Environmental risks of two chitin synthesis inhibitors on *Gambusia affinis*: Chronic effects on growth and recovery of biological responses

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ABSTRACT

To reduce the impact of conventional insecticides, new more selective chemicals need to be developed. Insect growth regulators such as chitin synthesis inhibitors seem promising because of their specific mode of action on insect and their lower toxicity against non-target organisms than conventional insecticides. Dimilin (25% WP) and andalin (25% EC), the respective trade names of diflubenzuron (DFB) and flucycloxuron (FCX), are two chitin synthesis inhibitors belonging to the class of benzoylphenylurea. In the present study, the toxicity of these insecticides was evaluated on growth and metric indexes in adult females of a larvivorous fish, *Gambusia affinis* (Cyprinodontiformes, Poeciliidae), extensively used in biological control of mosquitoes. In addition, the compounds were tested on amount of glutathione (GSH) and on glutathione S-transferase activity (GST). The exposure regime included an insecticide exposure period (28 days) and a recovery period in untreated water (8 days). The compounds were tested separately. Each compound was added in rearing water at two concentrations corresponding to LC_{50} and LC_{90} against fourth instar larvae of *Culex pipiens* (Diptera, Culicidae). The adult females were exposed in a continuous treatment for 28 days. The growth, metric indexes, GSH and GST activities were determined at different exposure times (0, 7, 14, 21 and 28 days). The results showed that DFB and FCX had no significant ($p > 0.05$) effects on growth, hepato-somatic (HSI) and gonadal-somatic (GSI) indexes, while only FCX at LC_{90} reduced significantly ($p < 0.05$) the condition factor starting day 21 of exposure. Furthermore, treatment affected both GSH amounts and GST activities. The observed effects varied as function of tested insecticide, exposure time and concentration. The response appeared more marked with FCX. The recovery study showed that this fish species was able to overcome relatively rapidly the stress induced by these insecticides. The overall results suggested that FCX and to a lesser extent also DFB exhibited slight toxic effects on this non-target fish species, and can be used for controlling of mosquitoes in an integrated manner.

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1. Introduction

Mosquito play an important role in global disease transmission (Nuttall, 1997) and are generally controlled by conventional insecticides (Casida and Quistad, 1998). However, these conventional neurotoxins possess strong secondary effects on the environment (Paoletti and Pimentel, 2000). In this context, there is search for new insect-selective insecticides with minimal toxic effects on the environment and human health. To reduce the impact of pesticides, new more selective chemicals are developed. Insect growth regulators (IGRs) seem promising because of their specific mode of action on insects and their lower toxicity against non-target organisms than conventional insecticides. The benzoylphenylurea (BPU)

insecticides such as diflubenzuron (DFB) and flucycloxuron (FCX) inhibit chitin synthesis, thereby causing abnormal endocuticular deposition and abortive moulting (Soltani et al., 1984, 1996; Rehimi and Soltani, 1999; Dhadialla et al., 2005). In addition, chitin synthesis inhibitors (CSIs) like DFB and related compounds affect reproduction in several insect orders, primarily by causing a reduction in egg hatch (Soltani and Soltani-Mazouni, 1992; Kellouche and Soltani, 2006). Many biochemical effects of DFB have also been reported on the metabolism of carbohydrates (Soltani, 1990) and lipids (Khebbeb et al., 1997). FCX is a BPU derivative controlling mites and insects (Scheltes et al., 1988) by interference with chitin biosynthesis as demonstrated in *Spodoptera littoralis* (Grosscurt et al., 1988) and in *Tenebrio molitor* (Soltani et al., 1993). This compound was also evaluated on growth, development and cuticle secretion in *Ephestia kuehniella* (Bendjedou et al., 1998). The relatively high accumulation rate of these IGRs in the adult reproductive organs (ovaries and testis) helps to explain their strong reproductive effects (Chebira et al., 2006).

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The intensive use of insecticides becomes environmentally hostile and ecologically unsafe since the main side effect of the application is expressed by the extinction of natural enemies of mosquitoes such as odonates, beetles and fishes in water pools. In Algeria, DFB is widely used against pest insects in forestry, while FCX is not yet approved. Moreover, previous bioassays conducted under laboratory conditions have shown that DFB and FCX are two potent IGRs for mosquito control (Soltani and Rehimi, 2001; Rehimi, 2004). Prior to implementation of DFB and FCX as chemical agents for mosquito control, knowledge on their potential effects on non-target organisms is needed. Several fish species have been tested against mosquitoes and *Gambusia affinis* (Cyprinodondiformes, Poeciliidae) was found very efficient as compared to two autochthonous *Cyprinidae* species, *Pseudophoxinus callensis* and *P. guichenoti* (Bendali et al., 2001). *G. affinis* remains one of the best candidates for biological control programs against mosquitoes (Kumar and Hwang, 2006; Walton, 2007). This mosquitofish has been the subject of several studies including behavioral ecology, histopathology, reproductive biology, endocrinology and toxicology (Tolar et al., 2001; Koya et al., 2003; Venkateswara Rao et al., 2005; Smith and Sargent, 2006). The normal development of the ovaries has already been described (Drardja-Beldi, 1993; Beldi, 2001), and data obtained provide an experimental basis to study the activity of IGRs on females. Moreover, Soltani et al. (1999) reported that triflumuron, another CSI, reduced the number of juveniles per brood in *G. affinis*.

For environmental risk assessment, a comprehensive understanding on adaptation and/or a recovery is important (Wu et al., 2005; Du et al., 2009). Therefore, the aims of the present study are (1) to investigate potential hazards of DFB and FCX on growth of *G. affinis* females under chronic exposure to estimate their ecological risks, (2) to test the detoxification system by measuring the glutathione (GSH) amount and glutathione S-transferase (GST) activity, and (3) to evaluate the ability of this fish to overcome the stress induced by these two IGRs. The data obtained permit us to evaluate the possible field application of these potent chemicals for mosquito control strategies.

2. Materials and methods

2.1. Fish rearing

G. affinis was collected from Kherraza River (4° 04′ N, 04° 02′ E) located at the west of Annaba city (Northeast Algeria). This river was previously described (Drardja-Beldi, 1993). Briefly, this seasonal river is fed from the Edough Mountain, its current speed is rather slow beyond the rainful periods. The river pH was found to be slightly basic (pH 7.5–8.3) in winter and acid (pH 6) in summer. The water temperature reaches 32 °C in summer and 8 °C in winter. Important species of diversity in fauna and flora have been observed: Insecta (Odonata, Diptera), Crustacea (Daphnia, Cyclops), diatoms, plants (sedge, reeds, Elodea), vertebrates (amphibians, freshwater turtle, mosquitofish). The sediment consists mainly of mud-rich dark organic material containing abundant Oligochaeta (Tubifex). Collected fishes were acclimatized for at least 15 days prior to exposure. In a first series of experiments, only the females were considered as Soltani et al. (1999) reported on a reduction in numbers of juveniles per brood in *G. affinis* upon treatment with the CSI triflumuron. Adult females (mean length: 35.4 ± 3.2 mm, mean weight: 425.8 ± 4.8) were placed in 50 L glass aquaria (length: 60 cm, width: 30 cm and height: 30 cm) with aerated water and fed commercial dry feed (Tetramin®, Germany). The natural photoperiod of 14:10 (L:D) was maintained. Exposed and control fishes were reared in aquaria (50 fishes per aquarium) under laboratory conditions: temperature

21.03 ± 0.31 °C; salinity 242.00 ± 33.57 mg/L; pH 8.07 ± 0.09; dissolved oxygen 2.88 ± 0.12 mg/L.

2.2. Insecticides and treatments

Dimilin (25% wettable powder, WP) and andalin (25% emulsifiable concentrate, EC), kindly provided by Pr. G. Smagghe (Ghent University, Belgium), were tested separately. The compounds were added to the rearing water to obtain two final concentrations (DFB: 16 and 78 ng/L, and FCX: 35 ng/L and 1.9 µg/L) which correspond to the LC_{50} and LC_{90} for fourth-instar larvae of *Culex pipiens*, respectively, as previously determined (Soltani and Rehimi, 2001; Rehimi, 2004). Previous assays were done with newly moulted fourth-instar larvae for 24 h under standard laboratory conditions and mortality was scored until adult emergence. Fishes being starved for 2 days were exposed to each insecticide at these two above concentrations for 28 days. For each concentration, three aquaria were used and each contained 50 fishes. Fishes that survived after 28 days of exposure were transferred into untreated water (8 days) to evaluate the recovery pattern of environmental biomarkers. In each experiment, untreated animals were used as controls.

2.3. Morphometric measurements

At appropriate times, fishes were randomly collected and anesthetized in 0.04% MS-222 (3-aminobenzoic acid ethyl ester methanesulfonate salt). Their standard length (*L*), body weight (BW), hepatopancreas weight (HW) and gonad weight (GW) were measured at various times during the experiment. The hepato-somatic (HSI = HW × 100/BW) and gonadal-somatic (GSI = GW × 100/BW) indexes and the condition factor (K = BW (mg) × 100/L (mm³)) were calculated.

2.4. Biochemical procedure

GSH and GST were determined individually in hepatopancreas as previously described (Habbes et al., 2006). Briefly, GSH was measured spectrophotometrically according to Weckbercker and Cory (1988). Each hepatopancreas was homogenized in EDTA (0.02 M; pH 6) and subjected to a deproteinisation with sulfosalylic acid (SSA) at 0.025%. Then the homogenates were maintained for 15 min on ice and centrifuged (3000g, 15 min). The resultant supernatant was used as enzyme source for the GSH estimation. The absorbance was read at 412 nm. GST activity was assayed by the method of Habig et al. (1974) using two substrates, 1-chloro-2,4-dinitrobenzene (CDNB) and reduced GSH. The hepatopancreas were homogenized in sodium phosphate buffer (0.1 M, pH 6) and centrifuged (14,000g, 30 min). The GST activity in the resulting supernatant was measured at 340 nm. The protein concentration was determined according to Bradford (1976) and specific activity of GST was expressed as µM/min/mg protein while GSH amounts as µM/mg protein. For each time, the mean value of GSH and GST was established on measures from 8 to 10 fishes.

2.5. Statistical analysis

The normality of data was verified using the Kolmogorov–Smirnov test, and the homogeneity of variances was checked by Levene's test. Data have been expressed by the mean ± standard deviation (m ± SD). Data were subjected to analysis of variance (ANOVA) followed by a Tukey test. The relation between HIS, GSI and CF with the values of GSH and GST for the fishes was also examined. All statistical analyses were performed using MINITAB software (Version 13.31, Penn State College, PA, USA) and $p < 0.05$ was considered to be a statistically significant difference.

The numbers of fishes used for each time during treatment and the recovery period are given with the results.

3. Results

3.1. Effects on growth and metric indexes

The chronic effects by DFB (16 and 78 ng/L) and FCX (35 ng/L and 1.9 μg/L) have been examined on the growth and metric indexes of adult females of *G. affinis*. No fish mortality was observed during the exposure period. The data revealed that DFB and FCX had no significant ($p > 0.05$) effect on both length (LC_{50}: $F = 1.26$, $df = 2.105$, $p = 0.287$; LC_{90}: $F = 1.07$, $df = 2.105$, $p = 0.348$), weight (LC_{50}: $F = 0.98$, $df = 2.105$, $p = 0.378$; LC_{90}: $F = 0.04$, $df = 2.105$, $p = 0.962$), HSI (LC_{50}: $F = 0.27$, $df = 2.105$, $p = 0.766$; LC_{90}: $F = 2.81$, $df = 2.105$, $p = 0.064$) and GSI (LC_{50}: $F = 1.09$, $df = 2.105$, $p = 0.339$; LC_{90}: $F = 0.79$, $df = 2.105$, $p = 0.459$) during treatment, and the recovery period was similar as in the controls (Tables 1–4). Concerning the condition factor, treatment with DFB and FCX at their respective LC_{50} had no significant effect ($F = 2.16$, $df = 2.105$, $p = 0.121$) (Fig. 1A). In contrast, FCX at its LC_{90} reduced significantly ($p < 0.001$) the condition factor starting from day 21 of exposure onwards compared to controls of the same age (Fig. 1B). ANOVA showed a significant effect of treatment at LC_{90} ($F = 4.42$, $df = 2.105$, $p = 0.014$). The reduction caused by FCX at LC_{90} persisted during the recovery period only with this high toxic concentration.

3.2. Effect on GSH amounts and GST activities

As depicted in Figs. 2 and 3, DFB and FCX affected the GSH amount and GST activity. Data show a significant ($p < 0.01$) reduction in GSH amount in treated females with DFB at LC_{50} starting from day 14 onwards. This effect was more marked ($p < 0.01$) with the LC_{90} and happened earlier during exposure (starting day 7). FCX at the two tested doses reduced significantly ($p < 0.05$) the amount of GSH starting from day 7 onwards and the effect was concentration-dependent (Fig. 3). Two way ANOVA indicated significant ($p < 0.001$) effects of both treatment (LC_{50}: $F = 91.78$, $df = 2105$; LC_{90}: $F = 844.25$, $df = 2105$) and exposure time (LC_{50}: $F = 68.57$, $df = 4105$; LC_{90}: $F = 543.33$, $df = 4105$), and also a significant ($p < 0.001$) time × treatment interaction (LC_{50}: $F = 11.03$, $df = 8105$; LC_{90}: $F = 117.71$, $df = 8105$), at the two tested concentrations. The Tukey test revealed that there was a significant difference in GSH amount between concentrations. During the recovery period, the amount of GSH was found lower in DFB-treated series at the two tested concentrations only at days 1 and 2 compared to in controls ($p < 0.05$). In contrast, there was a significant difference in GSH amounts between FCX-treated series and controls for all times periods during the recovery period. In addition, the values recorded in treated groups increased progressively during the recovery period. Fig. 3 summarized the impact of DFB and FCX on GST activities in females of *G. affinis*. Data indicated that DFB at LC_{50} had no significant effect on the GST activity. In contrast, fish that were exposed to FCX at LC_{50} exhibited a significant ($p < 0.05$) induction in GST activity at day 14, 21 and 28 as compared to controls of the same age (Fig. 3A). A significant ($p < 0.01$) induction in GST activity was recorded starting day 7 with the two insecticides administered at their LC_{90} (Fig. 3B). Two-way ANOVA indicated significant ($p < 0.001$) time (LC_{50}: $F = 235.40$, $df = 4105$; LC_{90}: $F = 311.66$, $df = 4105$) and treatment (LC_{50}: $F = 798.17$, $df = 2105$; LC_{90}: $F = 1066.29$, $df = 2105$) effects, and a significant ($p < 0.001$) time × treatment interaction (LC_{50}: $F = 61.96$, $df = 8105$; LC_{90}: $F = 88.72$, $df = 8105$). A comparison using the Tukey test demonstrated that there was a significant difference in GST activity between concentrations of two compounds. During the recovery period, the GST activity remained higher at day 1 with the LC_{90}-DFB and at days 1 and 2 with LC_{90}-FCX as compared to controls. Finally, at the end of the recovery period (8 days) there was no significant ($p > 0.05$) difference between control and treated series.

Table 1
Effect of diflubenzuron (DFB) and flucycloxuron (FCX) on length (mm) of *G. affinis* adult females during the exposure (E) and recovery (R) periods (m ± SD; $n = 8$–10; for each exposure time, mean values followed by the same letter are not significantly different at $p > 0.05$).

Period	Time (days)	Control	LC_{50}		LC_{90}	
			DFB	FCX	DFB	FCX
E	0	33.1 ± 1.6	–	–	–	–
	7	37.6 ± 1.3a	36.1 ± 2.6a	35.1 ± 3.2a	36.1 ± 3.0a	36.8 ± 5.0a
	14	34.5 ± 2.0a	35.0 ± 3.2a	33.9 ± 3.0a	34.7 ± 3.3a	33.0 ± 2.1a
	21	37.0 ± 1.7a	34.0 ± 2.3a	34.5 ± 4.2a	34.0 ± 1.4a	34.0 ± 3.0a
	28	34.8 ± 0.5a	33.5 ± 2.1a	34.5 ± 3.4a	32.1 ± 1.0a	33.0 ± 3.2a
R	1	35.2 ± 0.70a	34.7 ± 2.01a	34.5 ± 3.3a	33.1 ± 1.5a	34.1 ± 1.8a
	2	35.4 ± 1.02a	34.5 ± 1.07a	34.0 ± 1.7a	33.5 ± 1.7a	34.0 ± 1.9a
	4	34.9 ± 0.90a	34.0 ± 0.9a	34.0 ± 2.7a	33.4 ± 0.9a	33.99 ± 2.1a
	8	34.5 ± 1.50a	34.1 ± 1.10a	33.9 ± 2.5a	33.5 ± 0.99a	34.01 ± 2.0a

Table 2
Effect of diflubenzuron (DFB) and flucycloxuron (FCX) on weight (mg) of *G. affinis* adult females during the exposure (E) and recovery (R) periods (m ± SD; $n = 8$–10; for each exposure time, mean values followed by the same letter are not significantly different at $p > 0.05$).

Period	Time (days)	Control	LC_{50}		LC_{90}	
			DFB	FCX	DFB	FCX
E	0	415.3 ± 9.3	–	–	–	–
	7	420.8 ± 9.2a	417.4 ± 10.7a	419.7 ± 14.5a	418.6 ± 13.4a	419.6 ± 19.3a
	14	425.5 ± 9.1a	424.3 ± 11.1a	425.2 ± 19.4a	424.8 ± 14.0a	424.4 ± 24.3a
	21	426.8 ± 10.7a	427.6 ± 19.8a	424.6 ± 28.4a	425.8 ± 40.0a	425.7 ± 23.0a
	28	429.0 ± 15.7a	427.2 ± 7.5a	426.4 ± 14.5a	426.1 ± 32.1a	427.7 ± 15.5a
R	1	429.7 ± 10.7a	427.8 ± 7.5a	427.0 ± 13.1a	426.2 ± 17.9a	427.5 ± 24.3a
	2	428.1 ± 9.5a	428.0 ± 8.9a	427.2 ± 12.9a	426.4 ± 19.7a	427.9 ± 21.2a
	4	430.1 ± 12.7a	427.9 ± 9.1a	427.8 ± 11.7a	427.0 ± 17.1a	427.8 ± 20.9a
	8	430.2 ± 11.2a	429.2 ± 9.5a	428.1 ± 15.1a	427.9 ± 20.1a	428.0 ± 10.1a

Table 3
Effect of diflubenzuron (DFB) and flucycloxuron (FCX) on hepato-somatic index of *G. affinis* adult females during the exposure (E) and recovery (R) periods (m ± SD; *n* = 8–10; for each exposure time, mean values followed by the same letter are not significantly different at *p* > 0.05).

Period	Time (days)	Control	LC_{50}		LC_{90}	
			DFB	FCX	DFB	FCX
E	0	0.05 ± 0.00	–	–	–	–
	7	0.06 ± 0.01a	0.06 ± 0.00a	0.05 ± 0.01a	0.06 ± 0.01a	0.05 ± 0.00a
	14	0.07 ± 0.01a	0.07 ± 0.02a	0.07 ± 0.02a	0.06 ± 0.00a	0.06 ± 0.01a
	21	0.07 ± 0.00a	0.07 ± 0.02a	0.07 ± 0.02a	0.06 ± 0.01a	0.06 ± 0.01a
	28	0.07 ± 0.01a	0.06 ± 0.00a	0.06 ± 0.01a	0.06 ± 0.01a	0.06 ± 0.01a
R	1	0.07 ± 0.01a	0.06 ± 0.01a	0.06 ± 0.01a	0.06 ± 0.01a	0.06 ± 0.01a
	2	0.07 ± 0.01a	0.06 ± 0.01a	0.06 ± 0.01a	0.06 ± 0.01a	0.06 ± 0.01a
	4	0.08 ± 0.02a	0.07 ± 0.02a	0.07 ± 0.01a	0.07 ± 0.01a	0.06 ± 0.01a
	8	0.08 ± 0.02a	0.07 ± 0.02a	0.07 ± 0.01a	0.07 ± 0.02a	0.07 ± 0.02a

Table 4
Effect of diflubenzuron (DFB) and flucycloxuron (FCX) on gonad-somatic index of *G. affinis* adult females during the exposure (E) and recovery (R) periods (m ± SD; *n* = 8–10; for each exposure time, mean values followed by the same letter are not significantly different at *p* > 0.05).

| Period	Time (days)	Control	LC_{50}		LC_{90}	
			DFB	FCX	DFB	FCX
E	0	0.85 ± 0.06	–	–	–	–
	7	0.81 ± 0.07a	0.80 ± 0.03a	0.80 ± 0.05a	0.81 ± 0.07a	0.79 ± 0.0a
	14	0.82 ± 0.08a	0.79 ± 0.07a	0.81 ± 0.10a	0.79 ± 0.20a	0.81 ± 0.09a
	21	0.81 ± 0.01a	0.79 ± 0.12a	0.78 ± 0.08a	0.78 ± 0.08a	0.80 ± 0.06a
	28	0.84 ± 0.16a	0.81 ± 0.07a	0.80 ± 0.12a	0.78 ± 0.12a	0.81 ± 0.10a
R	1	0.84 ± 0.10a	0.81 ± 0.10a	0.80 ± 0.14a	0.79 ± 0.14a	0.81 ± 0.14a
	2	0.84 ± 0.09a	0.81 ± 0.12a	0.80 ± 0.10a	0.79 ± 0.10a	0.81 ± 0.14a
	4	0.84 ± 0.12a	0.82 ± 0.08a	0.81 ± 0.13a	0.80 ± 0.08a	0.82 ± 0.12a
	8	0.84 ± 0.12a	0.82 ± 0.08a	0.82 ± 0.13a	0.81 ± 0.12a	0.82 ± 0.10a
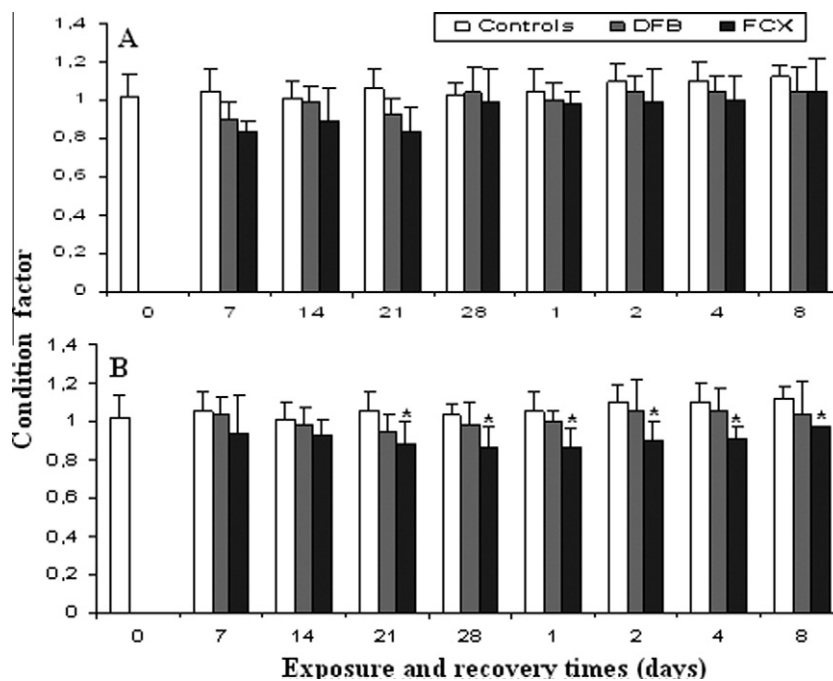
Fig. 1. Effect of diflubenzuron (DFB) and flucycloxuron (FCX) at LC_{50} (A) and LC_{90} (B) on condition factor of *G. affinis* adult females during the exposure and recovery periods (m ± SD; *n* = 8–10; asterisks above treated series indicated a significant difference at *p* < 0.05 with controls of the same exposure time).

Statistical analyses on the relation between the CF, HIS and GSI with the values of GSH and GST recorded during the exposure and recovery periods were also made. During the exposure period we observed a significant correlation only in DFB-treated series between HIS and GST at LC_{50} (*R* = 0.350, *p* = 0.027), and GSI and GSH (*R* = 0.343, *p* = 0.030) at LC_{90} (Table 5). Concerning the recovery period, a high significant correlation (*R* = 0.499, *p* = 0.001) was found only at LC_{90}-DFB treated series between HIS and GSH (Table 6).

4. Discussion

The intensive use of conventional insecticides caused secondary effects on the environment and subsequently alternative methods are developed (Frank, 2009). In this context, there is search for new insect-selective insecticides with minimal ecotoxicological risks. The IGRs are quite selective in their mode of action and potentially act only on target species (Isshaya, 1990; Dhadialla et al., 2005). In
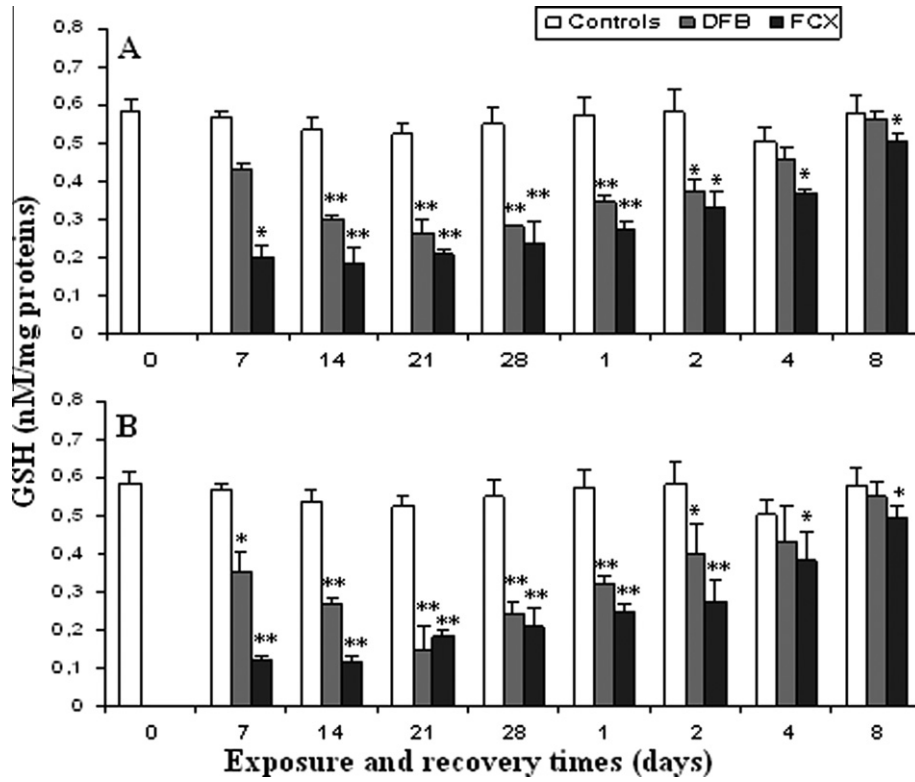
Fig. 2. Effect of diflubenzuron (DFB) and flucycloxuron (FCX) at LC$_{50}$ (A) and LC$_{90}$ (B) on amount of GSH (µM/mg of proteins) of *G. affinis* adult females during the exposure and recovery periods (m ± SD; *n* = 8–10; asterisks above treated series indicated a significant difference with controls of the same exposure time; *significant difference at *p* < 0.05; **significant difference at *p* < 0.01).
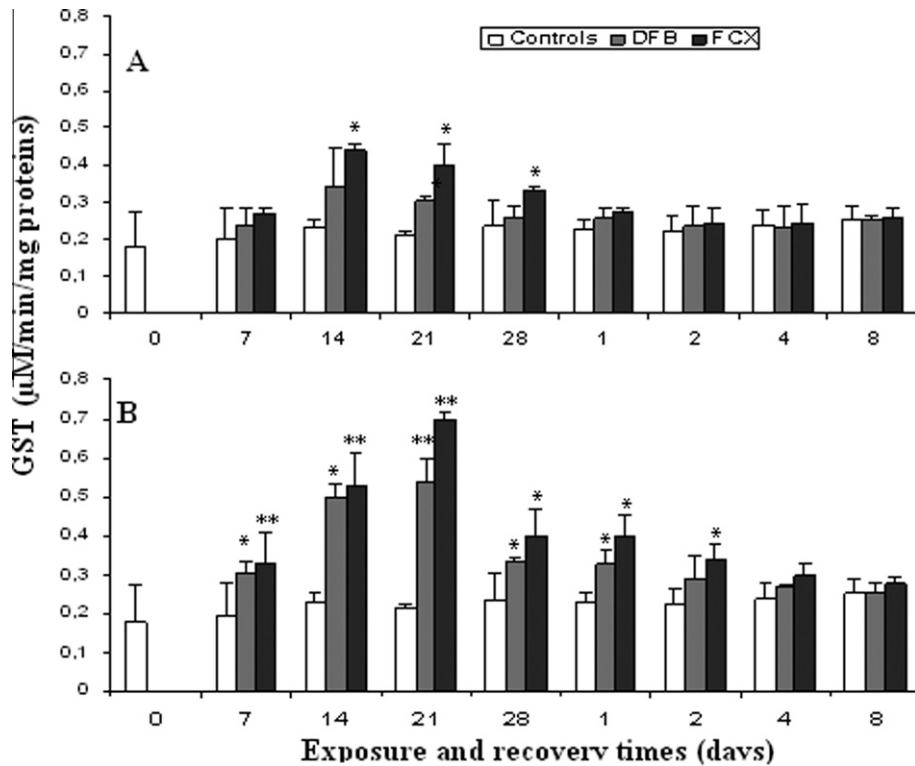

Fig. 3. Effect of diflubenzuron (DFB) and flucycloxuron (FCX) at LC$_{50}$ (A) and LC$_{90}$ (B) on activity of GST (µM/mn/mg of proteins) of *G. affinis* adult females during the exposure and recovery periods (m ± SD; *n* = 8–10; asterisks above treated series indicated a significant difference with controls of the same exposure time; *significant difference at *p* < 0.05; **significant difference at *p* < 0.01).

Table 5
Relation between the condition factor (CF), hepato-somatic index (HIS) and gonad-somatic index (GSI) with the values of GSH and GST during the exposure period of *G. affinis* to insecticides (DFB, FCX) at LC_{50} and LC_{90} (R = coefficient of correlation, p = significance level).

LC	Y	X	DFB			FCX		
			Regression	R	p	Regression	R	p
LC_{50}	CF	GSH	$y = 0.768 + 0.111x$	0.186	0.252	$y = 0.758 + 0.151x$	0.285	0.075
	CF	GST	$y = 0.846 - 0.134x$	−0.134	0.400	$y = 0.812 - 0.031x$	−0.038	0.818
	HSI	GSH	$y = 0.074 - 0.030x$	−0.203	0.209	$y = 0.066 - 0.013x$	−0.086	0.596
	HSI	GST	$y = 0.039 + 0.089x$	0.350	0.027	$y = 0.045 + 0.051x$	0.209	0.196
	GSI	GSH	$y = 0.768 + 0.111x$	0.186	0.252	$y = 0.772 + 0.124x$	0.226	0.160
	GSI	GST	$y = 0.846 - 0.134x$	−0.134	0.410	$y = 0.792 + 0.049x$	0.057	0.726
LC_{90}	CF	GSH	$y = 0.757 + 0.155x$	0.280	0.080	$y = 0.784 + 0.103x$	−0.172	0.055
	CF	GST	$y = 0.867 - 0.158x$	−0.278	0.083	$y = 0.834 - 0.059x$	0.305	0.289
	HSI	GSH	$y = 0.063 - 0.014x$	−0.163	0.314	$y = 0.058 - 0.003x$	−0.038	0.814
	HSI	GST	$y = 0.055 + 0.011x$	0.125	0.442	$y = 0.053 + 0.008x$	0.098	0.547
	GSI	GSH	$y = 0.742 + 0.185x$	0.343	0.030	$y = 0.784 + 0.103x$	0.305	0.055
	GSI	GST	$y = 0.862 - 0.162x$	−0.292	0.067	$y = 0.834 - 0.059x$	−0.172	0.289

Table 6
Relation between the condition factor (CF), hepato-somatic index (HIS) and gonad-somatic index (GSI) with the values of GSH and GST during the recovery period of *G. affinis* (R = coefficient of correlation, p = significance level).

LC	Y	X	DFB			FCX		
			Regression	R	p	Regression	R	p
LC_{50}	CF	GSH	$y = 0.955 + 0.077x$	0.048	0.769	$y = 0.861 + 0.397x$	0.172	0.288
	CF	GST	$y = 0.995 - 0.025x$	−0.015	0.929	$y = 1.567 - 2.211x$	−0.275	0.086
	HSI	GSH	$y = 0.068 - 0.006x$	−0.047	0.775	$y = 0.047 + 0.047x$	0.175	0.281
	HSI	GST	$y = 0.0595 + 0.022x$	0.148	0.363	$y = 0.111 - 0.183x$	−0.194	0.231
	GSI	GSH	$y = 0.794 + 0.049x$	0.091	0.575	$y = 0.799 + 0.014x$	0.013	0.935
	GSI	GST	$y = 0.882 - 0.233x$	−0.407	0.009	$y = 0.679 + 0.498x$	0.134	0.408
LC_{90}	CF	GSH	$y = 1.027 + 0.027x$	0.034	0.836	$y = 0.813 + 0.292x$	0.130	0.425
	CF	GST	$y = 1.045 - 0.022x$	−0.012	0.943	$y = 0.934 - 0.056x$	−0.026	0.873
	HSI	GSH	$y = 0.027 + 0.094x$	**0.499**	**0.001**	$y = 0.042 + 0.058x$	0.215	0.183
	HSI	GST	$y = 0.074 - 0.033x$	−0.075	0.648	$y = 0.061 + 0.003x$	0.014	0.934
	GSI	GSH	$y = 0.845 - 0.122x$	−0.168	0.299	$Y = 0.816 + 0.011$	0.012	0.941
	GSI	GST	$y = 0.809 - 0.040x$	−0.024	0.885	$y = 0.815 + 0.013x$	0.015	0.925

general, these compounds have a good margin of safety to most non-target biota, including invertebrates, fish, birds and another wildlife (Mulla, 1995). Although considerable research has evaluated the efficacy of these compounds against target pest populations, relatively little information is available concerning their toxicity on non-target organisms. *G. affinis* is widely used for biological control programs against mosquitoes (Kumar and Hwang, 2006). A recent publication provides an overview and highlights some of the important advances in our knowledge on mosquitofish (Walton, 2007). Both the two tested IGRs (Rehimi, 2004; Rehimi and Soltani, 1999) and mosquitofish (Bendali et al., 2001) are useful in a control strategy of mosquitoes. In situations where the chemicals will be present, this might interfere with the physiology of the mosquitofish and influence the success of mosquito control. Toxicological studies have focused on diverse subjects, including accumulation in the body, behavioral effects, physiological (enzyme, hormonal, biochemical) effects, morphological effects (growth and tissue studies), and reproductive effects especially those resulting from chronic exposure, population and ecosystem studies (Walton, 2007).

Growth has been used as an indicator for pollution stress in marine invertebrates (Widdows et al., 1995). In the present study, exposure to DFB and FCX has no significant effect on both growth, HSI and GSI in adult females of *G. affinis* during the exposure period. DFB at 0.2 ppm caused a temporary hyperactivity in mosquitofish (Ellgaard et al., 1979). Colwell and Schaeffer (1983) found that treatment of ponds with SIR-8514, a substituted benzamide, at a concentration of 7 ppb had no significant effect on growth rates or conditions factors in adult mosquitofish, but it altered their diets

and offspring. Miura and Takahashi (1974) reported that insect developmental inhibitors as TH-6040, Altosid and H-24108 affected some zoological groups of non-target organisms commonly found in mosquito breeding habitats, and *G. affinis* showed no effect at high dose levels. In contrast, treatment with 4-nonylphenol reduced significantly the growth of males and females of *Gambusia holbrooki* (Drèse et al., 2000). DFB was found to affect growth of juveniles of *G. affinis* (Drardja-Beldi and Soltani, 2003). The toxicity of DFB to *G. affinis* juveniles is comparatively less than the carbamates, organophosphorus compounds and pyrethroids (Walton, 2007); however, this insecticide is extremely toxic to aquatic invertebrates (Miura and Takahashi, 1974). When applied to the Kokawa river at the concentration of 1.25 ppm for 1 h to control simuliid larvae, DFB eliminated most of the invertebrates and no mortality was observed on fish (Satake and Yasuno, 1987). Recently, cadmium and another IGR (halofenozide) were found to affect the metric indexes after a 60 days exposure period in adult females of *G. affinis*, but the effects recorded are more marked on the HSI (Chouahda and Soltani, 2009). The condition factor helps to assess the experimental improvements in an environment for an existing fish and for the purpose of new stocking (Wootton, 1998). Data obtained in our study demonstrated that only FCX at LC_{90} reduced significantly the condition factor starting from day 21 of exposure onwards.

GSH is considered as one of the most important antioxidant agents involved in the protection of cell membranes against free radicals damage (Sies and Akerboom, 1984; Martinez-Alvarez et al., 2005; Lam, 2009). GSTs are a family of dimeric multifunctional enzymes that have been shown to be involved in

detoxification of xenobiotics, protection from oxidative damage, and the intracellular transport of hormones, endogenous metabolites and exogenous chemicals in diverse organisms (Zhou et al., 2009). DFB and FCX affected the GSH amount and GST activity. The observed effects varied as function the tested insecticide and the concentration. The response appeared more marked with FCX. The decrease in hepatic GSH might be explained by the existence of detoxification reactions against insecticide metabolites. Similar results have been obtained in fish and mussel exposed to different heavy metals or organic compounds (Regoli and Principato, 1995; Caseni et al., 1999; Drardja-Beldi and Soltani, 2003). The induction of GST activities was also reported in several fish species: *G. affinis* treated with cadmium (Souissi et al., 2008) or with halofenozide (Chouahda and Soltani, 2009), and *Anguilla anguilla* exposed to naphtalene (Teles et al., 2003) and *Jenynsia multidentata* to endosulfan (Ballesteros et al., 2009). In *G. affinis*, monocrotophos, an organophosphorous insecticide, induced an oxidative stress as measured by several antioxidant enzymes and affected the locomotor behavior (Kavitha and Venkateswara Rao, 2007). Recently, it has been reported that two commercial formulations of herbicides, containing clomazone and propanil, affected oxidative stress (acetylcholinesterase, catalase, thiobarbituric acid-reactive substances) and metabolic parameters (glycogen, lactate, glucose, proteins) in teleost fish (*Leporinus obtusidens*) after 90 days of exposure (Moraes et al., 2009).

The recovery study showed that this fish species was able to overcome relatively rapidly the stress induced by these insecticides. Fishes exposed to the high concentration and then transferred to untreated water, recovered with normal GST values after 1 day for DFB and 2 days for FCX. The amount of GSH required 2 days of recovery for DFB and more than 8 days for FCX. These pesticides induced an oxidative stress in *G. affinis*. Subsequently, this rapidly stimulated the antioxidant defences as evidenced by changes in biomarkers measured during the treatment and the recovery period. The response of *G. affinis* varied as function the concentration, the exposure time and the insecticide. The effects were relatively more marked with FCX as compared to DFB. This differential biological response of *G. affinis* may probably be due to a difference in penetration and/or metabolism of the two tested insecticides in the fish body. Indeed, in a topical bioassay with *T. molitor* using these two chitin synthesis inhibitors, the penetration rate through the cuticle was highest for FCX (Chebira et al., 2006) and this explained its higher toxicity (Soltani et al., 1993). In *C. pipiens* larvae, DFB appeared more toxic than FCX (Rehimi, 2004). As suggested by Schneider et al. (2003, 2004), the toxicity of these IGRs can be related to a high retention and stability as active compound in the body.

In conclusion, the results obtained on the effects by DFB and FCX in the non-target organism *G. affinis* revealed that these two compounds exhibited a slight toxicity. Only FCX at its LC_{90} affected the condition factor. Moreover, these compounds stimulated the detoxification system as evidenced by an increase of GST activities and an inhibition of GSH amounts. The recovery study showed that this fish species was able to overcome relatively rapidly the stress induced by these insecticides. The overall results indicated that these two IGRs exhibited only minor secondary effects on *G. affinis*, and so they have potential for controlling of mosquitoes in an integrated manner. However, before implementation, further studies for assessing the impact of these IGRs on the other non-target organisms are needed.

Acknowledgments

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