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Research Articles

Evaluation of Nile tilapia (*Oreochromis niloticus*) fingerlings exposed to the pesticide pyriproxyfen

Fabio Francisco da Silva[1,2], Jaqueline Maria da Silva[2], Themis de Jesus da Silva[1]
Bruno Mendes Tenorio[3], Fernanda das Chagas Angelo Mendes Tenorio[4], Elton Lima Santos[1]
Sonia Salgueiro Machado[2] & Emerson Carlos Soares[1]
[1]Aquaculture and Water Quality Laboratory, Agricultural Science Center
Federal University of Alagoas, Rio Largo, Alagoas, Brazil
[2]Laboratory of Biotechnology and Enzymology, Institute of Chemistry and Biotechnology
Federal University of Alagoas, Maceió, Alagoas, Brazil
[3]Department of Morphology, Health Sciences Center, Federal University of Paraíba
João Pessoa, Paraíba, Brazil
[4]Department of Histology and Embryology, Bioscience Center, Federal University of Pernambuco
Recife, Pernambuco, Brazil
Corresponding author: Emerson Carlos Soares (soaemerson@gmail.com)

ABSTRACT. *Oreochromis niloticus* (Nile tilapia) is one of the most produced fish for human consumption globally; however, these fish are susceptible to exposure to toxic chemicals in the water. Pyriproxyfen is a pesticide widely used to control mosquitoes in drinking water supplies and pests in crops. The present study aimed to examine pyriproxyfen's effects on fish *O. niloticus* fingerlings exposed using an acute 96 h and subchronic tests for 28 days. The lethal concentration LC_{50}-96 h for *O. niloticus* was 2.77 mg L^{-1}, no observed effect concentration (NOEC), and the lowest observed effect concentration (LOEC) was 1.0 and 2.5 mg L^{-1}, respectively. The hepatic catalase activity was significantly changed after exposure to pyriproxyfen above 0.4 mg L^{-1}. Pyriproxyfen also induced histopathological lesions in the hepatic tissue at 28 days in a dose-dependent pattern at concentrations above 0.4 mg L^{-1}. Pyriproxyfen above 1.2 mg L^{-1} reduced the locomotor behavior, distance traveled inside the tank, mean speed, and angular variation. This pesticide also decreased weight gain, food conversion, and specific growth rate at concentrations above 1.2 mg L^{-1}. In conclusion, exposure to pyriproxyfen in water from 0.4 mg L^{-1} may impair *O. niloticus* development; consequently, pyriproxyfen may affect this fish's production and quality at higher concentrations than commonly used in water (0.01 mg L^{-1}).

Keywords: *Oreochromis niloticus*; fish; pyriproxyfen; LC_{50}; locomotor behavior; catalase; histopathology

INTRODUCTION

The worldwide use and adverse consequences attributed to pesticides have increased in the last few decades (La Verda *et al*., 2015; Legrand *et al*., 2017). Also, the indiscriminate use of pesticides has risen in tropical areas due to the epidemic of dengue, chikungunya, and zika, mainly due to the climate and inappropriate sanitary conditions (Marcondes & Ximenes, 2016).

The widespread dispersion of *Aedes aegypti* and effective adaptation to urban areas enhanced the need for more efficient pesticides, among this pyriproxyfen (4-phenoxy phenyl (RS)-2-(2-pyridyloxy) propyl ether). Pyriproxyfen is a pyridine-based larvicide used directly in water supplies to control the proliferation of mosquito larvae (Caixeta *et al*., 2016; Dzieciolowska *et al*., 2017; Peterson *et al*., 2017; Maharajan *et al*., 2018). This larvicide is an analog of a juvenile hormone that acts to inhibit the mosquito's metamorphosis and embryogenesis (Ohba *et al*., 2013). Previously investigators reported adverse effects following exposure to low concentrations of pyriproxyfen (Truong *et al*., 2016; Dzieciolowska *et al*., 2017). Due to low solubility, high partition coefficients, and hydrophobicity of pyriproxyfen, this pesticide displays the potential to be environmentally persistent, and precautions need to be taken when applying pyriproxyfen to or near water

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bodies in order to avoid contamination of water. When exposed to natural light in river water, the half-live of 21 days was observed for pyriproxyfen, more stable in dark conditions (Sullivan & Goh, 2008). Besides, pyriproxyfen was used as a pesticide in households for California red scale, silver leaf whitefly, and red fire ant in citrus, cotton, vegetable, and peanut crops (Legrand *et al*., 2017; Maharajan *et al*., 2018). Pesticides applied to crops may inadvertently reach aquatic ecosystems and accumulate in sediment and living organisms such as fish (Tenorio *et al*., 2017). The guideline value for daily intake of pyriproxyfen is 100 mg kg^{-1} of body-weight per day for a lifetime, and the recommended use of pyriproxyfen in the drinking water sources is 0.01 mg L^{-1}, the dose also used to control mosquito *A. aegypti* (Caixeta *et al*., 2016; Maharajan *et al*., 2018). Due to its very low mammalian toxicity, pyriproxyfen is approved by the World Health Organization (WHO) to treat potable water against mosquitoes. However, studies have observed increased mosquitoes resistance to the pesticide pyriproxyfen (Maoz *et al*., 2017).

Oreochromis niloticus (Nile tilapia) is produced in more than 140 countries; it is very versatile in fish farming (Meurer *et al*., 2005; Vieira *et al*., 2018) and is the second most widely cultivated fish group globally. Its production occurs mainly in net-tanks in dammed water bodies (Furuya *et al*., 2004; FAO, 2010). Tilapia production has quadrupled over the past decade because of its suitability for aquaculture, marketability, and stable market prices. Tilapia continued its rapid increase in global production with 5,576,800 mt, clearly emerged as a very promising group in aquaculture (Prabu *et al*., 2019).

Since pyriproxyfen exhibits the potential to be environmentally persistent in water, this study's objective was to determine the 50% lethal concentration (LC_{50}), NOEC, LOEC, and catalase activity 96 h after exposure of *O. niloticus* to a commercial formulation of pyriproxyfen. These commercial formulations have been recommended to be used in water supplies to control mosquitoes' proliferation in the country. This study, based upon LC_{50} value, also determined the effects of these concentrations of pyriproxyfen formulations on biometric performance, hepatic microstructure, and locomotor behavior of fish under subchronic toxicity tests for 28 days.

MATERIALS AND METHODS

Experimental methodologies were approved by the Ethics Commission on the Use of Animals of the Federal University of Alagoas (protocol no. 57/2016), in accordance with the principles for research using animals.

Reagents

All chemicals, including 5,5-dithio-bis-nitrobenzoic acid (DTNB; 98%), acetylthiocholine iodide (ASCh; 97%), bovine serum albumin (BSA; 98%), sodium chloride, were purchased from Sigma - Aldrich (St. Louis, MO, USA). Pyriproxyfen was obtained from commercial formulation Sumitomo Tiger100EC (Japan).

Oreochromis niloticus and fish acclimatization

Fish fingerlings were produced and supplied from the pisciculture station of the Agriculture Science Center of the Federal University of Alagoas - CECA/UFAL. Before initial biometry, fingerlings of *O. niloticus* were acclimated to lab conditions in 240 L tanks containing dechlorinated water with air recycling system aerator, a biological filter containing oysters, bio-balls, porcelain, PVC screens, bacteria placed exogenously, and UV filter (Jebo, China) for ten days at room temperature (26 ± 1°C) under a 12 h photoperiod, and then transferred to the experimental tanks (20 L). During all experimental periods, water quality parameters were monitored by a multiparameter probe (HANNA Instruments, model 9828, Woonsocket, USA). Temperature was 24.3 ± 0.1ºC; pH 7.1 ± 0.2; dissolved oxygen 6.5 ± 0.2 mg L^{-1}; total ammonia-nitrogen <0.01 mg L^{-1}; total dissolved solids (542 ± 21 mg L^{-1}); conductivity (863 ± 63 Ω m^{-1}). According to the manufacturer's specification, fish were fed three times per day with extruded commercial feed (3 mm), containing 45% (minimum) crude protein.

Acute toxicity assay (96 h)

Fish were submitted to biometry (mean weight 3.5 ± 0.5 g; mean length 4.5 ± 0.5 cm), and then assigned to control or groups exposed to different concentrations of pyriproxyfen for 96 h. *O. niloticus* fingerlings were distributed into nine groups of 10 animals each according to the exposure concentration of pyriproxyfen (0.0 (control); 0.25, 0.5, 1, 2.5, 3, 4, 5 or 10 mg L^{-1}). Pyriproxyfen stock solutions were prepared (100 g L^{-1}) and diluted appropriately to reach each treatment's concentrations. Each assay was replicated three times, and fish remained unfed during the assay period.

The number of deaths in each treatment was recorded at 24 h intervals. If the death occurred, it was noted, and fish were immediately removed from the experimental tanks. After 96 h, the number of dead fish in each group was recorded, and the 96 h-LC_{50} values (with 95% confidence intervals; 95% CI) for pyriproxyfen were calculated based on cumulative mortality data using the GraphPad 5.0 prism (Graph Pad Software Inc., San Diego, CA, USA) and Probit model, according to Silva *et al.* (2015). The lack of

movement and no response to tactile stimuli were used to verify mortality. Non-observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were computed with an R-script from mix to 1.3.2 with a significance level of 0.01 (Zhu *et al*., 2016).

Subchronic toxicity tests (28 days)

Fish were submitted to biometry (mean weight 3.5 ± 0.5 g; mean length 4.5 ± 0.5 cm). *O. niloticus* fingerlings were exposed to pyriproxyfen concentrations 0.0 (control), 0.4, 0.8, 1.2 and 1.8 mg L^{-1} with three replicas each for 28 days (n = 6). The range of concentrations tested was selected to be below the 96 h-LC_{50} values obtained in the acute toxicity test described above (highest concentration is around 65% of the LC_{50}).

During the subchronic toxicity test, animals were fed three times a day (08:00, 12:00 and 16:00 h) *ad libitum*. The experimental tanks were maintained under constant aeration, siphoned daily with water, and pyriproxyfen replacement (20%). The water quality, temperature, dissolved oxygen, and pH indicators were monitored daily at 8:00 and 16:00 h, using a multiparameter probe (Hanna Instruments, model 9828, Woonsocket, USA). Total ammonia (mg L^{-1}) was weekly measured spectrophotometrically at 450 nm (spectrophotometer Hanna Instruments, model HI 83203, Belgium).

After experimental treatment, animals were anesthetized with 150 mg L^{-1} Tricaine MS222, producing unconsciousness to be sacrificed by cranial perfusion. Tissues were removed and stored for later lab analysis (Silva *et al.*, 2015; Chaves *et al*., 2017).

Liver extract preparation and catalase activity

Crude liver extracts from animals exposed to the subchronic toxicity tests were prepared using a liver from a pool of three animals for each tested concentration. Liver tissues were suspended in a 20 mM potassium phosphate buffer (pH 7.4, 0.1% Triton X-100 and 150 mM NaCl, 1:20 dilution) and further homogenized with a Potter-Elvejhem glass homogenizer. Catalase activity in the liver extract was assayed spectrophotometrically at 240 nm using hydrogen peroxide (H_2O_2) as a substrate (Moraes *et al*., 2007). One-Unit (U) of CAT activity was calculated in terms of μmol H_2O_2 consumed min^{-1}.

Evaluation of the fish biometric performance

The parameters examined included: 1) total length (cm) (TL); 2) daily weight gain (g) (DWG): mean final weight minus the initial mean weight divided by the number of days; 3) apparent feed conversion (AFC): the total amount of dry feed supplied to the fish divided by the total weight gain (mean final weight minus mean initial weight); 4) specific growth rate (SGR): SGR = 100 × (ln final weight / ln initial weight) / experiment time.

Locomotion behavior analysis

Control and treated fish were filmed after 28 days using a digital camera Finepix 4500 (Fujifilm, Brazil) positioned 30 cm from experimental tanks. The locomotor behavior was assessed as described by Tenorio *et al.* (2017). Briefly, the videos were recorded at 320×240 pixels and 30 frames per second (FPS) for 10 min. This video resolution was sufficient to track the fish. After, the videos were transformed into 4 FPS using Virtual Dub 1.10.4 software (Avery Lee); therefore, four images per sec were employed to analyze locomotor behavior, using a total of 2,400 points of coordinate X, Y, which described the locomotor behavior of each fish. The video tracking was performed using the software Image J 1.49v (National Institutes of Health-NIH, USA) with the plug-in MTrackJ (Meijering *et al*., 2012; Erasmus University Medical Center, Netherlands), obtaining the following parameters: 1) traveled distance (cm): quantify the length of fish trajectory in the tank, starting from the first coordinate point of the trajectory to its last point, 2) D2S (cm): average distance from the first coordinate point to the current point, 3) D2P (cm): mean distance from the current coordinate point to the previous point, 4) θ (degrees): angle of the in-plane components of the track points vector, 5) Δθ (degrees): angular change between the in-plane components of the track points vector, 6) mean speed (cm s^{-1}): traveled distance per time.

Histopathological analysis of the liver

The fish liver was removed, dissected, and weighed. Fragments of hepatic tissue with 3 mm thickness were cut and then immersed in formalin solution in phosphate buffer (10%) for 24 h. Subsequently, the fragments were immersed in 70% alcohol and stored. After fixation, liver samples were dehydrated by increasing ethanol concentrations to absolute ethanol, diaphanized in xylol, infiltrated, and embedded in paraffin. The obtained blocks were cut utilizing a microtome to obtain 4 μm cross-sections and stained with hematoxylin-eosin. The histological sections were photographed with an optical microscope (Diagtech, Brazil) attached to a camera (Motic 2300, Hong Kong) connected to a computer and analyzed using the biometric software Motic Images Plus 2.0 (Hong Kong). The microscopic descriptions of *O. niloticus* liver structure were performed as described by Vicentini *et al.* (2005).

Statistical analyses

The homogeneity of the animals was confirmed by the Cochran test ($P < 0.05$). The results obtained in this study were evaluated using the software Systat 13.0, SPSS package, utilizing analysis of variance (ANOVA), and Tukey's tests for parametric data in with a full random experimental design, as well as the Kruskal-Wallis and Dunn tests for non-parametric data. Results were expressed as mean ± standard deviation. A *P*-value < 0.05 was considered statistically significant.

RESULTS

The water quality parameters measured during the ten days acclimation period, acute toxicity assay (96 h), and subchronic toxicity tests (28 days) did not show significant changes (temperature 24.3 ± 0.1ºC; pH 7.1 ± 0.2; and dissolved oxygen 6.5 ± 0.2 mg L^{-1}).

Acute toxicity assay (96 h)

After 96 h exposure to pyriproxyfen, the fish mortality observations occurred between concentrations 2.5 to 10 mg L^{-1} (Fig. 1). A 100% mortality rate occurred after exposure above 4 mg L^{-1} pyriproxyfen. The 96 h-LC_{50} value of pyriproxyfen was 2.77 mg L^{-1}, the 95% confidence interval was 1.381 to 1.412 (Fig. 1). Data also demonstrated the influence of exposure time on mortality frequency. The longer the exposure time, the higher the rate of fish mortality. The no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) for *Oreochromis niloticus* fish exposed to pyriproxyfen were 1 and 2.5 mg $L^{-1,}$ respectively.

Catalase (CAT) activity

Compared to the control, the hepatic CAT showed significant change after exposure to each concentration of pesticide pyriproxyfen tested in the subchronic experiment (Fig. 2).

Subchronic toxicity tests (28 days)

Since exposure concentrations used in this experiment were below the LC_{50} for pyriproxyfen, no mortality was observed in the control group during the experimental period.

Evaluation of the fish biometric performance

Values of the zootechnical parameters: total length (TL), daily weight gain (DWG), specific growth rate (SGR), and apparent feed conversion (AFC), increased after exposure to pyriproxyfen above 1.2 mg L^{-1} (Table 1).

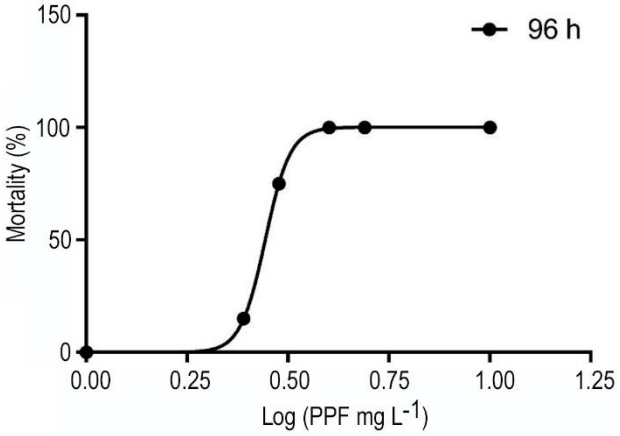

Figure 1. Percentage of mortality of *Oreochromis niloticus* fingerlings exposed to different concentrations of pyriproxyfen (PPF) for 96 hours.

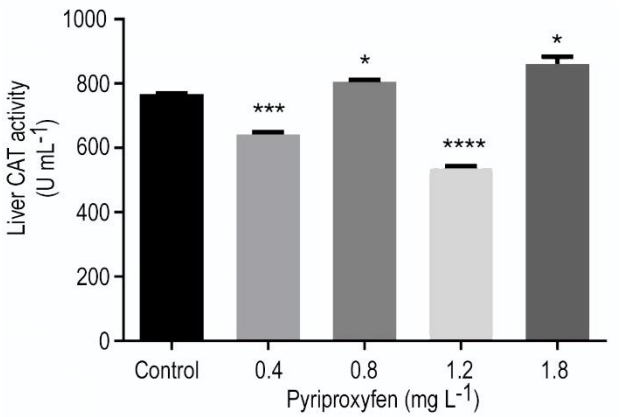

Figure 2. Catalase (CAT) activity in the liver of *Oreochromis niloticus* fingerlings exposed to different concentrations (mg L^{-1}) of pyriproxyfen (PPF) for 96 hours. *Indicates a statistically significant difference.

Locomotion behavior analysis

Fish exposed to pyriproxyfen above 1.2 mg L^{-1} for 28 days exhibited a reduction in the traveled distance inside the tank, mean speed, path parameter D2P, as well as the parameter angular variation of the trajectory (Table 2). Limitations in the video tracking of fish exposed to 1.8 mg L^{-1} pyriproxyfen did not allow an adequate quantitative analysis. Fish crowded, making individual locomotor behavior analysis unviable in these fish.

Histopathological analysis of the fish liver

The liver of control fish showed normal structure for the *O. niloticus* (Fig. 3). The hepatocytes displayed cordonal distribution forming plaques. The hepatocytes were rounded with normal size; nuclei were spherical and central with a prominent nucleolus. The cytoplasm was clear and rich in lipids removed by histological

Table 1. Biometric performance of *Oreochromis niloticus* fingerlings exposed to pyriproxyfen concentrations 0.4, 0.8, 1.2, and 1.8 mg L^{-1}. TL: total length, DWG: daily weight gain, AFC: apparent feed conversion, SGR: specific growth rate, after exposure to pyriproxyfen, mean ± standard deviation, CV: coefficient of variation. Different superscript letters in the same line indicate a statistically significant difference ($P < 0.05$).

Variables	Control	0.4 mg L^{-1}	0.8 mg L^{-1}	1.2 mg L^{-1}	1.8 mg L^{-1}	CV%
TL	7.27 ± 0.24^{a}	7.55 ± 0.44^{a}	7.33 ± 0.52^{a}	6.74 ± 0.21^{ab}	5.88 ± 0.49^{b}	5.2
DWG	6.80 ± 0.81^{a}	7.40 ± 1.30^{a}	6.84 ± 1.19^{a}	5.30 ± 1.25^{a}	3.52 ± 0.94^{b}	24.6
AFC	1.20 ± 0.32^{a}	1.36 ± 0.3^{a}	1.22 ± 0.29^{a}	1.47 ± 0.38^{b}	5.17 ± 0.42^{c}	17.1
SGR	1.26 ± 0.2^{a}	1.39 ± 0.19^{a}	1.26 ± 0.14^{a}	0.86 ± 0.1^{b}	0.24 ± 0.03^{b}	12.6

Table 2. Locomotor behavior of *Oreochromis niloticus* exposed to pyriproxyfen 0.8 and 1.2 mg L^{-1} for 28 days. Distance (cm), D2S (cm), D2P (cm), θ (degrees), Δθ (degrees), and mean speed (cm s^{-1}). Mean ± standard deviation. Different superscript letters in the same column indicate a statistically significant difference ($P < 0.05$).

	Distance	D2S	D2P	θ	Δθ	Speed
Control	4164.8 ± 991.0^{a}	11.5 ± 4.2	1.7 ± 0.4^{a}	1.0 ± 2.8	-1.0 ± 1.4^{a}	6.9 ± 1.6^{a}
0.8 mg L^{-1}	3369.0 ± 593.1^{ab}	15.5 ± 2.4	1.4 ± 0.2^{ab}	-0.8 ± 1.8	0.5 ± 1.1^{ab}	5.6 ± 0.9^{ab}
1.2 mg L^{-1}	2785.8 ± 721.5^{b}	15.9 ± 7.6	1.1 ± 0.3^{b}	1.4 ± 4.0	1.2 ± 0.7^{b}	4.6 ± 1.2^{b}

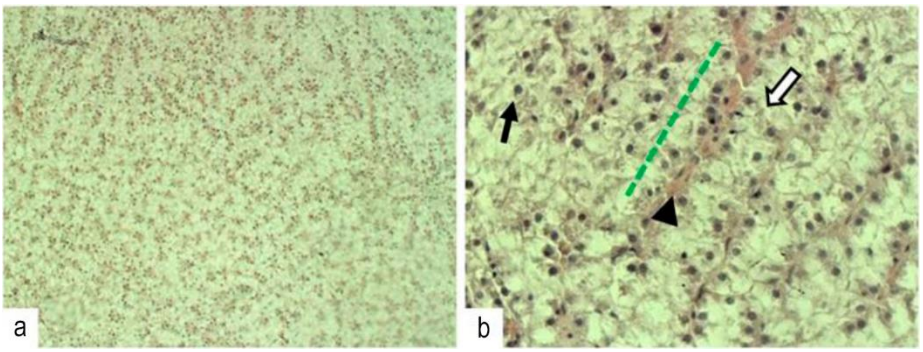

Figure 3. Photomicrographs of the liver in *Oreochromis niloticus* fingerlings (control group). a) Liver tissue without histopathological changes (100x), b) cordonal arrangement of hepatocytes (green trace), spherical and centralized nucleus (arrow); hepatic sinusoid capillary (arrowhead) and organization in a double strand of hepatocytes between the sinusoids (large white arrow) (400x).

processing, showing a light background (negative staining). The central vein appeared normal with small sinusoidal capillaries in direct contact with hepatocytes. These sinusoids were abundant, lined by normal endothelial cells, and filled with erythrocytes showing eosinophilic staining. The liver also contained exocrine pancreatic tissue, with an acinar organization, light brown granules, and diffuse localizations.

The fish *O. niloticus* exposed to 0.4 mg L^{-1} pyriproxyfen exhibited some morphological alterations in the liver (Figs. 4a-b), areas with loss of the hepatocytes' cordial organization, with nucleus displaced to the periphery, the cytoplasm was more stained and less vacuolated. The liver also showed small areas of inflammatory infiltrate and sinusoidal capillaries with mild congestion.

Exposure to 0.8 mg L^{-1} pyriproxyfen (Figs. 4c-d) induced loss of hepatocytes' cordonal organization in several areas, with a peripheral nucleus and greater vacuolization. The hepatic parenchyma contained increased multifocal congestion of the sinusoids and an increased amount of immune cells. Areas of inflammatory infiltration and fibrosis in hepatic tissue were also observed. Exposure to a concentration of 1.2 mg L^{-1} pyriproxyfen induced even more severe pathological lesions, wide areas of vacuolization in the hepatocytes, and greater congestion in the sinusoidal capillaries. Many hepatocytes also exhibited nucleus displaced to cellular periphery and loss of cordonal arrangement. Several dispersed immune cells were detected among hepatocytes and sinusoids, and several inflammatories infiltrate and areas of fibrosis (Figs. 4e-f).

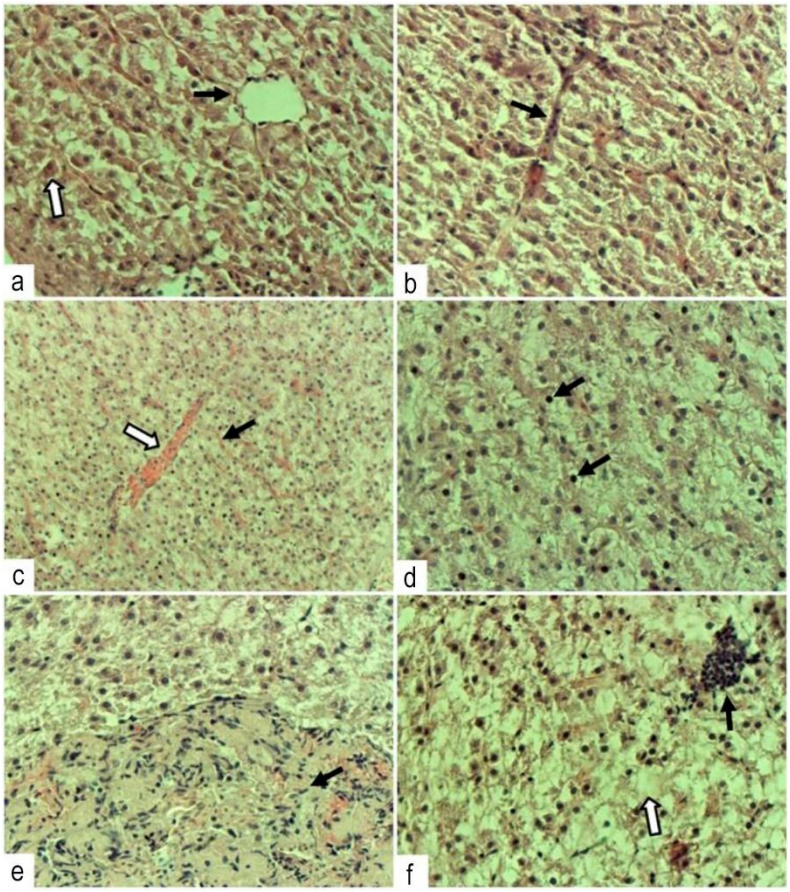

Figure 4. Photomicrographs of the liver in *Oreochromis niloticus* fingerlings exposed to pyriproxyfen concentrations: a-b) 0.4 mg L^{-1}, c-d) 0.8 mg L^{-1}, e-f) 1.2 mg L^{-1} for 28 days. a) Liver tissue showing the central vein (black arrow) and hepatocyte with nucleus displaced to the periphery (white arrow), 400x; b) note congestion of the blood vessel (black arrow), 400x; c) central vein in longitudinal section (white arrow) from which the sinusoidal capillaries proceed in radial pattern (arrow), 200x; d) liver tissue showing the greater presence of immune cells (black arrow), 400x; e) areas of fibrosis in the liver (black arrow), 400x; f) inflammatory infiltrate in the hepatic tissue (black arrow) and wide areas of vacuolization (white arrow), 400x.

DISCUSSION

Many chemicals are released continuously into the environment, including endocrine disruptors, heavy metals, oils, personal care products, pharmaceuticals, and pesticides. Pyriproxyfen is a pesticide widely used in tropical areas to control the proliferation of mosquitos larvae in aquatic habitats; besides, it is used as a pesticide in citrus, cotton, vegetable, and peanut crops; this pesticide can be carried by rain to aquatic environments, exposing non-target species like fish to this pesticide (Caixeta *et al.*, 2016; Legrand *et al*., 2017; Peterson *et al.*, 2017; Tenorio *et al*., 2017).

Studies report concerns about pyriproxyfen inducing adverse effects in aquatic ecosystems at concentrations required to control mosquitos' larvae (Caixeta *et al*., 2016). Toxicity tests for pyriproxyfen at different concentrations using *Daphnia magna* and *Artemia salina* showed high toxicity in both microcrustaceans; the lowest-observed-effect concentration of pyriproxyfen was 1.25 µg L^{-1} (Santos *et al*., 2017). Pyriproxyfen's toxicity was also investigated in estuarine copepods *Eurytemora affinis*, illustrating 48 and 96 h-LC_{50} of 73.24 and 29.70 µg L^{-1}, respectively (Legrand *et al.*, 2017).

Pyriproxyfen exhibits low solubility, high partition coefficients, and hydrophobicity; therefore, this pesticide is consistent with chemical characteristics that are environmentally persistent (Sullivan & Goh, 2008). When comparing the pyriproxyfen LC_{50} for larvivorous fish *Pseudomugil signifier* (0.84 mg L^{-1}) (Brown *et al*., 1998), bluegill sunfish *Lepomis macrochirus* (0.27 mg L^{-1}), and rainbow trout *Oncorhynchus mykiss* (0.33 mg L^{-1}) (WHO, 2006); in the present study, the *Oreochromis niloticus* fingerlings LC_{50} at 2.77 mg L^{-1} was higher indicating greater resistance to this pesticide. In

agreement with this observation, FAO (2010) noted that *O. niloticus* fish were known to be resistant to various adverse conditions such as poor water quality and disease.

Pyriproxyfen 1.2 mg L^{-1} altered the locomotor behavior of fish *O. niloticus*. These findings are novel as no apparent studies were found examining behavioral changes in *O. niloticus* after exposure to the pesticide pyriproxyfen. Also, *O. niloticus* seem to be more resistant to behavioral changes produced by this pesticide since *Xiphophorus maculatus* fish exposed to pyriproxyfen concentration close to the dosage used to control mosquito *A. aegypti* (10 μg L^{-1}) did not markedly affect the rate of mortality. However, this concentration may decrease the swimming performance, as evidenced by erratic swimming and loss of equilibrium (Caixeta *et al*., 2016). Truong *et al.* (2016) demonstrated adverse behavioral effects in embryonic zebrafish *Danio rerio* exposed to pyriproxyfen 6.4 μM. They raised concerns as locomotor behavior changes impair feeding ability, reproduction, and survival in aquatic environments (Tenorio *et al*., 2017). Behavioral responses were used to assess toxicological changes in aquatic animals (Peterson *et al*., 2017), including fish (Renick *et al*., 2016). Therefore, toxicity tests using behavioral changes may represent a reliable tool to detect toxicity attributed to contaminants in animals and aquatic organisms (García de la Parra *et al*., 2006; Tenorio *et al*., 2017).

Recent studies showed that pyriproxyfen did not induce developmental abnormalities in zebrafish embryo at concentrations equivalent to levels used in mosquitoes control practice; however, doses higher than 1.0 mg L^{-1} induced marked teratogenic effect on zebrafish embryos (Dzieciolowska *et al*., 2017). The concentration of pyriproxyfen, resulting in 50% of zebrafish presenting adverse morphological effects, was 5.2 μM (Truong *et al*., 2016).

Catalase is a key enzyme that plays an essential role in cell defense agains t oxidative stress. Several investigators noted changes in liver CAT activity in fish exposed to pesticides, and thus considered this enzyme a useful marker of chemical-mediated tissue oxidation (Moraes *et al*., 2007; Clasen *et al*., 2018). Catalase activity is higher in organs with high oxidative potential such as the liver, kidney, and erythrocytes (Glorieux *et al*., 2015). Several studies have shown changes in liver catalase of fish exposed to pesticides, and catalase has been considered a useful marker of liver changes due to damage by toxic substances (Moraes *et al*., 2007; Clasen *et al*., 2018).

Although no clear pattern was observed, the present study showed changes in CAT activity in the liver of fish *O. niloticus* exposed to commercial formulation of pyriproxyfen decreasing at 0.4 and 1.2 mg L^{-1} and increasing at 0.8 and 1.8 mg L^{-1}, suggesting a role for catalase in the defense mechanism to reduce oxidative stress that needs to be better understood. Other studies also showed variation in liver CAT activity of *O. niloticus* exposed to pesticides, such as methomyl (Meng *et al*., 2014). A decrease in CAT activity indicates a reduced capacity to scavenge hydrogen peroxide produced in the liver, and this inhibition caused by pesticide was reported in several fish species (Ural, 2013). In the present study, the variation found in catalase activity after exposure to pyriproxyfen showed a pattern similar to that observed previously in the catalase of fish *Catla catla* after exposure to the pesticide methyl parathion (Abhijith *et al*., 2016). Moreover, zebrafish embryo exposed to 1.66 μg mL^{-1} pyriproxyfen showed increased catalase activity (Maharajan *et al*., 2018).

The biphasic liver CAT response, together with the histopathological tissue changes, indicates oxidative stress in the liver of fish *O. niloticus* after exposure to pyriproxyfen at the lowest dose employed 0.4 mg L^{-1}. It is worth noting that in our study, changes were observed in fish at doses of pyriproxyfen close to that used directly in water reservoirs to control mosquitoes proliferation (10 μg L^{-1}), especially when one considers the persistence of the pesticide in the environment and the tendency to bioconcentrate (Ose *et al*., 2017) in the animal organism several-fold higher than that found in the water where these animals live (Caixeta *et al*., 2016; Tenorio *et al*., 2017).

The present study also demonstrated changes in the liver microstructure of *O. niloticus* exposed to pyriproxyfen, including an increase of inflammatory infiltrate, fibrosis, elevated amount of immune cells, vacuolization in the hepatocytes, and congestion in the sinusoidal capillaries. These hepatic histopathological alterations in the liver increased in a dose-dependent pattern. Few reports examined the effects of pyriproxyfen on the microstructure of fish organs. Maharajan *et al.* (2018) found that histopathological analysis of zebrafish following exposure to pyriproxyfen 1.66 μg mL^{-1} resulted in thinning of heart muscles, pericardial edema, and hyperemia. However, no apparent data on pyriproxyfen's influence on liver microstructure in fish *O. niloticus* was available. Corroborating, pyriproxyfen metabolites are widely distributed in the liver after exposure to this pesticide (Ose *et al*., 2017).

CONCLUSIONS

Exposure to the pesticide pyriproxyfen at concentrations above 1.2 mg L^{-1} impaired growth and behavior

of fish *Oreochromis niloticus* and damaged the liver. These parameters are important for the feeding and reproduction of these fish. Considering that these fish are raised in lakes and dams, aquatic contamination with pyriproxyfen may hinder its breeding for human consumption, thus reducing fish farming efficiency. It is noteworthy that minor damages were observed in fish liver at a concentration of 0.4 mg L^{-1}, representing potential harm to these fish, especially with prolonged exposures. However, *O. niloticus* exhibits greater resistance to pyriproxyfen than other fish previously reported, which may be useful for fish breeding in areas of mosquito infestations where this pesticide is widely applied directly into the water reservoirs. Pyriproxyfen may affect this fish's production and quality at higher concentrations than commonly used in water (0.01 mg L^{-1}).

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