52	131	0	0	0	0	-
		T53	122	0	0	0	0	

hpf= hours post-fertilization
hpf= heures post-fertilisation

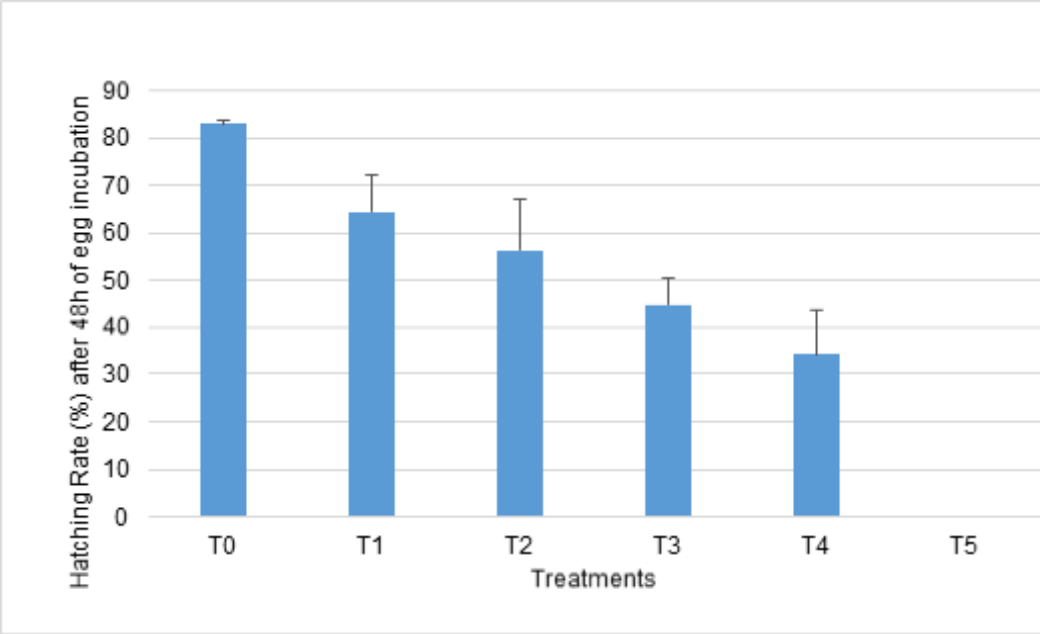

Figure 2: Effect of increasing the acute concentrations of Thalis 112 EC on the hatching rate of eggs of Clarias gariepinus. Values are mean ± SD (n = 3). * Significantly different from the control treatment ($p<0.05$, Dunnett's test).

*Effet de l'augmentation des concentrations aiguës de Thalis 112 EC sur l'éclosion des œufs de Clarias gariepinus. Les valeurs sont moyennes ± SD (n = 3). * Significativement différent du traitement témoin (p<0,05, test de Dunnett).*

DEFORMITY AND BEHAVIORAL ABNORMALITIES RATES

Two types of malformation (short-tailed vesiculated embryos and lordosis) and three types of abnormal behavior (dead embryos in the egg, black pigmentation on yolk sac, and intense lethargy) were observed (Table 4). These deformations and behaviors increased with the increase of Thalis in the environment. No abnormal behavior was noted in the controls.

Table 4: Rates of morphological and behavioral abnormalities caused by Thalis 112 EC in Clarias gariepinus.

Taux d'anomalies morphologiques et comportementales causées par Thalis 112 EC chez Clarias gariepinus.

	T0	T1	T2	T3	T4	T5
Deformity (%)						
Short-tailed vesiculated embryos	01.68 ± 0.01	10.22 ± 3.08*	19.44 ± 2.22*	12.85 ± 2.0*	21.55 ± 5.99*	0
Lordosis	0	08.98 ± 1.10*	16.63 ± 1.68*	24.16 ± 3.61*	20.66 ± 5.83*	0
Behavioral abnormalities (%)						
Dead embryos in the egg	0	0	5.9 ± 2.48*	17.45 ± 2.55*	24.93 ± 4.77*	100*
Black pigmentation on yolk sac	0	0	06.14 ± 1.2*	15.85 ± 2.36*	36.66 ± 5.15*	0
Intense lethargy	0	100*	100*	100*	100*	0

Mean ± SD (n = 3)

* Significantly different from the corresponding control treatment (Dunnett's test, $p<0.05$)

* *Significativement different du traitement témoin (Test de Dunnett, $p<0,05$)*

DISCUSSION

The study was undertaken to determine the effects of acute concentrations of Thalis 112 EC (Emamectin benzoate 48 g.L-1 and Acetamiprid 64 g.L-1) on the survival, hatching, and malformations of embryos of C. gariepinus.

It has been reported by Ansari and Ahmad (2010) that chorion of fish does not protect the developing embryo from pesticides in a contaminated environment. But 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). Emamectin benzoate is lipophilic with an octanol/water partition coefficient equal to 5 (Agritox, 2014). Acetamiprid is hydrophilic with a very low log Kow = 0.8 (Annabi *et al.,* 2019). Thus, from the incubation of the fertilized eggs in the test solutions, Emamectin benzoate quickly penetrated inside eggs by the chorion which has a lipoprotein nature. Acetamiprid will pass more slowly. 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 observed a 100% of eggs/ embryos mortality in the highest concentration (T5) of Thalis. This is also justifies that the mortalities of eggs/embryos increase as the concentration of the pollutant climbs in the environment. These results are consistent with those of Rahman *et al.* (2020) who exposed eggs/embryos of the Zebrafish Danio rerio to Sumithion, by Agbohessi *et al.* (2013) with C. gariepinus embryonated eggs exposed to Endosulfan and Tihan 175 OTEQ, and Tyor and Harkrishan (2016) who exposed the Common carp Cyprinus carpio embryos to Imidacloprid. According to the latter authors, increase in mortality with an increase of the concentrations of pollutant may be due to rapid absorption of pesticides and rapid onset of action. Malone and Blayloc (1970) had moreover reported that at a 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 leveling off. This means that as exposure progressed, the sensitivity of the embryos increased to Thalis. 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 explains the decrease in the LC50 during exposure. Tyor and Harkrishan (2016) obtained similar results when fertilized eggs of C. carpio was exposed to Imidacloprid. Similar findings were reported by Agbohessi *et*

al. (2013) who showed that Flubendiamide (log Kow = 4.14) a fat-soluble molecule becomes more toxic to embryos with time after entering the chorion. The 48h-LC50 obtained in the present study is 117.58 ppm. This value is very high compared to 78.0 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). The differences observed are linked to the difference in the molecules. The high value of the LC50 obtained in this study is surely linked to the combined effect of the two molecules (Emamectin benzoate and Acetamiprid) which constitute the pesticide tested.

In the control group, the hatching rate was 82.96 ± 0.76 %, a value in agreement with those recorded by Kucharczyk *et al.* (2019) (87.9 - 97.1 %), but very high to 11.8 - 66.2% found in natural substrates by Macharia *et al.* (2005). The hatching rates of 0.0 to 64.56% obtained in the Thalis treatments in the present study are similar to the rates of 0.12 to 68.9% in eggs/embryos of C. gariepinus subjected to Buprofezin (Marimuthu *et al.,* 2013) and the values of 3.3 to 73.3% 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 the hatching gland cells of the embryo (Marimuthu *et al.*, 2013). Pollutant exposure might delay, prevent, or stimulate hatching by acting on the secretion of the hatching enzyme. The embryo itself by its movements inside the chorion can favor hatching (Agbohessi *et al.*, 2013). In the present study, it noted a decrease in the hatching rate with the increase in the pesticide concentrations in the environment. These findings are consistent with those of Tyor and Harkrishan (2016) who showed a decrease 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. Sreedevi *et al.* (2014) also reported a similar finding on the reduced hatching success of D. rerio embryos due to Chlorpyrifos toxicity. Reduced hatching success was observed in Eastern rainbowfish Melanotaenia splendida exposed to Chlorpyrifos (Humphrey and Klumpp, 2003). Agbohessi *et al.* (2013) also reveal a delay of hatching in C. gariepinus due to Spirotetramat, Flubendiamide, Tihan, and Endosulfan. Unfortunately, there are few exposure studies of Emamectin benzoate or Acetamiprid to fish eggs/embryos in the literature for comparison. However, about the physicochemical characteristics of each of these constituent molecules of Thalis, the effects observed in the present study on hatchability are probably induced by all of the two molecules but much more by Emamectin benzoate which is liposoluble.

Several deformities and behavioral abnormalities in the embryos of C. gariepinus were evident after exposure to different concentrations of Thalis. Similar deformities were reported in D. rerio embryos and larvae exposed to different concentrations of Cypermethrin (Shi *et al.*, 2011), in C. gariepinus following exposure to Buprofezin (Marimuthu *et al.,* 2013), in T. fasciata when exposed to Chlorpyrifos (Sumon *et al.*, 2016) and in Stinging catfish, Heteropneustes fossilis when exposed to Sumithion (Shahjahan *et al.*, 2017). The present study is also supported by previous findings on D. rerio exposed to Sumithion (Rahman *et al.*, 2020), on C. gariepinus subjected to Endosulfan, Spirotetramat, Flubendiamde, and Tihan (Agbohessi *et al.*, 2013). Dead embryos in the egg were increased with increasing concentrations of Thalis with 100% in T5, possibly due to the energy depletion, at a level insufficient to allow escape from the eggshell (Varo *et al.*, 2006). Koprucu and Aydin (2004) observed the death of embryos in eggs of C. carpio at concentrations of Deltamethrin >0.005 ppb. Thalis might also have caused energy depletion, albeit to a lesser extent, thus explaining the observation of intense lethargy of newly hatched larvae at concentrations >10.0 ppm. Beyger *et al*. (2012) observed nonmotile larvae of Florida flagfish Jordanella floridae after its exposure to 10 ppb of Endosulfan for 96 h. Globally, lethargy precedes the death of the embryos, which explains the death of all the free embryos at 48 hpf. Black pigmentation on embryo yolk sac was increased with increasing concentrations of Thalis, possibly due to the

accumulation of residues of Emamectin benzoate and Acetamiprid in the vitelline reserve of embryos, which affects the quality of these reserves (Agbohessi *et al.*, 2013). Rahman *et al.* (2020) observed similar findings in D. rerio embryos/larvae exposed to Sumithion. Short-tailed larvae were increased with increasing concentrations of Thalis, probably due to apoptosis in the tail area, decreased cardiac output, and changes in the muscle fibers of the tail (Hagenaars *et al.*, 2011). Agbohessi *et al.* (2013) observed similar results in C. gariepinus embryos/larvae exposed to Flubendiamide. Curvature of the spine (lordosis), a deformity that frequently occur in C. gariepinus embryos/ larvae exposed to toxic substances could result from differential accumulation of toxic substances and lack of neuromuscular coordination. (Rahman *et al.,* 2020). Moreover, spinal curvature might be the consequence of decreased collagens in the spinal column, changing amino acid composition (Ekrem *et al.*, 2012) or due to down regulations of pkt7 gene, a critical regulator of wnt signaling (Hayes *et al.*, 2014). Rahman *et al.* (2020) observed similar findings in D. rerio embryos/larvae exposed to Sumithion.

CONCLUSION

The present study revealed that Thalis 112 EC, a binary insecticide used extensively in the fields against insect pests of cotton during the flooding period of reproduction of C. gariepinus, negatively affects the survival and the hatching success of eggs/embryos and induces deformities. This means that in the natural environment, Thalis contributes to affecting the renewal of stocks of C. gariepinus, as already demonstrated for many pollutants. 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 reduced effect on the larvae hatching of Thalis.

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The authors declare no competing interests.

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