Acute Toxicity of Chlorpyrifos (CPF) to Juvenile Nile Tilapia (*Oreochromis niloticus*): Genotoxicity and Histological Studies

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ABSTRACT

Chlorpyrifos (CPF) has recently become a controversial agricultural chemical in Thailand, with proposals to ban it due to public health concerns. Currently (2021), its use is still allowed as an insecticide in ricefields, but there is concern that CPF in the aquatic environment may impact non target species such as Nile tilapia (*Oreochromis niloticus*). This study was conducted to evaluate the standard 96-h LC_{50} for CPF in juvenile tilapia and to detect any DNA damage and tissue alterations caused by exposure to CPF. The 96-h LC_{50} was calculated using probit analysis and then used in an acute exposure test. The 96-h LC_{50} was 22.0±6.0 $\mu g \cdot L^{-1}$. The comet assay was conducted to detect any DNA damage, and histological techniques were used to observe tissue alterations. The mean percentage of tail DNA detected in the organs of treated fish showed significant difference ($p<0.05$) from the control fish, and differences were also found among tissues. Several alterations were observed in the organs of treated fish. Gill histology showed hyperplasia and fusion of secondary epithelial cells, damage of columnar tip cells, and detachment of secondary epithelium. Damage in the stratified squamous epithelium was detected in the oesophagus, and the intestines showed numerous goblet cells with expanded size and fusion of columnar epithelium and villi. The study indicates that CPF is an unsafe insecticide. It causes mortality, DNA damage and tissue alterations at low concentrations. Rice fields and freshwater aquaculture share the same water sources, and so CPF is likely to find its way from paddy fields to both subsistence and commercial aquaculture operations.

Keywords: Chlorpyrifos, Genotoxicity, Histology, Nile tilapia, *Oreochromis niloticus*, Toxicity

INTRODUCTION

Pesticides are widely used and disposed of, and subsequently cause pesticide contamination in the atmosphere, soils and water and the food chain. These are hugely threatening to both human health and the ecosystem (Elibariki and Maguta, 2017). The production of pesticides continuously rises and the adverse effects of these pesticides on aquatic organisms are increasingly reported (Altun *et al*., 2017).

Chlorpyrifos (CPF) is a well-known chlorinated organophosphate insecticide, widely used in agriculture and households to control insect pests, although its application is currently limited for use in residential areas due to its moderate toxicity to humans (Altun *et al*., 2017; Gómez-Canela *et al*., 2017). It is a non-systemic insecticide, being effective by ingestion, inhalation, and direct contact (Singh *et al*., 2017). Chlorpyrifos is known to inhibit acetylcholinesterase, which is important in neurotransmission by non-vertebrates by quickly

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hydrolysing the neurotransmitter acetylcholine to choline and acetates at cholinergic synapses (Deb and Das, 2013). Its inhibition results in an accumulation of the neurotransmitter acetylcholine in the synapse junctions. This leads to excessive stimulation of postsynaptic cells, inducing cholinergic toxicity (Mansour and Mossa, 2010). Generally, acetylcholinesterase inhibitors are less toxic to vertebrates compared to invertebrates, thus they are used as insecticides. Nevertheless, CPF exposure in non-target aquatic organisms, including vertebrates, produces a wide range of negative effects including histopathological alterations, oxidative stress, nephrotoxicity, genotoxic and mutagenic effects, as well as changes in swimming behaviour and embryo development (Kavitha and Rao, 2008; Ali *et al*., 2009; Altun *et al*., 2017; Gómez-Canela *et al*., 2017). Chlorpyrifos is biodegradable in the laboratory in a matter of hours by pseudomonads, but biodegradation is slower under field conditions; CPF can persist up to three weeks in the field in temperate climates (Sud *et al*., 2020). The chlorination of CPF slows its biodegradability.

Chlorpyrifos is extensively used by Thai farmers, but due to the health concerns, it has recently been the subject of protests, and the Ministry of Public Health and some NGOs have proposed a ban on its use (Bangkok Post, 2018). The use of CPF in rice farming is a matter of particular concern because water from the rice farms reaches irrigation canals and rivers, which are, in turn, used by the aquaculture industry. Currently, CPF and two chemically similar chemicals continue to be used in Thailand, but with tighter controls and limits on their usage (Nation Multimedia, 2018).

Nile tilapia (*Oreochromis niloticus*) is one of the main freshwater fish species used in commercial and subsistence aquaculture in Thailand (Jiraungkoorskul *et al*., 2002). Tilapia culture has grown extensively since the 1960s, and it is now the most important aquaculture fish species in the country (Bhujel, 2013). Nile tilapia has shown strong adaptability to most environmental conditions in tropical and subtropical regions and has come to be the most important fish commercially cultured in freshwater. Hence, the response of Nile tilapia to pesticides is of critical importance for toxicological research and aquatic toxicology in tropical and subtropical regions (Toledo-Ibarra *et al*., 2016).

In Thailand, research on the effects of pesticides on Nile tilapia is limited. Several studies have described the biochemical and histopathological effects of pesticides on Nile tilapia (Jiraungkoorskul *et al*., 2002; Jiraungkoorskul *et al*., 2003; Thanomsit *et al*., 2020). However, a study on its genotoxicity has not yet been attempted. In the present study we determined the acute toxicity (LC_{50}) of CPF to juvenile Nile tilapia using the standard 96-h test protocol (USEPA, 2002). We also assessed DNA damage in gill, liver and kidney, and examined histological alterations in gill, liver, oesophagus, and intestine of juvenile Nile tilapia exposed to an acute concentration of CPF.

MATERIALS AND METHODS

Chemicals

Chlorpyrifos (40% purity) was purchased from a market in Bangkok, Thailand, and imported by S&P Formulator Co., Ltd with chemical abstract name O, O-diethyl O-3,5,6,-trichloro-2-pyridyl phosphorothioate, and registration No. 2212-2556. We deliberately chose the commercial product for the project to make our findings environmentally relevant. A stock solution of CPF was prepared by dilution in acetone to make it dissolve more readily in water (<0.05% in pesticide solution) (Xing *et al*., 2012; Altun *et al*., 2017). Normal-melting agarose (NMA) and low-melting agarose (LMA) were obtained from Invitrogen and HydraGene Co., Ltd., respectively. SYBR Safe 10,000X, DMSO and Paraplast-Plus were from Sigma-Aldrich.

Experimental fish

Juvenile Nile tilapia were purchased from a local aquaculture farm in Krabi Province, Thailand. The fish were 6.13±0.79 cm in total length and 4.76±1.14 g in body weight. Fish were acclimated to laboratory conditions for a period of two weeks in glass fish tanks (60×30×40 cm) before starting the tests. The tanks contained 50 L of dechlorinated

tap water, with 30 fish per tank. Water quality was maintained throughout the experiment at pH of 7.0-7.6, conductivity of 200-250 μmhos·cm^{-1}, alkalinity of 62-65 mg·L^{-1}, total hardness of 50-70 mg·L^{-1}, and non-detected level of ammonia (<0.15 mg·L^{-1}). The sediment in each tank was removed daily by siphoning, then water was added (approximately 10 %) to maintain the water level. A diffusion air system supplied dissolved oxygen throughout the acclimatized period. The fish were fed at 2 % body weight once a day with standard aquaculture pelleted feed containing 35 % protein. Fish were carefully observed, and any signs of stress, physical damage, disease or mortality were recorded. Fish that showed unhealthy signs, e.g., loss of equilibrium, abnormal swimming or physical damage were removed (Bunthawin *et al*., 2011) (Thai Ethics Authorisation: U109406-2564, Project No 2564-14-015).

Acute toxicity

The acute toxicity test was performed according to the standard USEPA procedure for the static non-renewal technique (USEPA, 2002). The range-finding procedure consisting of down-scaled tests (chemical concentrations of 100, 10, 1, 0.1, 0.01 mg·L^{-1} and a control for a period of 96 h) was conducted to determine the appropriate test scale for the definitive test. The definitive test was conducted in five different concentrations of CPF (0.01, 0.02, 0.04, 0.06 and 0.08 mg·L^{-1}) and a control without CPF, for a period of 96 h, with three replicates per concentration. The pH was 7.0-7.2 and the dissolved oxygen was 4.5-6.0 mg·L^{-1} throughout the study.

In each replication, five fish of similar size were randomly selected and transferred into a glass chamber (35×20×23 cm) filled with 10 L of deionized water. Fish were not fed for 48 h before and for 96 h during the test (as per the standard USEPA [2002] protocol). Abnormalities and mortalities were carefully recorded. In addition, any dead fish were removed immediately.

Acute exposure test

Based on the result from the definitive test, the 96-h LC_{50} value of CPF was calculated by Probit analysis using IBM SPSS 20 software, and then the results were used to design the acute exposure test (Jiraungkoorskul *et al*., 2003). The test consisted of control and treatments, conducted for 96 h with three replications. Five fish were used at each concentration. At the end of the experiment, the fish were euthanised and dissected, and gills, liver, kidneys, oesophagus, and intestine were removed for genotoxic and histological studies.

Comet DNA assay

The comet assay was performed according to Lu *et al*. (2017) and Pandey *et al*. (2018) with slight adjustment. The gill, liver, and kidney tissues were homogenized in ice-cold physiological phosphate buffer saline (NaH_2PO_4/Na_2HPO_4 0.1 M - PBS, pH 7.4) and centrifuged at 3000 rpm at 4 °C for 5 min. The cell pellets were then resuspended in cold PBS for the comet assay.

The microscope slides were dipped in ethanol and burned to remove oil and dust. The slide was coated with 1% normal-melting agarose (NMA) as the base layer by dipping the slide in the agarose gel and laid on a tray with a flat surface to dry at room temperature. The mixture of the single cells solution (50 μL) with 0.5 % low-melting agarose (LMA) (450 μL) and SYBR Safe 100X (5 μL) was dropped onto the slide already coated with 1% NMA. After solidification, the slides were immersed in lysis buffer (2.5 M NaCl, 100 mM Na_2-EDTA, 10 mM Tris, 10% DMSO (dimethyl sulfoxide) and 1% Triton X-100 added fresh, pH 10) for 1 h at 4 °C. The slides were then immersed in the fresh cold electrophoretic buffer (300 mM NaOH, 1 mM Na_2-EDTA, pH 13) in the gel electrophoresis chamber for 30 min at 4 °C for DNA unwinding. Electrophoresis was carried out in the same buffer for 20 min at 4 °C using 0.8 V/cm and 300 mA. The slides were neutralized in cold distilled water for 5 min twice and then completely dried in an incubator. The slides were visualized under fluorescence microscope, and images were analysed using the Comet Assay Software Project protocol (CASP) (Konca *et al*., 2003). The %Tail DNA was selected as the parameter to quantify DNA damage.

Histological studies

Tissue processing was performed according to Humason (1979) and Suvarna *et al*. (2019). The gill, liver, oesophagus, and intestine tissues were fixed in Bouin's solution and dehydrated in 50% and 70% alcohol, through a series of alcohol (70%, 95%, and 100%), and lastly in isopropanol. The tissues were cleared in xylene and embedded in Paraplast-Plus. Sections of 3-5 µm were cut using a rotary microtome and stained with haematoxylin and eosin. Histological alterations were examined under a compound light microscope.

Statistical analysis

Zar (2014) was used as the standard statistical reference text. The 96-h LC_{50} value of CPF was calculated by Probit analysis in IBM SPSS 20. One-way ANOVA was performed followed by LSD (Least-Significant-Difference) (Zar 2014) to compare mean %Tail DNA among tissue types and between control and treatments (Zar 2014). A probability level of $p<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

96-h LC_{50} and fish behaviour observations

The 96-h LC_{50} of CPF for juvenile Nile tilapia was 22±6 µg·L^{-1} (Table 1). The mortality in the control treatment was zero. CPF was found to be highly toxic to the fish at a very low concentration. The 96-h LC_{50} of the present study is in agreement with Muttappa *et al*. (2015). In contrast, Gül (2005) reported the 96-h LC_{50} of chlorpyrifos-methyl for Nie tilapia larvae to be 1.57 mg·L^{-1}, and Taufik *et al*. (2020) reported a 96-h LC_{50} of 4 to 9 mg·L^{-1} for freshwater catfish. Their LC_{50} values are quite high compared to the present study. However, Gül (2005) also reported that although the chlorpyrifos-methyl form was toxic to juvenile and adult fish, it was less toxic to Nile tilapia larvae than to most of the adult fish (0.1-0.678 mg·L^{-1}). Chlorpyrifos (analytical grade, 99% pure) toxicity reported by Rao *et al*. (2003) in juvenile *O. mossambicus* (3.0 ±1.0 g) was found to be 0.0259 mg·L^{-1} (or 25.9 µg·L^{-1}), which is closer to that found in the present study. Oruç (2010) reported the 96-h LC_{50} of CPF in juvenile and adult Nile tilapia to be 98.67 µg·L^{-1} and 154.01 µg·L^{-1}, respectively, which indicates that CPF is highly toxic to fish at slightly higher concentrations than found in the present study. Most studies disagree with the high concentrations required for toxicity reported by Rao *et al*. (2003) and Muttappa *et al*. (2015). The variation in reported 96-h LC_{50} for CPF might be due to the quality of the pesticide used in the experiments (and whether carrier or non-carrier-free), test species and quality of the fish, and test conditions, particularly water quality (dissolved ions, dissolved organic matter and turbidity). Like many pesticides, chlorpyrifos binds strongly to clay and sediment in water, lowering its effective concentration to

Table 1. Lethal concentration values and 95 % confidence limits of chlorpyrifos in juvenile Nile tilapia (*Oreochromis niloticus*) at different exposure times.

Exposure time	Lethal concentration mg·L^{-1}			
	LC_{25} (±95 % C.L.)	LC_{50} (±95 % C.L.)	LC_{75} (±95 % C.L.)	LC_{100} (±95 % C.L.)
24 h	0.020 (0.012-0.021)	0.032 (0.024-0.044)	0.050 (0.038-0.087)	0.151 (0.087-0.627)
48 h	0.015 (0.009-0.021)	0.023 (0.017-0.032)	0.036 (0.027-0.047)	0.100 (0.060-0.369)
72 h	0.015 (0.010-0.019)	0.023 (0.017-0.028)	0.033 (0.027-0.047)	0.088 (0.059-0.207)
96 h	0.014 (0.009-0.019)	0.022 (0.016-0.028)	0.032 (0.026-0.046)	0.087 (0.057-0.219)

the test animals, and without a detergent carrier is very insoluble in water (~1.4 mg·L^{-1}, Tomlin, 2003). Activated carbon filtration (using carbonised wood) is an effective means to remove CPF from the water used by fish farms (Taufik *et al*., 2020), and it can reduce the toxicity by 91.9 %.

Observation of behavioural response of juvenile Nile tilapia was recorded at 1, 2, 4, and 8 h, and then subsequently every 24 h until the end of the study. The treatment fish showed noticeable abnormal behaviour immediately after exposure to the pesticide. They were less active and moved slowly and sluggishly. They showed erratic swimming and most of the time, they gathered at the water surface. Fish also showed rapid operculum movement with mouths open, fin rot, and their colour turned noticeably paler than the control fish. These abnormal behaviours were found in the first 8 h of exposure, but the erratic swimming was noticeable throughout the study. In contrast, no such abnormal behaviours were observed in the control groups. Fish behaviours observed during the acute toxicity test were in agreement with those recorded by Gül (2005), Deb and Das (2013), Singh *et al*. (2017) and El-Bouhy *et al*. (2018). Erratic swimming and loss of equilibrium after exposure to the pesticide were probably due to the inhibition of acetylcholinesterase (AChE) activity (Singh *et al*., 2017). Chlorpyrifos is well known as an AChE inhibitor, which is essential for normal behaviour and muscle function; the latter is mainly a cholinergic target in fish (Deb and Das, 2013).

Genotoxicity

The use of %Tail DNA data has been recommended for regulatory purposes and for inter-laboratory comparisons (Kumaravel and Jha, 2006). This parameter represents the amount of DNA that has migrated out of the nucleus compared to the total cellular DNA content, expressed as a percentage. It is directly proportional to the amount of damaged DNA (Kumaravel and Jha, 2006). Hence, if the %Tail DNA is high, it means the nuclear DNA is heavily damaged.

In the present study, a significant increase in %Tail DNA was detected in fish exposed to the 96-h LC_{50} dose of CPF (22±6 µg·L^{-1}) (Table 2) in kidney and liver samples, but not in gills. Additionally, %Tail DNA differed significantly ($p<0.05$) among organs of treated fish. The kidney showed the highest degree of DNA damage followed by gills and liver, respectively. The results are shown in Table 2. As far as we know, genotoxic effects of CPF on target organs (gills, oesophagus, intestine, liver and pancreas) of juvenile tilapia have not been previously determined. Therefore, a direct comparison of its toxicity cannot be made herein. The %Tail DNA from various organs in the present study is quite low compared to the study of Ali *et al*. (2009), who detected DNA damage in the gills of the freshwater fish *Channa punctatus* exposed to CPF at higher concentrations than our study. El-Bouhy *et al*. (2018) found DNA damage in the gills of juvenile Nile tilapia exposed to CPF to be 22.13±0.35 %,

Table 2. Percentage of tail DNA detected in different organs of juvenile Nile tilapia (*Oreochromis niloticus*) exposed to acute concentration of chlorpyrifos.

Organ		Percent Tail DNA (Mean±SD)
Gill	Control	$5.606±4.556^{ax}$
	Treated	$5.127±2.289^{ax}$
Liver	Control	$0.000±0.00^{ay}$
	Treated	$0.006±0.008^{by}$
Kidney	Control	$4.221±0.978^{ax}$
	Treated	$15.027±3.148^{bz}$

Note: Values with different letters are significantly different at $p<0.05$ (a, b indicate differences between the control and treated groups; x, y, z show differences between organs).

which is higher than that found in the present study. The results of the present study are comparable to a study by Zeid and Khalil (2014), who detected DNA damage in the gills of fingerling (juvenile) Nile tilapia exposed to fenitrothion (another type of organophosphate insecticide).

The low proportion of damaged DNA found in the present study might be due to several factors including high tolerance of the fish, antioxidant factors in the diet of the fish, and a possible detoxification mechanism. Antioxidant enzymes probably play an important role in the neutralization of reactive oxygen species (ROS) and detoxification of toxicants to protect cells from severe damage. Xing *et al*. (2012) state that several enzyme systems cause reactions to neutralize free radicals and ROS including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase, and catalases (CAT). They provide chemical protection against harmful effects of reactions that cause excessive oxidation, DNA damage, and cell death in biological systems (Ansari *et al*., 2014). Their activation controls the oxidative damage caused by pesticides, including CPF (Zahran *et al*., 2018). The activity of antioxidant enzymes may be reduced or induced depending on several factors including duration of exposure, xenobiotic type, and tolerance of the species (Zahran *et al*., 2018). Research has generally emphasized two factors determining the impact of potential carcinogens: (i) the relative level of uptake and metabolic activation of carcinogens following the formation of DNA adducts, and (ii) the mechanisms of detoxification. DNA repair is a third possibility, but that has not been extensively reported in fish (Espina and Weis, 1995). Decrease of DNA damage in fish tissues after exposure to different concentrations of CPF has been recorded, although the decrease was non-linear (Ali *et al*., 2009). The fndings may indicate the repair of damaged DNA, loss of severely damaged cells, or both.

High tolerance of Nile tilapia might effectively induce the activity of antioxidant enzymes against free radicals. According to Bhujel (2013), Nile tilapia has become the cultured fish of choice for many subsistence farmers as it grows well under adverse environmental conditions. Based on the behavioural observations during the toxicity test, fish demonstrated a high level of tolerance to adverse environmental conditions, which might be a key to the low level of DNA damage recorded in our study.

Histological observations

The histological study of juvenile Nile tilapia exposed to 96-h LC_{50} of CPF (22±6 $\mu g \cdot L^{-1}$) showed several alterations as described below.

Gills

In fish, the gill is the essential organ in the transport of respiratory gases, regulation of osmotic and ionic balance, and tolerance to ammonia. Toxic chemicals might induce damage to gill tissue, reducing the gas exchange and disrupting their osmoregulatory function in fish (Peebua *et al*., 2008). In the present study, control fish showed normal structures of healthy gills (Figure 1a). Primary lamellae had numerous secondary lamellae. Normal epithelial cells and pillar cells of lamellae were observed. In the acutely exposed fish, after 96 h of exposure, the gills showed detachment of epithelial cells and hyperplasia and fusion of lamellar epithelium (Figure 1b). Damage to the lamellar tips were also observed in the gills (Figure 1b). The observed hyperplasia and fusion of secondary lamellar epithelium are in agreement with Zhang *et al*. (2019), who studied histopathological changes in the gill of zebrafish exposed to chronic toxicity to dichloroactamide, and with Zahran *et al*. (2018), who reported histological alterations in Nile tilapia exposed to CPF. Stalin *et al*. (2019) also reported fusion in gill lamellae of *Channa punctatus* that had been exposed to sublethal levels of CPF. Alterations included hyperplasia and detachment of epithelium in the secondary lamellae, which increases the distance between blood and water, thereby keeping toxic agents away from the circulatory system, but this decreases gas exchange efficiency (Campos-Garcia *et al*., 2016).

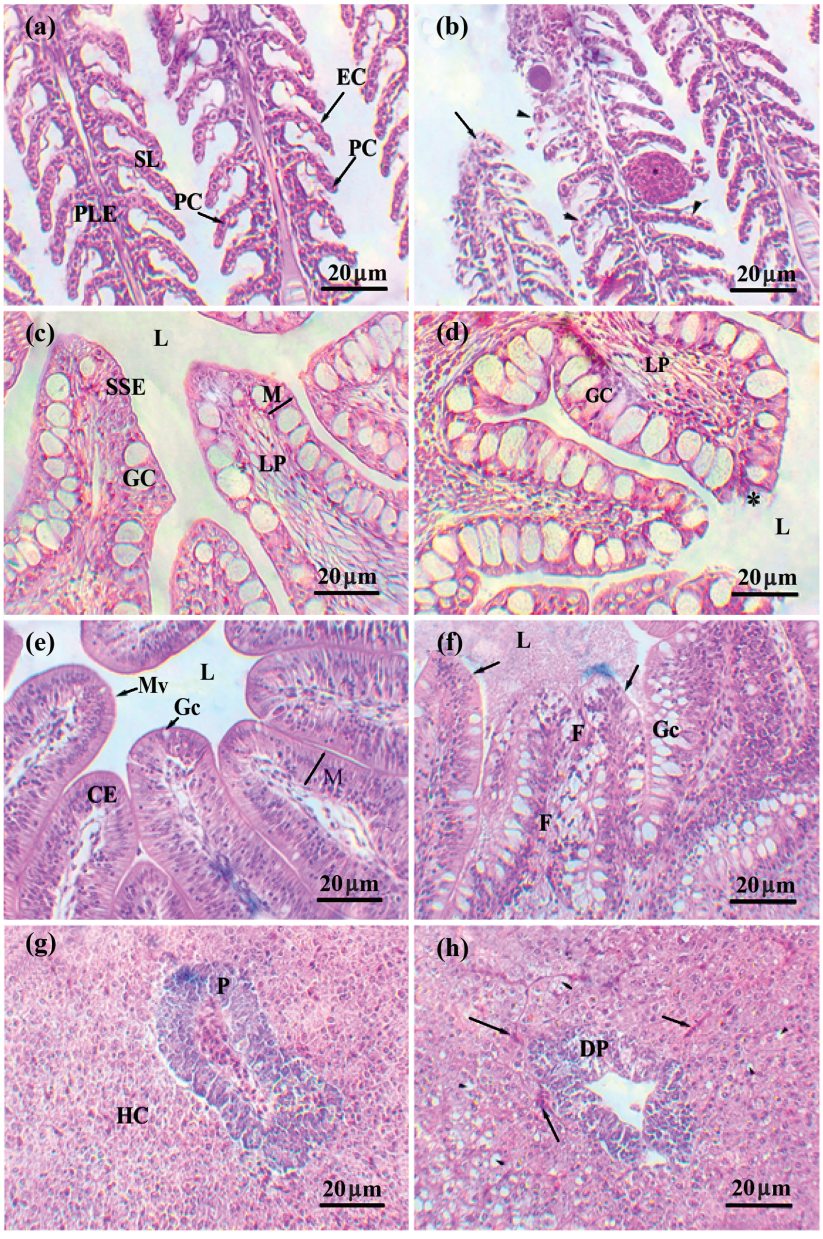

Figure 1. Gill, oesophagus, intestine and liver tissues of control and chlorpyrifos-treated Nile tilapia *Oreochromis niloticus*: (a) control gill showing normal primary lamellar epithelium (PLE), secondary lamellae (SL), epithelial cells (EC), and pillar cells (PC); (b) gill of treated fish showing damage to the columnar tip (arrow), detachment of secondary epithelium (arrowhead), and hyperplasia and fusion of lamellar epithelium (asterisk); (c and e) oesophagus and intestine of control fish showing normal mucosa (M), goblet cells (GC), stratified squamous epithelium (SSE), lamina propria (LP), microvilli (Mv) and lumen (L); (d) oesophagus of treated fish showing damage to stratified squamous tissue (asterisk); (f) intestine of treated fish showing fusion of columnar epithelium and villi (F), numerous goblet cells with larger sizes (GC) and loss of microvilli (arrow); (g) liver of control fish showing normal hepatocytes (HC) and pancreas (P); (h) liver of treated fish showing blood cell infiltration (arrow), vacuolizations (asterisk), and destruction of the pancreas (DP).

Oesophagus and intestine

Toxicants get into the digestive tract of a fish through water and food consumption, leading to a decline of the physical structure and function of the intestine (Younis *et al*., 2015). Although the fish were not fed during the toxicity tests, CPF may have still entered their digestive tract through water consumption and unpreventable feeding on debris in the aquaria. In the control fish, the oesophagus and intestine showed normal structure of the mucosa and lamina propria (Figures 1c and 1e). Stratified squamous epithelial tissues of the oesophagus and the columnar epithelium of the intestine were the major components of the mucosal layers, which were interspersed with numerous goblet cells. Lamina propria layers contained connective tissue, and skeletal muscle was found in the muscularis layers. The outer layers called serosa were covered by loose connective tissue. After 96 h of treatment with CPF, the oesophagus showed slight damage to stratified squamous tissue (Figure 1d). In the intestines, numerous larger goblet cells were observed (Figure 1f). Moreover, the intestine showed fusion of columnar epithelium and villi. The fusion of villi and epithelial cells was in agreement with Stalin *et al*. (2019), who also found alterations in the intestine of *C. punctatus* treated with CPF. Elsayed *et al*. (2015) and Bhattacharjee and Das (2015) reported numerous goblet cells present in the intestines of fish after treatment with cadmium chloride and lindane (an organochlorine insecticide), respectively.

Liver and pancreas

In the control fish, the liver showed polyhedral hepatocyte cells with central nuclei. Sinusoids were lined and surrounded by hepatocyte cells. The pancreas was histologically normal (Figure 1g). After 96 h of acute exposure, there was vacuolization in hepatocytes and pancreas. The destruction of pancreas cells was also observed (Figure 1h). Vacuolization in the hepatocyte cells was similarly reported by Muttappa *et al*. (2015), who studied *O. mossambicus* treated with sublethal CPF, and Khatun *et al*. (2016), who studied a catfish (*Heteropneustes fossilis*) treated with sublethal CPF. Similar effects were also reported in the study conducted by Stalin *et al*. (2019) in *C. punctatus* treated with sublethal CPF. The destruction of pancreas cells found in the present study is in agreement with Zahran *et al*. (2018). In fish, the liver is an organ that plays a very important role in the biotransformation of organic xenobiotic substances and probably in the excretion of harmful trace metals, storage and digestion of food, and sex hormone metabolism (Peebua *et al*., 2008). Histological observations in the liver of fish are highly sensitive and accurate in evaluating the effects of toxic and sub-toxic external substances in experimental and field studies (Hadi and Alwan, 2012).

CONCLUSION

Chlorpyrifos was found to be highly toxic to juvenile Nile tilapia. This agricultural chemical was found to cause DNA damage and various tissue alterations, which indicates that CPF has adverse effects on fish health and might cause fish mortality and inhibited growth.

Pesticide use is intermittent rather than continuous. This raises the important follow-up question of whether the damaging effects of exposure is permanent or temporary. For example, we do not know if the histological effects documented in the present study are reversible in surviving fish, but the lack of DNA effects suggests that the toxic effects of CPF might not be permanent. Longer-term effects of an episodic exposure to CPF (or indeed most other pesticides) are poorly documented (Deb and Das, 2013). The use of CPF in rice farming is of particular concern because of the links between rice farming, irrigation water and both subsistence and commercial aquaculture. Hence, aquaculturists would be particularly interested not only in the immediate toxicity of CPF but also whether or not fish recover from episodic or periodic exposure(s) to the insecticide. It is degradable, but this can take up to three weeks, at least in temperate climates (Sud *et al*., 2020). Importantly, it is removable from fish farm water using activated carbon filtration (Taufik *et al*., 2020). Where CPF is banned, it will most likely be replaced by another acetylcholinesterase-class insecticide, and so the techniques, results and

findings in the present study will remain relevant for assessing the environmental effects of other pesticides. This study focused on the 96-h standard protocol test. Follow-up studies on more long-term effects of the tissue damage reported in this study are warranted.

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