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Hellenic Plant Protection Journal **16:** 67-78, 2023
DOI 10.2478/hppj-2023-0009

Behavioral and histopathological changes of *Clarias gariepinus* as a predatory fish against *Culex pipiens* larvae following exposure to sublethal concentration of quinclorac and bensulfuron-methyl based herbicide

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Summary *Clarias gariepinus* is one of the widespread culturable freshwater fish species in Africa, which is prevalent in various natural and human-made aquatic habitats including rice-fish system. This fish species displays predation potential on the aquatic stages of mosquitoes. Bensulfuron-methyl and quinclorac are herbicide active substances that have been extensively applied in rice culture in Egypt and other countries worldwide. This study assessed the adverse effects of sublethal concentration of a commercial herbicide formulation containing quinclorac and bensulfuron-methyl on the predation potential of *C. gariepinus* female and male predatory fish on *Culex pipiens* mosquito larvae. Also, stomach and intestine histopathology of the treated fish was investigated. The exposure of *C. gariepinus* to sublethal concentration of quinclorac and bensulfuron-methyl based herbicide produced detrimental effects on prey consumption and histopathological changes in the stomach and intestine of the fish. The mosquito consumption by the treated female and male fish decreased significantly compared to the untreated fish of both sexes. The histological changes in the intestines were hyperplasia of the intestinal epithelium and goblet cells; edema of lamina propria and broad intestinal villi, and distortion in intestinal villi in comparison to control. The stomach histopathology changes were necrosis and sloughing of mucosal epithelium with severe damage of sub-mucosa. Thus, the tested herbicide at sublethal concentration on *C. gariepinus* decreased the prey consumption on mosquito larvae and caused histopathological alterations in the fish that may impair its digestive physiology. These findings suggest a threat of the tested herbicide to *C. gariepinus* survival and potential as a native successful biocontrol agent against *Cx. pipiens* larvae.

Additional keywords: *Clarias gariepinus*, *Culex pipiens*, herbicide, histopathology, predatory fish, prey

Introduction

Several fish species have been used successfully as effective, low-cost, and eco-friendly biocontrol approaches against aquatic stages of mosquitoes (Chandra *et al.,* 2008; Bhattacharjee *et al.,* 2009). Since 1900, *Gambusia* spp. and *Poecilia* spp. are native larvivorous fish in American regions and have been widely used as exotic predators against mosquitoes in different aquatic habitats all over the world (Pyke *et al.,* 2008). However, negative impacts of *Gambusia* spp. on several aquatic beneficial invertebrates, amphibians, and other native fish species have been recorded in many countries (Pyke *et al.,* 2008). This has led researchers to look for different indigenous predatory fish species in local aquatic environments in Africa, Asia, and South America (Ghosh and Dash, 2007; Chandra *et al.,* 2008). Therefore, about 315 different fish species, from many different countries, have been recorded as being effective and potentially suitable indigenous predatory fish for mosquito control (Ghosh and Dash, 2007). For example, many edible fish such as *Clarias gariepinus* (Burchell), *Ore-*

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ochromis mossambica (Peters), *Oreochromis spilurus* (Gunther), *Oreochromis niloticus* L., *Tilapia zillii* (Gervais), and *Ctenopharyngdon idella* (Valenciennes) are farmed in natural habitats or human-made aquatic culture for human consumption and they also provide effective control of mosquito larvae and pupae of *Anopheles*, *Aedes*, and *Culex* species (Chandra *et al.,* 2008; Chala *et al.,* 2016; Abebe *et al.,* 2018; Das *et al.,* 2018; Mohamed *et al.,* 2021).

Clarias gariepinus is a native African freshwater fish, which is prevalent in various natural and human-made aquatic habitats or even in sewage systems in urban regions worldwide, especially in African and Asian countries (Ponzoni and Nguyen, 2008). It is an omnivore fish that regularly feeds on a large variety of aquatic invertebrates, aquatic insect larvae and pupae, small fishes, algae, and aquatic plants (Ghosh *et al.,* 2008; Ponzoni and Nguyen, 2008). It is a hardy fish and tolerant to difficult environmental conditions (e.g., polluted water) (Ponzoni and Nguyen, 2008). The immature stages of mosquitoes may occur in aquatic habitats of *C. gariepinus* (Gashaw *et al.,* 2008). All these reasons make *C. gariepinus* a suitable candidate biocontrol agent against aquatic animal pests, mainly mosquitoes. In Ethiopia, *C. gariepinus* has the potential to control the larvae of *Culex* spp. and *Anopheles arabiensis* Patton (Chala *et al.,* 2016) and the aquatic snail *Biomphalaria pfeifferi* Krauss (Gashaw *et al.,* 2008).

Egypt is the largest producer of various edible fish mainly *O. niloticus*, *C. gariepinus*, and *Mugil cephalus* L. in Africa (Soliman and Yacout, 2016). In Egypt, rice-fish farming produced about 51.31% of *O. niloticus* and 29.93% of *C. gariepinus* from the total production of rice-fish system in 2012 (Soliman and Yacout, 2016). However, according to the best of our knowledge, limited studies have been published assessing the predatory efficiency of *C. gariepinus* against aquatic stages of mosquitoes, and no studies on the mosquito *Culex pipiens* L. *Culex pipiens* is a main efficient vector of lymphatic filariasis worms and several serious arboviruses to humans and is the most widely distributed mosquito in temperate regions worldwide, including Egypt (Mohamed *et al.,* 2021).

Predation of larvivorous fishes may be affected by different biotic and abiotic factors, which are related to the fish (predator), mosquito (prey), and its aquatic habitats (Yildirim and Karacuha, 2007; Chala *et al.,* 2016; Mohamed *et al.,* 2021). Chala *et al.* (2016) indicated that the predation efficacy of *C. gariepinus* against mosquito larvae was significantly affected by the mosquito genera, larvae number exposed, hours of feeding, and the size of predatory fish. Interestingly, predation of larvivorous fishes is also negatively impacted by the presence of toxicant chemical residues, mainly pesticides (insecticides and herbicides) in their aquatic habitats (Kerby *et al.,* 2012). Sublethal concentrations of different chemical pesticides induced larger adverse effects on the behavior of predatory fish than on their prey (Kerby *et al.,* 2012). For example, the insecticide diazinon at sub-lethal concentrations significantly reduced the activity and attack rates of *Gambusia affinis* (Baird and Girard) on *Pseudacris regilla* (Baird and Girard) tadpole prey which likely reflects the negative effects of the pesticide on the predatory behavior of *G. affinis* (Kerby *et al.,* 2012). Also, several herbicides produced adverse effects on different freshwater fish such as changes in mobility, feeding, orientation behavior, enzymes, biochemical and hematological modifications, histopathological disturbances, oxidative stress, genotoxicity, endocrine toxicity, and neurotoxicity (Ullah *et al.,* 2014; Stanley and Preetha, 2016; Fathy *et al.,* 2019; Saleh *et al.,* 2022).

Bensulfuron-methyl (sulfonylurea) and quinclorac (quinoline) are among the most commonly and widely applied selective herbicides in rice, various agricultural crops, and turfgrass lands worldwide. The residues of both herbicides are persistent in different aquatic systems that may result in adverse effects on aquatic biota, mainly fish (Okamoto *et al.,* 1998). Bensulfuron-methyl has been detected in water of rice fields at a range of <0.01–139.97 µg/L (Parveen *et al.*, 2005),

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while quinclorac was present at 1.34–6.97 µg/L (Resgalla *et al.,* 2007). Recently, both herbicides are mixed in a post-emergence herbicide (trade name Repare®), which has also been extensively applied in rice culture in Egypt and other countries worldwide (APC 2021). Exposure of *O. niloticus* to a sublethal dose of bensulfuron-methyl and quinclorac individually altered the behavior of fish, induced oxidative stress, and damaged the liver and spinal cord (Fathy *et al.,* 2019; Saleh *et al.,* 2022). The 96-h exposure of silver catfish, *Rhamdia quelen* to LC_{50} of quinclorac (395 mg/L) elicited adverse behavioral changes, oxidative stress, biochemical alternations, and induced histological lesions in the liver, kidney, and gill on the fish (Miron *et al.,* 2005; Persch *et al.,* 2017; Persch *et al.,* 2018). Histopathological examination variables are frequently used as potential biomarkers in the pesticide toxicological studies to provide information about organ damage and the physiological, functional, and health status of fish after exposure to these toxic agents (Vander Oost *et al.,* 2003; Fathy *et al.,* 2019; Saleh *et al.,* 2022). *C. gariepinus* is continuously exposed to a great range of pesticide residues (e.g. herbicides), in its aquatic habitats, especially present in or surrounding agricultural regions like rice-fish culture (Chala *et al.,* 2016).

This study was designed in order to evaluate the effects of sublethal concentration of a quinclorac and bensulfuron-methyl based commercial herbicide on the predation potential of *C. gariepinus* against *Cx. pipiens* larvae under laboratory conditions and to assess the histopathological changes in the stomach and intestine of the fish.

Materials and Methods

Collection and maintenance of the fish *Clarias gariepinus*

Females and males of *C. gariepinus* fish were collected from a private local fish farm in Assiut Governorate, Egypt, and transferred to the Environmental Toxicology laboratory in the Plant Protection Department, Faculty of Agriculture, Assiut University. The female and male fish were stocked separately in 70 L boxes and acclimated for 4 weeks under laboratory conditions at 25 ± 1°C and 12:12 h (L:D) photoperiod.

Culex pipiens larvae

Field populations of *Cx. pipiens* larvae were collected from the sewage treatment plant in the Arab El-Madabegh region, Assiut city, Egypt. The samples were transfered to the laboratory and kept at 25 ± 1°C. The collected *Cx. pipiens* larvae were fed on a diet containing fine ground dry bread and yeast and then late third to early fourth instar larvae were used in this study.

Determination of sublethal concentration of herbicide on *C. gariepinus*

The tested commercial mixture herbicide formulation was Repare® 18% TB (16.5% quinclorac and 1.5% bensulfuron-methyl; Starchem Chemical Manufacturing, Egypt). A preliminary experiment was conducted to define the sub-lethal concentration of the herbicide on *C. gariepinus* fish. In this experiment *C. gariepinus* fish (72 fish, 28.5–30.0 cm and 167–180 g) were divided to six experimental groups (12 fish/group), the non-exposed fish (control) group and five experimental groups exposed to different concentrations of the tested quinclorac and bensulfuron-methyl herbicide. Each group of *C. gariepinus* fish was kept in three separate 70 L boxes (4 fish/box). In the non-exposed control group, the fish remained in water, while in the herbicide treatment groups, the fish were exposed to 390, 780, 1560, 3120 and 6240 mg of Repare® 18% TB/L water for 96 hours. Fish were maintained in the laboratory at the same conditions as described above. Mortality was recorded every 24 hours over 96 h and dead fish were removed from boxes immediately. Finney's probit analysis (Finney, 1971) was used to estimate lethal concentration (LC_{50} and LC_{90}) values of *C. gariepinus* after 96 h exposure using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA, 2).

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Predatory potential of *C. gariepinus* fish on *Culex pipiens* larvae after exposure to sublethal concentration of herbicide

After the acclimatization, eighteen females (30.0–30.76 cm and 180.00 ± 2.77 g) and eighteen males (29.50–31.00 cm and 162.5 ± 2.51 g) of *C. gariepinus* were selected for the experiments. The aforementioned female and male fish were divided into two groups (9 fish/sex/group) as follows: the first group was the non-exposed fish (control) and the second group was exposed to the determined sub-lethal concentration of quinclorac and bensulfuron-methyl based mixture herbicide in water (390 mg of Repare® 18% TB/L) for 15 days and placed in 70 L boxes. The water in boxes was changed every 48 h and the herbicide was again added to maintain the concentration constant as described by Doherty *et al.* (2016) and Hamed and Osman (2017). Fish were fed once a day with commercial dry food pellets (25% crude protein, Al-Salam Company, Egypt). On the day 14 of exposure, all *C. gariepinus* fish in all groups were left over without food for 24 h to starve. After the exposure period and before the predation experiment, the treated fish of each sex were transferred carefully to separate boxes containing clean water to wash the fish off herbicide before starting the predation experiments. Each fish was placed in 8 L water with 600 mosquito larvae for 12h, from 7:30 a.m. to 7:30 p.m. (day time predation experiment) and then the same fish was transferred to new box with fresh 8 L water and new batch of 600 larvae from 7:30 p.m. to 7:30 a.m. (night time predation experiment). This procedure was repeated every 12 hours for a total period of 5 days (120 hours).

The total number of *Cx. pipiens* larvae consumed by both pre-treated and untreated female and male fish groups was recorded every 12 h over 120 hours. The means of *Cx. pipiens* larvae consumption by pre-treated and untreated female and male *C. gariepinus* fish groups were estimated and expressed as mean ± SE. The t-test for independent samples was performed to compare the mean number of consumed larvae between treated and untreated group at a= 0.05 signifficance level.

Histopathological studies

Three *C. gariepinus* fish from each of the pre-treated and untreated (control) groups were randomly selected after 15 days of exposure. Tissue samples of the stomach and intestine were anatomically dissected from each fish in both groups, rapidly removed, washed with neutral saline, and fixed in neutral buffered formalin 10%. Post-fixed tissue specimens of each group were dehydrated in a graded alcohol series, cleared with methyl benzoate, and then embedded in a paraffin wax (Bancroft and Stevens, 1982). Thin sections were cut at 5 µm thickness and stained with the hematoxylin–eosin stain (HE) technique (Bancroft *et al.,* 1996). All sections in each group were histopathologically examined and photographed using an Olympus CH30 microscope.

Results

Sublethal concentration of tested herbicide on *C. gariepinus*

All fish exposed to 3120 and 6240 mg Repare® 18%/L water died within 24 h, but for those exposed to 390 and 780 mg of the herbicide there was no mortality after 24 h and even after 96 h of treatment, respectively. The concentration of 1560 mg/L produced 41.67% mortality after 24 h and 96 h of treatment. The LC_{50} of the herbicide against *C. gariepinus* at 96 h was 1607 mg/L (Table 1). The selected sublethal concentration of Repare® herbicide in our study was 390 mg/L, which is almost the 1/4 LC_{50} value at 96 h and approximately 1/27 from the recommended application rate (10416.67 mg Repare® 18%/L) of the herbicide for rice weeds in Egypt (APC, 2021).

Predatory potential of *C. gariepinus* fish on *Culex pipiens* larvae after exposure to sublethal concentration of herbicide

The data regarding the predatory efficacy of the treated female and male of *C.*

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gariepinus fish after exposure to sublethal concentration of quinclorac and bensulfuron-methyl based herbicide (390 mg Repare® 18%/L) and the control against third to fourth -instar larvae of *Cx. pipiens* under laboratory conditions are showed in Table 2. The sublethal concentration of the tested herbicide affected negatively the feeding potential of the female and male of *C. gariepinus*. The daily consumption (sum of day and night time records) of pre-treated and untreated females of *C. gariepinus* on *Cx. pipiens* larvae was 669.17 ± 3.72 and 1018.17 ± 10.27 larvae, respectively, i.e., significantly reduced by 34.28%. Also, the daily consumption of pre-treated and untreated males of *C. gariepinus* on *Cx. pipiens* larvae was 471.25 ± 10.78 and 595.92 ± 24.92 larvae, respectively, i.e., significantly reduced by 20.92%. The daily larval consumption by the pre-treated and untreated *C. gariepinus* was 570.21 ± 2.68 and 794.65 ± 5.93, respectively, regardless the gender (Table 2), hence, the sublethal concentration of the herbicide reduced the consumption potential of *C. gariepinus* on *Cx. pipiens* larvae by 28.23%.

Larval consumption by the pre-treated and untreated female of *C. gariepinus* was higher than in the pre-treated male by 1.71 and the untreated male fish by 1.42 times. The larval consumption by the pre-treated and untreated male fish was also higher during the nighttime by 1.91 and 1.71 times than during the daytime, respectively.

Histopathological studies

In the present study, no visible changes or no pathologic changes were observed in

Table 1. Lethal concentrations (LC_{50} and LC_{90}) along with 95% confidence limits of quinclorac and bensulfuron-methyl based herbicide (Repare® 18%) on *Clarias gariepinus* after 96 h exposure.

Value	Concentration (mg/L)	95% confidence limits		Slope±S.E.
		Lower	Upper	
LC50	1607.0	1542.72	1794.84	0.004 ± 0.001
LC90	1941.0	1770.05	2957.69	0.004 ± 0.001

Table 2. Predation of untreated and treated females and males of *Clarias gariepinus* after exposure with sublethal concentration of quinclorac and bensulfuron-methyl based herbicide (390 mg Repare® 18%/L water) for 15 days against third-fourth instar larvae of *Culex pipiens*.

Fish sex	Treatment	Mean Fish size (±SE) (cm)	Mean Fish weight (±SE) (g)	Mean (±SE) number of consumed *Cx. pipiens* larvae per fish		
				Day time†	Night time†	Daily‡
Female	Untreated	30.00 ± 1.38	180.00 ± 2.77	461.50 ± 3.04	556.67 ± 10.97	1018.17 ± 10.27
	Treated	30.76 ± 0.33	180.00 ±2.77	309.67 ± 7.20	359.50 ± 5.79	669.17 ± 3.72
P-value				0.001*	0.0001*	0.0001*
Male	Untreated	29.50 ± 1.50	162.50 ± 2.51	196.38 ± 5.89	374.75 ± 1.25	571.13 ± 4.64
	Treated	31.00 ± 1.00	162.50 ± 2.51	174.13 ± 6.64	297.13 ± 4.14	471.25 ± 10.78
P-value				0.013*	0.0001*	0.001*
Adult	Untreated	29.75 ± 1.44	171.25 ± 2.64	328.94 ± 0.39	465.71 ± 5.70	794.65 ± 5.93
	Treated	30.88 ± 0.67	171.25 ± 2.64	241.90 ± 2.51	328.31 ± 4.63	570.21 ± 2.68
P-value				0.001*	0.0001*	0.0001*

Signifficant differences between means using t-test ($p < 0.05$) are indicated with an asterisk (*). † Day time (from 7.30 am to 7.30 pm) and night time (from 7.30 pm to 7.30 am). ‡ Daily is the sum of day and night time records.

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the stomach (normal histological structure in Figure 1A, 1B) and the intestine (Figure 2A) of C. gariepinus in the control group. Exposure for 15 days to 390 mg Repare® 18%/L altered the stomach histology resulting in a focal area of damage in stomach mucosa (Figure 1C, 1D), accompanied by necrosis and sloughing of mucosal epithelium that causes severe damage to sub-mucosa (Figure 1C, 1D). After 15 days, the fish intestinal in the treated group showed: hyperplasia of intestinal epithelium and goblet cells (Figure 2B), edema of lamina propria and broad intestinal villi in some cases (Figure 2C), and distortion in intestinal villi (Figure 2D) in comparison to control.

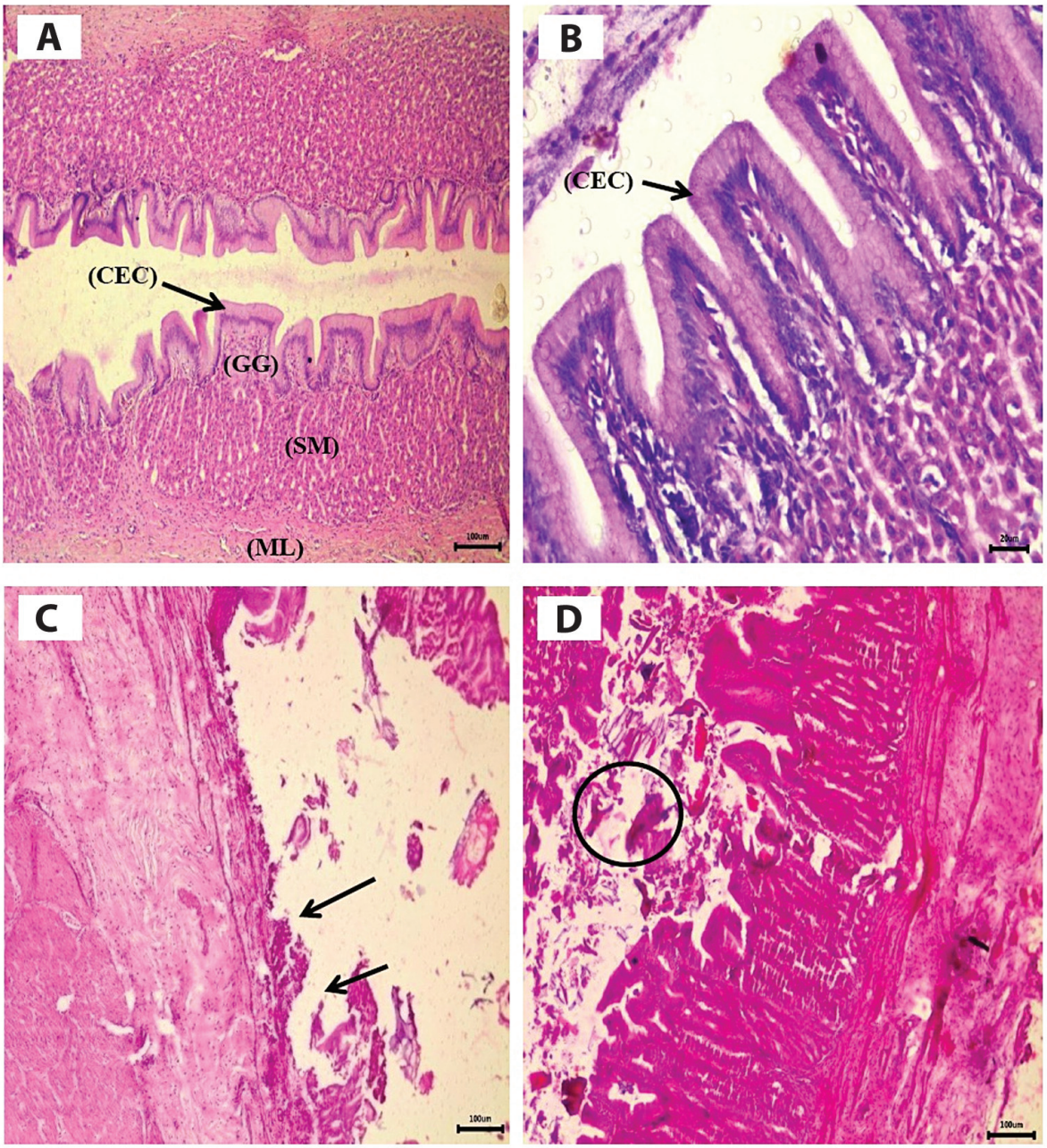


Figure 1. Stomach sections of *Clarias gariepinus* (H & E stain, 400×): A and B) Control stomach showing normal mucosa with a single layer of columnar epithelial cells (CEC) and gastric glands (GG), sub mucosa (SM), and muscle layer (ML). C) and D) Exposed to 390 mg Repare® 18%/L for 15 days showing necrosis (circle), sloughing of mucosal epithelium (arrow). Scale bars: A, C, D (100 µm) and B (20 µm).

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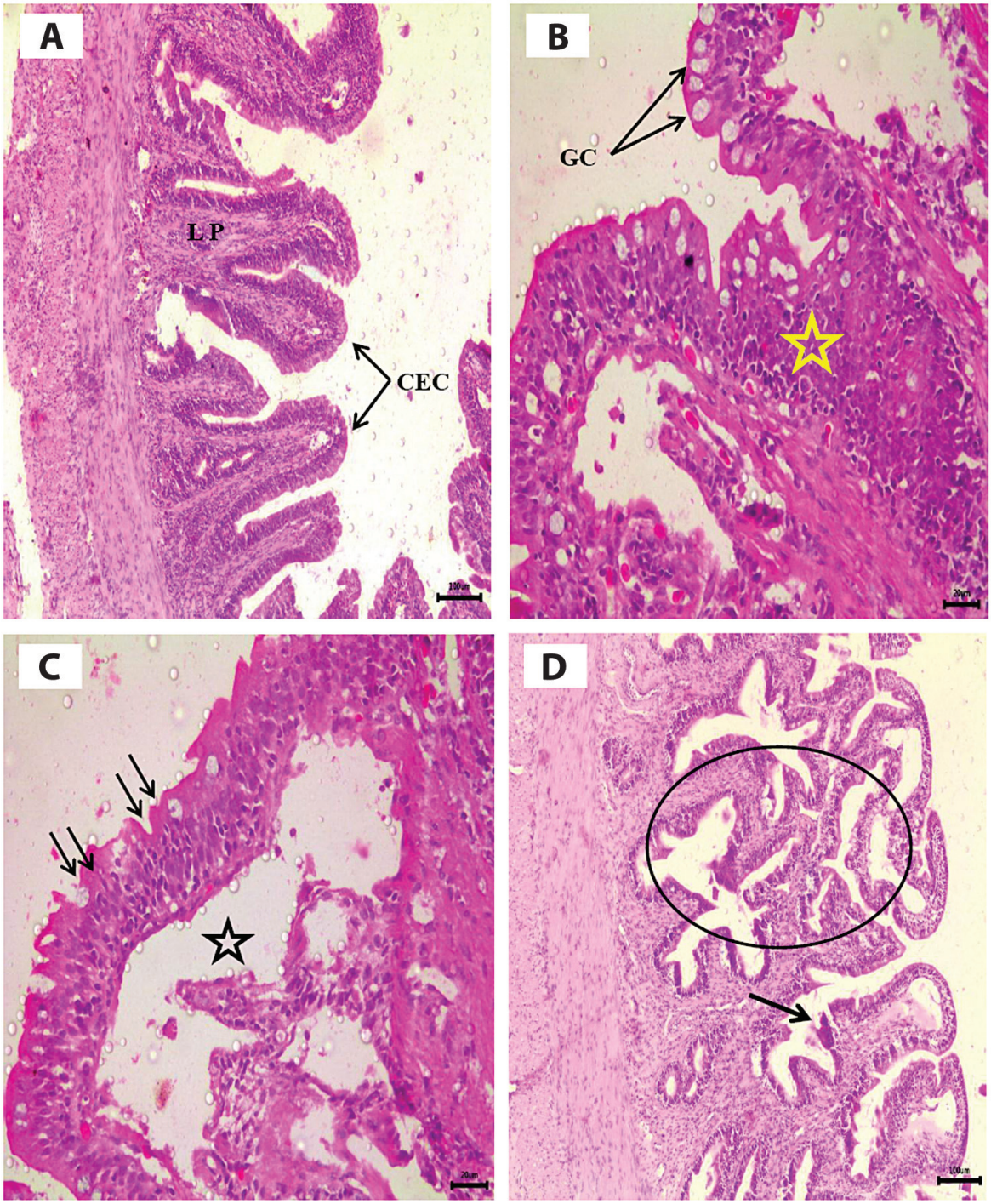


Figure 2. Intestine sections of *Clarias gariepinus* (H & E stain): A) Control intestine tissue showing normal lamina propria (LP), and normal columnar epithelial cells (CEC) with distinct nucleus. B, C, D) Exposed to 390 mg Repare® 18%/L for 15 days; B) Showing hyperplasia of intestinal epithelium (yellow star) with hyperplasia of goblet cells (GC) (arrow); C) Showing Edema of lamina propria (star) and broad intestinal villi (double arrow); D) Showing damaged CEC (arrow) and distortion of villi and lamina propria (oval). Magnification: A (100×) and B, C, D (400×). Scale bars: A, D (100 µm) and B, C (20 µm).

Discussion

This is the first report indicating that the exposure of *C. gariepinus* fish species to sublethal concentration (almost 1/4 of the LC_{50} concentration for 96 h exposure) of a com-

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mercial herbicide containing quinclorac and bensulfuron-methyl has negative effects on the predation potential of the fish, female and male. Kerby *et al.* (2012) indicated that exposure of *G. affinis* to sublethal concentrations (0.5 and 1.0 mg/L) of the pesticide diazinon for 48 h decreased its consumption by 90-100% on *P. regilla* tadpole prey, whereas the presence of the pesticide resulted in a significant reduction in activity and attack rates of *G. affinis* against the target prey.

In the case of bensulfuron-methyl and acetochlor mixture rice herbicide, the LC_{50} values against *Procambarus clarkii* fish were 191.25 and 145.24 mg/L at 24 and 96 h, respectively. Tthe herbicide at 1/2 of the 96-h LC_{50} (72.6 mg/L) caused behavioral and morphological changes and induced severe pathological alterations in the gill, heart, muscle, perigastric organ, midgut, and stomach of the fish (Yu *et al.,* 2017). The 96-h LC_{50} of bensulfuron-methyl on *Cyprinus carpio* was 1620 mg/L (Rahmani et al. 2020) and the 96-h LC_{50} of quinclorac on *Rhamdia quelen* fingerlings was 395 mg/L (Miron *et al.* 2005).

This is the first report for the predatory activity of *C. gariepinus* fish collected in Egypt against *Cx. pipiens* larvae under laboratory conditions, indicating its potential use as an eco-friendly and effectively indigenous biocontrol agent against the aquatic developmental stages of *Cx. pipiens*. The larvivorous efficiency of *C. gariepinus* has been reported in Ethiopia against larvae of both *An. arabiensis* and *Culex* sp. in laboratory and semi-field experiments; the larvae consumption by the fish was significantly increased during the nighttime than during the daytime (Chala *et al.,* 2016). In India, *C. gariepinus* exhibited a highest capacity to feed on *An. stephensi* (Liston) larvae compared with *C. idella*, *Cyprinus carpio* L., and *O. niloticus* (Ghosh *et al.,* 2005). Earlier studies demonstrated that the predation rates of *C. gariepinus* and other indigenous and exotic larvivorous fish species were greatly associated with different biological traits such as the biotype, size, developmental stages and sex of the larvivorous fish, and the genera, developmental stages and population density of mosquito prey (Seng *et al.,* 2008; Chala *et al.,* 2016; Mohamed *et al.,* 2021), or the presence of different chemical and physical stressors such as pesticides, light, and salinity in aquatic habitats (Kerby *et al.,* 2012; Yofukuji *et al.,* 2021).

In our study, the untreated female of *C. gariepinus* consumed more *Cx. pipiens* larvae than the untreated male fish. Moreover, the daily predation rates of the pre-treated and untreated groups of female *C. gariepinus* against *Cx. pipiens* larvae were higher than those of the male fish in both groups, indicating that the predation rates of *C. gariepinus* were strongly associated with the sex of fish. Similarly, mosquito larval consumption is strongly correlated with the sex of predatory fish *Poecilia reticulata* (Seng *et al.,* 2008; Saleeza *et al.,* 2014).

Female and male of *C. gariepinus* exposed to sublethal dose of the tested quinclorac and bensulfuron-methyl based herbicide showed decreased predation rates on *Cx. pipiens* larvae compared to non-exposed fish. This might be associated with the direct deleterious effect of the herbicide on the behavioral, physiological, pathological, and metabolic functions in the treated fish, e.g. to reduce the activity and change swimming speed behavior (rest and slow swim) that may decrease the attack capability rate on mosquito larvae. The presence of chemical contaminants such as heavy metals and pesticides in aquatic habitats may affect the survival and predatory ability of predatory fish by decreasing the predation rates, altering their swimming ability or reducing their vigor (Kerby *et al.,* 2012; Monde *et al.,* 2016; Yofukuji *et al.,* 2021). Changes in ecological functions of mobility and predation may be due to the inhibitory effect of the toxic compounds on fish's acetylcholinesterase (Banaee, 2012). The hybrid catfish, which was exposed to sub-lethal doses of endosulfan insecticide (0.03-1.0 µg/L) exhibited behavioral changes and decreased its predation capacity on *Bulinus globosus* (Morelet) prey (Monde *et al.,* 2016). Kerby *et al.* (2012) found that diazinon insecticide at low concentrations significantly decreased the predation rate of mosquitofish *G. affinis* against tad-

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poles by reducing the fish activity and vigor.

Pesticides and other chemical toxicants in water can enter the digestive tracts of fish during feeding and can also cause histopathological injuries in the digestive organs of fish that may negatively influence the fish feeding activity (Banaee 2012; Yu *et al.,* 2017; Saleh *et al.,* 2022). Histological analyses in the present study showed histological alternations in the stomach and the intestine tissue of *C. gariepinus* fish after exposure to the quinclorac and bensulfuron-methyl based herbicide at a sublethal concentration of 390 mg Repare® 18%/L water for 15 days. Similarly, Samanta *et al.* (2016a) reported that exposure of *O. niloticus* to Almix® herbicide (metsulfuron-methyl+chlorimuron-ethyl) for 30 days led to degenerative changes like distorted mucosal folds, damage in columnar epithelial cells and submucosa, and merged mucosal folds in stomach tissues. Pathological changes in the stomach, midgut, and intestine were also found in *Procambarus clarkia* (Girard) after exposure to the mixture bensulfuron-methyl+acetochlor (Yu *et al.,* 2017). Exposure of *Cirrhinus mrigala* (Hamilton) for 96 h to 1.5-3.0 µg/L fenvalerate insecticide altered intestine histology resulting in necrosis of the epithelial cells linning intestinal villi, sloughing of the mucosal epithelium and lymphocytic cell reaction in the lamina propria (Velmurugan *et al.,* 2007).

Fish stomach and intestine are primarily responsible for the digestion of ingested food materials and are vital organs, which are affected by different xenobiotic compounds, mainly pesticides (Braunbeck and Appelbaum, 1999). The histological changes in stomach and intestine of *C. gariepinus* exposed to quinclorac and bensulfuron-methyl based herbicide could be related to the deformity structures and functions of these organs due to the herbicide toxicity and cause a negative effect on the prey consumption by the fish. The prey consumption of a bug predator, *Podisus nigrispinus* (Dallas) was decreased significantly by prey exposure to permethrin, thiamethoxam, and *Bacillus thuringiensis* insecticides causing morphological and histological alternation in the midgut of the predator that impaired its digestibility (Silva *et al.*, 2021). Samanta *et al.* (2016b) described similar lesions formation in villi of *Anabas testudineus* (Bloch) after exposure to glyphosate herbicide.

In conclusion, this study showed that the fish *C. gariepinus* collected from Egypt can consume *Cx. pipiens* larvae, and fish females exhibit higher consumption capacity than the males. Our results suggest that the native to Egypt fish, *C. gariepinus* could be considered as a pioneer and a promising biocontrol agent against *Cx. pipiens* larvae. However, exposure of *C. gariepinus* to sublethal concentration of a herbicide containing quinclorac and bensulfuron-methyl (390 mg Repare® 18%/L water) for 15 days reduced the predatory potential of the fish female and male against mosquito larvae of *Cx. pipiens*. The herbicide also caused histopathological changes in the stomach and intestine of this predatory fish that may compromise predation and affect digestion and absorption of nutrients. These findings suggest the necessity of appropriate application of the tested herbicide according to the authorized uses indicated in the label of the product in agricultural lands and fish farms in order to prevent or reduce harmful effects on the fish. The effects of pesticides on predatory fish, their prey, and their interactions require further laboratory and field studies.

The authors present sincere thanks to Dr Youssef M. M. Omar (Plant Protection Department, Faculty of Agriculture, Assiut University, Egypt), for reviewing and improving the earlier version of the manuscript.

Compliance with ethical standards

All laboratory experiments were approved by the Committee of the Faculty of Veterinary of Assiut University, Egypt.

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Received: 27 September 2022; Accepted: 18 June 2023

Συμπεριφορικές αλλαγές και ιστοπαθολογικές αλλοιώσεις στο ψάρι *Clarias gariepinus* ως αρπακτικό προνυμφών του κουνουπιού *Culex pipiens* μετά από έκθεση σε υποθανατηφόρο συγκέντρωση ζιζανιοκτόνου με δραστικές ουσίες quinclorac και bensulfuron-methyl

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Περίληψη Το *Clarias gariepinus* είναι ένα καλλιεργούμενο είδος ψαριού του γλυκού νερού, ευρέως διαδεδομένο στην Αφρική, το οποίο κυριαρχεί σε διάφορα φυσικά και ανθρωπογενή υδάτινα οικο-

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συστήματα, συμπεριλαμβανομένου του μικτού συστήματος ιχυοκαλλιέργειας - καλλιέργειας ρυζιού. Αυτό το είδος ψαριού εμφανίζει αρπακτική ικανότητα στα υδρόβια στάδια ανάπτυξης των κουνουπιών. Οι bensulfuron-methyl και quinclorac είναι ζιζανιοκτόνες δραστικές ουσίες που έχουν εφαρμοστεί εκτενώς σε καλλιέργειες ρυζιού στην Αφρική και άλλες χώρες παγκοσμίως. Η μελέτη αυτή αξιολόγησε τις αρνητικές επιδράσεις υποθανατηφόρου συγκέντρωσης ενός εμπορικά διαθέσιμου ζιζανιοκτόνου που περιέχει τις δραστικές ουσίες quinclorac and bensulfuron-methyl, στην αρπακτική ικανότητα των θηλυκών και αρσενικών ατόμων του ψαριού *Clarias gariepinus* σε προνύμφες του κουνουπιού *Culex pipiens*. Επίσης, μελετήθηκε η ιστοπαθολογία του στομάχου και του εντέρου των ψαριών στα οποία έγινε η εφαρμογή του ζιζανιοκτόνου. Η έκθεση του *C. gariepinus* στην υποθανατηφόρο συγκέντρωση του ζιζανιοκτόνου με δραστικές ουσίες quinclorac και bensulfuron-methyl προκάλεσε αρνητικές επιδράσεις στην κατανάλωση λείας και ιστοπαθολογικές αλλοιώσεις στον στόμαχο και στο έντερο του ψαριού. Η κατανάλωση προνυμφών κουνουπιών από τα θηλυκά και αρσενικά ψάρια στα οποία είχε γίνει εφαρμογή του ζιζανιοκτόνου μειώθηκε σημαντικά σε σχέση με εκείνη των ψαριών και των δύο φύλων που δεν εκτέθηκαν στο φάρμακο. Οι ιστοπαθολογικές αλλοιώσεις στο έντερο ήταν υπερπλασία του επιθηλίου του εντέρου και των καλυκοειδών κυττάρων, οίδημα της υποβλεννογόνιου στοιβάδας και διεύρυνση των εντερικών λαχνών, και παραμόρφωση των εντερικών λαχνών, σε σύγκριση με το μάρτυρα. Οι ιστοπαθολογικές αλλοιώσεις του στομάχου ήταν νέκρωση και αποκόλληση του επιθηλίου του βλεννογόνου με σοβαρή βλάβη του υποβλεννογόνου. Ως εκ τούτου, το δοκιμαζόμενο ζιζανιοκτόνο σε υποθανατηφόρο συγκέντρωση στο ψάρι *C. gariepinus* μείωσε την κατανάλωση προνυμφών κουνουπιών και προκάλεσε ιστοπαθολογικές αλλοιώσεις στο ψάρι, οι οποίες ενδεχομένως επηρέασαν τη φυσιολογίας της πέψης. Τα ευρήματα της μελέτης δείχνουν ότι το δοκιμαζόμενο ζιζανιοκτόνο μπορεί να αποτελέσει απειλή για την επιβίωση του ψαριού *C. gariepinus* και τη δυνατότητα χρήσης του ως επιτυχούς ιθαγενή παράγοντα βιολογικής καταπολέμησης προνυμφών του κουνουπιού *Cx. pipiens*.

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