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RESEARCH ARTICLE

Cypermethrin Affects the Filtration Rate Ability and Oxidative Status of the Freshwater Mussel Unio Gibbus

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Abstract

This study aimed to investigate the harmful effects of insecticide Cypermethrin (CYP) on the filtration rate capacity and oxidative status and neurotoxicity of the freshwater mussel, *Unio gibbus*. The experiment was conducted in a laboratory setting using two CYP concentrations (C1 = 100 µg/L and C2 = 150 µg/L) over a 2-days period. Samples of the mussel's gills and digestive gland were taken after exposure. The levels of antioxidants (SOD, CAT), H_2O_2, and damage markers (MDA) were measured, along with the activity of the neurotoxicity enzyme, AChE. The results showed that CYP increased lipid peroxidation and antioxidant enzyme levels in the gills and digestive gland. The effects of different CYP concentrations on filtration rate of this mussel were determined in laboratory experiments by measuring the neutral red retention after 2, 4, and 72 hours of exposure. The results show that Cypermethrin impaired the valve activity in a concentration-dependent manner, leading to a reduction in the time of normal opening

Keywords: Pesticides, Cypermethrin, In vivo study, *Unio gibbus*, Biomarkers, Filtration rate

1. Introduction

Pyrethroids are a recent family of pesticides widely used in agricultural sectors (crop treatment and protection of stored products), public health (hygienic treatments), forestry, horticulture (to control ecto- and endoparasites), and some other sectors. This is due to their high insecticidal powers and low side effects on non-target organisms, particularly birds and mammals. Pyrethroids are characterized by their persistence for long periods in the environment and their resistance to photodegradation (Casida, 1980; Leng et al., 2005; Julien et al., 2008). Their persistence in the environment, coupled with their lipophilic nature, explains their bioaccumulation in wildlife through the food chain and their presence in several ecosystems (Feo et al., 2010; Hernán et al., 2013). The intensive use of these compounds is causing serious problems for aquatic fauna (Sereda et al., 2009; George et al., 2011). Therefore, environmental monitoring based on toxicological research has intensified in recent years in order to develop diagnostic strategies to understand the interactions between pesticides and aquatic biocenoses. Thus, research using contaminant analytical techniques has become increasingly reliable, achieving increasingly lower detection limits, regardless of the matrix considered (water, sediment, and living organisms) (Feo et al., 2010; Cayo et al. 2012; Celis et al., 2013). However, although these methods are essential for assessing the presence of contaminants in the environment, they do not provide information on the real impact of chemical molecules on living organisms. Therefore, the study of biological responses to pollutants seems essential in order to estimate the risks posed by pollution to the health of organisms and the integrity of the ecosystem. The use of chemical measurements combined with biological

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responses seems to be a promising alternative in understanding environmental problems caused by pesticides. Nowadays, this integrated approach has attracted the attention of several researchers (Amiard et al., 2006; Viarengo et al., 2007; Gagne et al., 2008, Buffet et al., 2012; Canesi et al., 2012). Among the biological responses used in the detection of environmental disturbances, biomarkers have been developed as tools for monitoring and evaluating the environment. Their use is closely linked to the knowledge of the molecular mechanisms and processes involved in the responses of organisms to contaminants. Biomarkers have the advantage of detecting early changes caused by a given toxicant in organisms. These modulations, which may be genetic, enzymatic, tissue or functional in nature, make it possible to predict the consequences of pollutants on the entire ecosystem. The study of the impact of pollutants on organisms requires the use of sentinel species such as bivalve mollusks (Sasikumar et al., 2006; Pan and Wang, 2012). In Tunisia, most coastal monitoring programs have used the marine mussel (Mytilus galloprovincialis), the clam (Ruditapes decussatus) (Dellali, 2001; Dellali et al., 2004; Sellami et al., 2014), Bolinus brandaris and Hexaplex trunculus (Abidli et al., 2012; Abidli et al., 2013). These studies have allowed the validation of these mollusks as highly relevant tools for detecting environmental disturbances affecting lagoon and coastal environments. In Tunisia, although several mollusk species have been validated as sentinels for environmental disturbances affecting marine environments, no species has been validated as a biosurveillance tool for continental aquatic ecosystems despite their rich fauna (Khalloufi and Boumaıza, 2005).

2. Materiels and Methods

2.1 Sampling Site Presentation (Fig. 1)

The Sedjenène river is a permanent endorheic watercourse that flows into Lake Ichkeul in northern Tunisia. It covers an area of 372 km2 (Ministry of Agriculture, 2000) between Cap Serrât and Garaat Ichkeul, with a length of 68.4 km (Zouabi, 2003). The river is located at an altitude of 7 m downstream from the Sedjenène dam on the MC51 road (37°11.603'N; 009°34.764'E), close to a settlement called "Sidi Salah Bougabrine". This watercourse has clear water, low salinity (1‰ < S < 2‰), and low current speed (current velocity < 10 cm/s) for a long period of the year (Khalloufi and Boumaïza, 2005). At the collection site (Fig.13), the substrate consists of mud, coarse sand mostly, gravel, and blocks in some places (Khalloufi and Boumaïza, 2005). The vegetation is composed of rushes, reeds, algae, and pondweeds. The nature of the substrate and the type of vegetation create a favorable biotope for the proliferation of bivalves, which benefit from the environmental conditions and find their ecological preferences (Khalloufi and Boumaïza, 2005).The upstream of the Sedjenène river comes from the Cap-Serrât region, an area with low agricultural and industrial activity and characterized by small settlements. This makes our site a relatively clean environment and far from any source of pollution. This advantage justifies the choice of the station and allows the bivalves from this environment to be considered as witnesses.

2.2 Mussel Sampling and Experimental Design

The individuals collected were transported in a cooler in order to be distributed into oxygenated aquariums

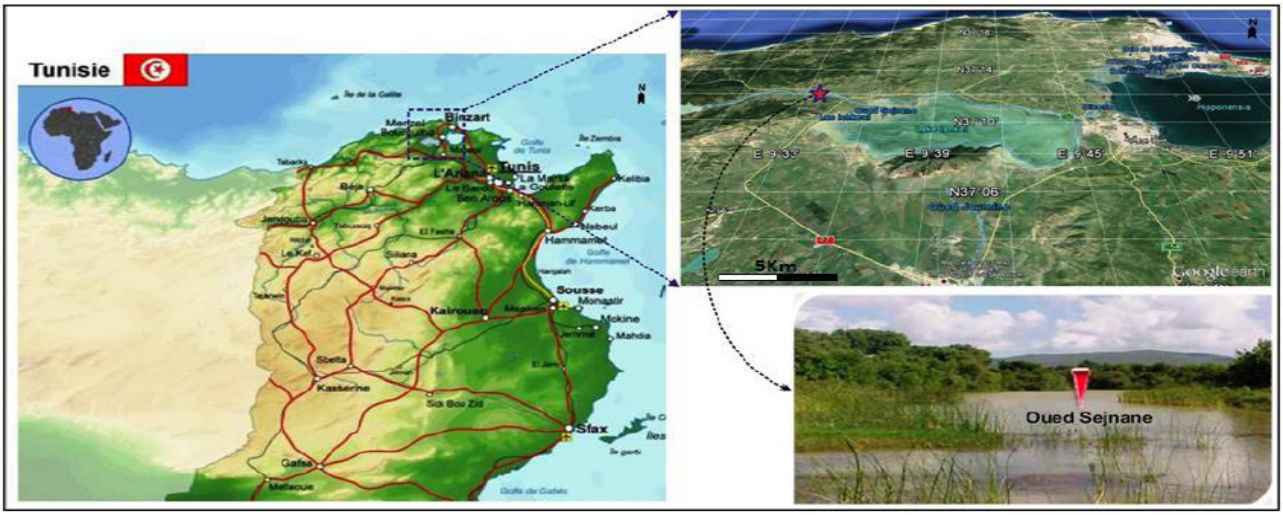

Figure 1. *Localization of the sampling site.*

upon their arrival at the laboratory (about 10 individuals per aquarium). The aquariums, containing 3 L of freshwater collected from the site, were maintained under controlled temperature conditions (19°C/day) and photoperiod (16 hours of light and 8 hours of darkness). Daily water renewal was carried out to prevent the accumulation of organic matter in the aquariums and the possible appearance of nitrogen compounds such as ammonium, nitrites, or nitrates.

After the acclimatization period, the specimens were distributed into 18 oxygenated aquariums (10 individuals per aquarium) using an air stone and at a temperature similar to that found in their natural environment at the time of collection. In order to determine the effect of cypermethrin concentration, two concentrations were tested: a low concentration C1 = 100 µg/L and a high concentration C2 = 150 µg/L. The effect of time was tested by considering two time intervals, 48 hours apart. At each water change, which was performed every two days, cypermethrin was administered again. Three replicates were considered for each condition tested, and the bivalves were distributed, for each time interval in this study, as follows:

- Three control aquariums containing only freshwater,
- Three aquariums contaminated with cypermethrin at a concentration of 100 µg/L,
- Three aquariums contaminated with cypermethrin at a concentration of 150 µg/L.

2.3 Measures of Biomarkers

The gills and digestive glands of 10mussels were separately placed on ice in stake boxes. Protein extraction was then carried out in a buffer composed of distilled water (500 mL), Tris/HCl (10 mM), sucrose (500 mM), EDTA (1 mM), and PMSF (1 mM) with a stabilized pH of 7.4. The tissues were homogenized in the buffer using an Ultra Turrax homogenizer (model : IKA T18 Basic) and then centrifuged (9,000 rpm, 4°C for 30 min) to estimate the "S9 post-mitochondrial fraction" containing the total proteins in the supernatant following the Bradford method (Bradford, 1976).

Neurotoxicity by iron ore was evaluated by measuring changes in acetylcholinesterase (AChE) activity, an enzyme responsible for the hydrolysis of acetylcholine into choline and acetic acid. AChE activity was assessed using the spectrophotometric method by reacting the thiol reagent 5,5'-dithiobis-2-nitrobenzoic acid with thiocholine to produce yellow-colored 5-thio-2-nitrobenzoic acid, as described by Ellman et al. (1961).

Superoxide dismutase (SOD) activity was assessed by the ability of the enzyme to inhibit auto-oxidation of pyrogallol (Marklund et al., 1982).

Wolff's method (1994) was utilized to ascertain the hydrogen peroxide (H2O2) levels. The supernatant (0.1 mL) was mixed with FOX1 reagent (900 mL) which includes 100 lM xylenol orange, 100 mM sorbitol, 250 mM ammonium ferrous sulfate, and 25 mM H2SO4. The solution was then vortexed and allowed to incubate at room temperature for 30 minutes. Subsequently, the sample was centrifuged at low speed for 3 minutes, and the absorbance of the supernatant was measured at 560 nm.

Catalase is an enzyme involved in cellular defense against oxygen-induced oxidative stress caused by radicals. Here, catalase activity was evaluated using the Aebi method (1984).

To determine the level of membrane lipid peroxidation, the method of Buege and Aust (1978) was utilized by measuring the amount of malonyl dialdehyde (MDA) produced as a result of lipid peroxidation. About 1 mL of the sample extract was combined with 2 mL of TCA-TBA-HCl reagent (15% (w/v) TCA, 0.375% (w/v) TBA, and 0.25 N HCl) and the mixture was then boiled for 15 minutes, allowed to cool, and subsequently centrifuged at 12,000 g to remove the precipitate. The absorbance was measured at 530 nm, and the concentration of MDA in the sample was calculated using the extinction coefficient of 1.56 × 105 M-1 cm. The lipid peroxidation was expressed as µmol of MDA per mg of protein

3. Estimation of Filtration Rate

Filtration rate was measured by tracking the disappearance of neutral red dye particles introduced into the water column in the presence of the animal (Coughlan, 1969). After exposure, two concentrations of cypermethrin (100 µg/L and 150 µg/L). Five mussels from each treatment were placed in 200 ml beakers (one mussel per beaker) containing 100 ml of a neutral red solution (1 mg/ml) protected from light. To monitor the filtration power, the measure of filtration rate was performed after 2, 4 and 72 hours.

3.1 Measurement of Filtration Capacity

To measure the filtration capacity, we adopted the method of (Coughlan, 1969). A water sample was previously taken from each beaker and condition to

determine the actual initial concentration of neutral red (C0). After each hourly interval, a water sample (10ml) was taken from each beaker and underwent acid attack (37% HCl). This acidification releases a pink coloration, and the remaining concentration of neutral red (Ct) was determined by measuring the absorbance at 530 nm. The filtration rate, expressed in mg/animal/h, was determined using the following formula (Coughlan, 1969):

$F = [M/n * t] * \log (C0/Ct)$

F : the filtration rate

M : the volume of the test solution

n : the number of mussel used

t : the duration of the experiment expressed in hours

C0 : the initial concentration (1mg/ml) of the neutral red dye

Ct : the final concentration of the neutral red dye

4. Data Analysis

The data was analyzed using a one-way ANOVA test, followed by a Tukey's post hoc test. Statistical significance was determined by setting $p < 0.05$. The data was expressed as the mean ± SE, and $p < 0.05$ was considered significant compared to the control mussel or CYP-treated mussels. The analysis was conducted using Graphpad Prism 5.

5. Results

5.1 Acetylcholinesterase (AChE) Activity

After 48 hours of exposure, the AChE activity recorded in the control mussels (Figure 2) was about 12.47 ± 1.34 and 11.67 ± 1.22 µmoles/min/mg of protein in the gills and digestive gland, respectively. In bivalves contaminated with both measured concentrations C1 = 70.22 or C2 = 112.5 µg/L, AChE activity was concentration-dependently inhibited in the gills. Thus, this activity was reduced to 6.51 ± 0.56 µmoles/min/mg of protein and 3.61 ± 0.18 nmoles/min/mg of protein in response to C1 and C2, respectively. The difference with controls was highly significant ($p<0.001$). Similarly, at the level of the digestive gland, AChE activity showed the same inhibition profile ; the difference with controls was significant ($p<0.05$).

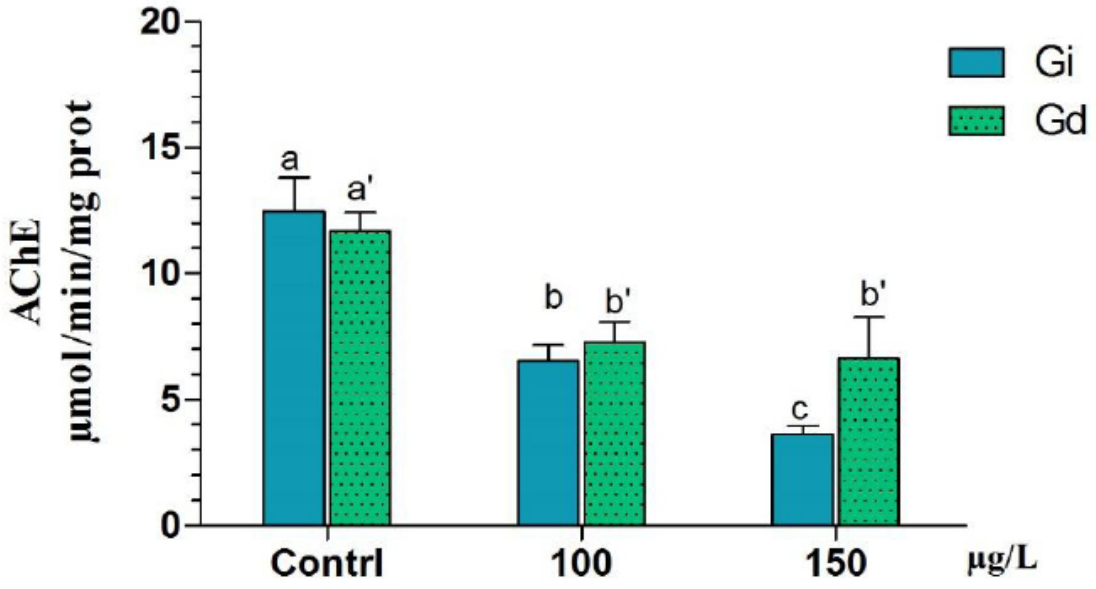

Figure 2. *Effects of cypermethrin concentrations on the specific activity of acetylcholinesterase (AChE) at the level of the gills (Gi) and digestive gland (Dg) of U. gibbus exposed, in vivo, for 48 hours. Data with different letters (a, a', b, b', c) represent significant differences compared to the control at $p < 0.05$.*

5.2 Superoxide Dismutase Activity (SOD)

Figure 3 illustrates the results related to the impact of cypermethrin on SOD activity recorded in gills and digestive gland after mussels were exposed for 48 hours. Indeed, in the gills, the activity increased from 23.17 Units/mg protein (value recorded in the controls) to 48.19 Units/mg protein (value recorded in those treated with C1). The exposure of mussels to C2 = 112.5µg/L decreases this activity compared to that recorded by C1, without exceeding the value recorded in the controls. The comparison of SOD activity in mussels treated with the controls by the Tukey HSD test reveals a significant increase ($p < 0.05$) in the activity of this enzyme after 48 hours of exposure.

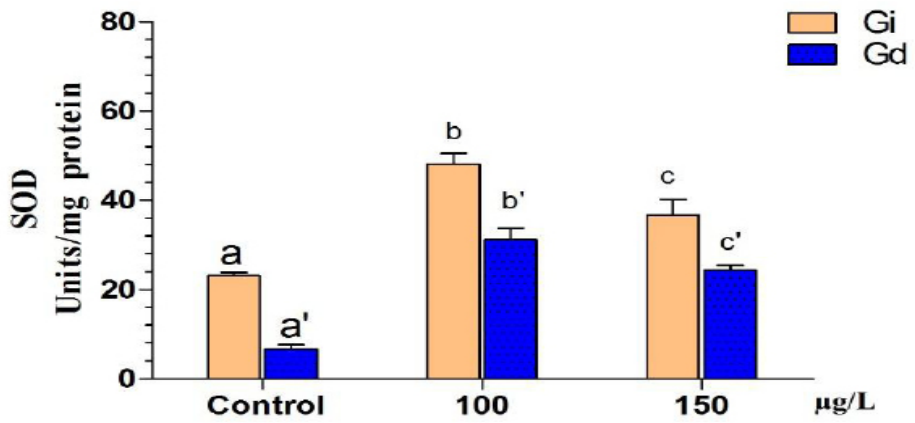

Figure 3. *Evolution of superoxide dismutase (SOD) activity as a function of cypermethrin concentrations in gills (Gi) and digestive gland (Dg) after 48 hours. Data with different letters (a, a', b, b', c, c') represent significant differences compared to controls at p < 0.05.*

5.3 Hydrogen Peroxide (H_2O_2) Levels

The estimation of hydrogen peroxide (H2O2) content by spectrophotometry was determined in gills and digestive gland (Figure 4). After 48 hours of exposure, the production of hydrogen peroxide increased by approximately 45% and 53%, respectively in the gills of mussels treated with 70.22 and 112.5 µg/L of cypermethrin compared to controls. In the digestive gland, the level of H2O2 increased by about 72% and 73% in mussels treated with 70.22 and 112.5 µg/L of cypermethrin, respectively.

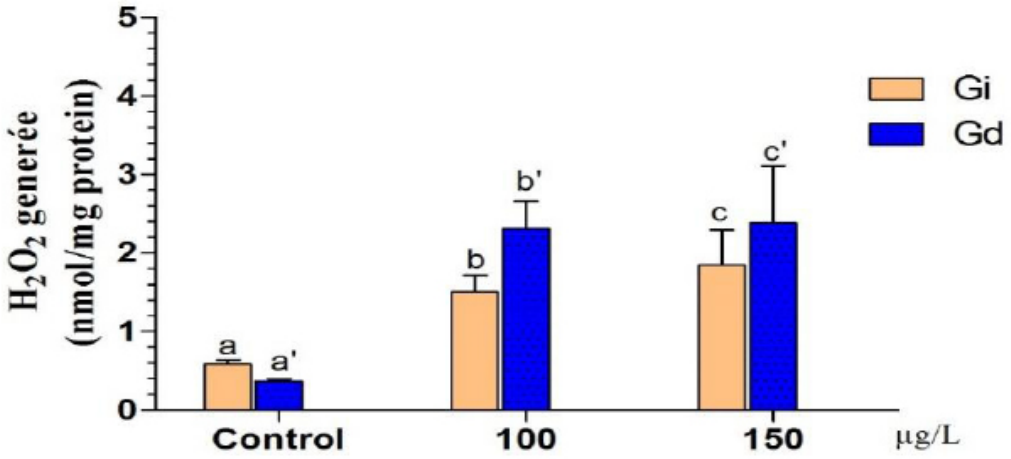

Figure 4. *Evolution of hydrogen peroxide (H2O2) concentration as a function of cypermethrin concentrations in gills (Gi) and digestive gland (Gd) after 48 hours. Data with different letters (a, a', b, b', c, c') represent significant differences compared to the control at p < 0.05.*

5.4 Catalase (Cat) Activity

After 48 hours of exposure, the CAT activity recorded in untreated mussels (controls) was around 6.287 ± 0.54 nmol/min/mg protein and 7.29 ± 0.32 nmol/min/mg protein in the gills and digestive gland, respectively (Fig.5). In contaminated individuals, CAT activity showed a modulation in both organs that followed a concentration gradient. Thus, this activity increased from 10.04 ± 1.56 nmol/min/mg protein to 13.51 ± 2.98 nmol/min/mg protein, respectively, in the gills and digestive gland in response to C1 = 70.22 µg/L of cypermethrin. The difference with controls was significant ($p < 0.05$). The activity of this biomarker showed a strong induction compared to controls following exposure to the concentration C2 = 112.5 µg/L of cypermethrin. Indeed, at the gill level, the C2 concentration induced catalase activity from 6.29 nmol/min/mg protein (the value recorded in control gills) to 20.09 nmol/min/mg protein in the gills of mussels contaminated with C2.

Similarly, for the digestive gland, this activity was 7.3 nmol/min/mg protein recorded for the controls, reaching a maximum value during exposure to the C2 concentration. The difference with controls tested by Tukey's HSD test is highly significant ($p < 0.001$) (Fig. 5).

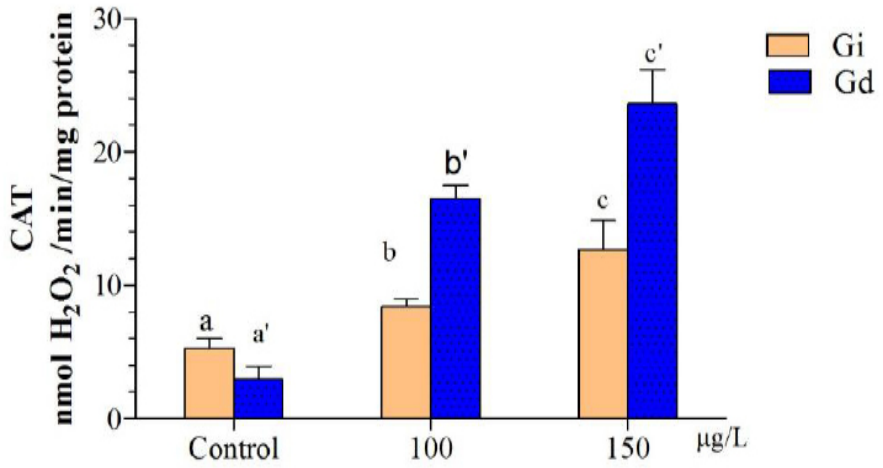

Figure 5. *Evolution of Catalase (CAT) activity as a function of cypermethrin concentrations in the gills (Gi) and digestive gland (Gd) of U. gibbus exposed in vivo after 48 hours. Data with different letters (a, a', b, b', c, c') represent significant differences compared to the control at p<0.05.*

5.5 Malondialdehyde (MDA) Levels

Lipid peroxidation has been widely defined as the oxidative degradation of polyunsaturated fatty acids in cell membranes. The formation of MDA as a secondary product of lipid peroxidation was monitored using substances reactive to thiobarbituric acid (TBARS). The results obtained after 48 hours of exposure are presented in Figure 6. For the gills, the MDA level increased in a concentration-dependent manner and the highest amount was measured in individuals exposed to C2 = 112.5 µg/L (10.07 nmole/mg of protein compared to 3.81 nmole/mg of protein recorded in control mussels) with a significantly high difference (p <0.001) between treated and control groups. In the digestive gland, only individuals exposed to C2 = 112.5 µg/L showed a 3-fold higher amount (p <0.001) compared to the digestive glands of control mussels with a level of 11.29 nmole/mg of protein compared to 3.60 nmole/mg of protein recorded in controls.

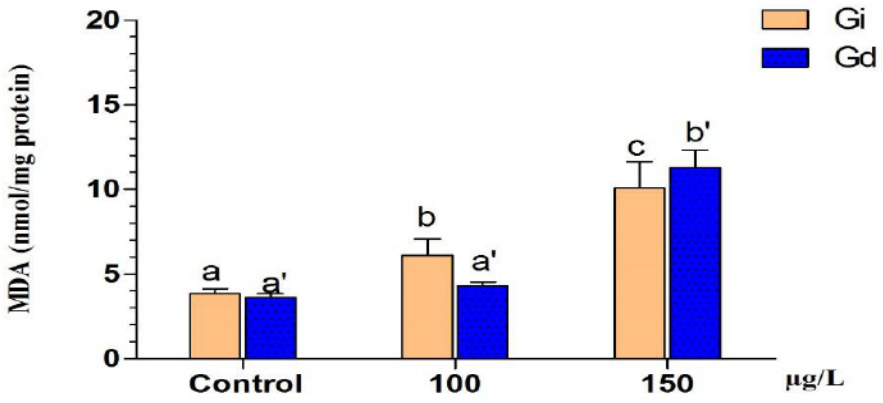

Figure 6. *The evolution of Malondialdehyde (MDA) levels as a function of concentrations of cypermethrin in the gills (Gi) and digestive gland (Gd) after 48 hours. Data with different letters (a, a', b, b', c, c') represent significant differences compared to controls at p<0.05.*

5.6 Effects of Cypermethrin on the Filtration Rate of Mussel

The monitoring of the potential impact of cypermethrin on the filtration capacity, which is an interesting parameter with strong ecological relevance, shows a susceptibility to influence the valvular activity (opening and closing movement of the valves) and ventilation of the mussel, which results in significant disturbances in the filtration rates. The monitoring of the filtration capacity in non-contaminated mussels (controls) showed a higher purification power amplitude, which varied very little over time, and the average filtration capacity was 173.9, 149.38, and 172.7 mg/animal/h respectively after 2, 4, and 72 hours.Exposure of mussels to a concentration of 100 µg/L is sufficient to disturb their filtration behavior, and a decrease in filtration capacity was observed after 4 hours of exposure. Subsequently,

when the cypermethrin concentration was increased to 150µg/L, filtration was still disrupted even with a short exposure time (2 hours), resulting in a decrease in filtration capacity that oscillated around an average of 127.15 to 45mg/animal/h, without showing a return to the filtration capacity observed in untreated mussels (Fig. 7).

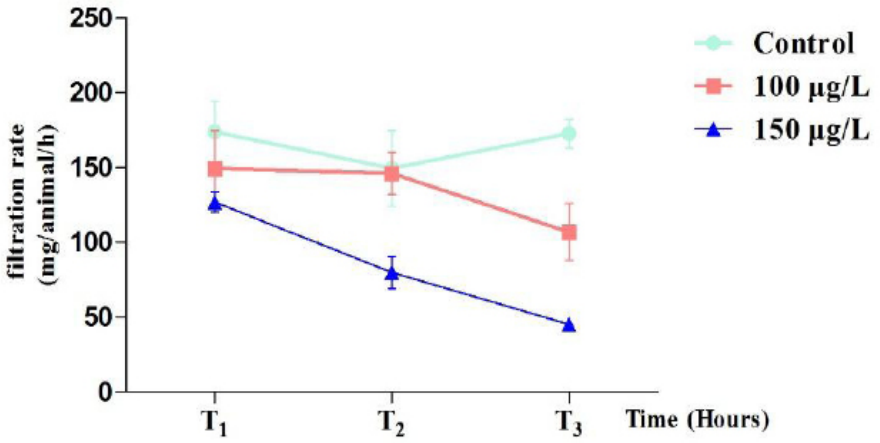

Figure 7. *Evolution of the filtration capacity in the freshwater mussel Unio gibbus following exposure to cypermethrin: Control ( ) ; 100 µg/L ( ) ; 150 µg/L( ). Filtration was measured after T1=2 hours, T2=4 hours, and T3=72 hours.*

6. Discussion

Studies of biological parameters involved in oxidative stress have shown the occurrence of oxidative stress in the intracellular environment following exposure to pesticides (Barzilai and Yamamoto, 2004). Therefore, the presence of these pollutants in the body is capable of inducing harmful effects on the actors of the cellular defense system. However, it is necessary to monitor the organism's response to this type of pollutant in order to highlight and track the different scenarios required in "biomonitoring" programs. Mussels are widely used in ecotoxicological studies and in biomonitoring programs of various aquatic environments (Halldórsson et al., 2007). Their sedentary nature, their considerable ability to concentrate pollutants in their tissues, and their mode of feeding through filtration justify their consideration as tools for evaluating pesticide toxicity (Carro et al., 2012; Suarez et al., 2013). In this study, we used the freshwater mussel U. gibbus to evaluate the effects of CYP on certain biological markers. The measurement of variations in enzymatic and non-enzymatic oxidative stress markers in this organism provides evidence of exposure to this compound and suggests a risk due to its toxic nature. It is therefore desirable to consider organs that allow extrapolation of the effect to the entire organism and consequently to the population. The gills are an important transfer pathway for cypermethrin in mussels as they are in direct contact with water and also play an important role in assimilating this compound. However, the storage and/or biotransformation of pollutants occurs mainly in the digestive gland. Antioxidants, in a broad sense, represent all molecules capable of directly inhibiting the production and limiting the propagation or destroying reactive oxygen species ROS (Lei et al., 2011). One of the first defense mechanisms available to mussels to counter exposure to CYP is the induction of their antioxidant defense system in these two organs. This self-protection mechanism thus increases the chance of neutralizing cypermethrin in the spaces where this compound diffuses and therefore reduces its toxic effect.Several studies have examined the impact of pyrethroids on AChE, and in general, a reduction in AChE activity has been observed after exposure to sublethal concentrations (Szegletes et al., 1995 ; Kumar et al., 2009). In this study, we have shown that AChE activity decreases gradually following exposure of the freshwater mussel Unio gibbus to different concentrations of CYP. Inhibition of AChE activity in the gills and digestive gland is time-concentration dependent. Our results are consistent with the work of Toumi et al. (2015), which showed inhibition of AChE activity in the crustacean Daphnia magna treated with deltamethrin. Similarly, inhibition was observed in the mussel Dreissena polymorpha treated with chlorpyrifos and terbutilazine (Binelli et al., 2006) and the rat treated with deltamethrin (Yousef et al., 2006; Hend et al., 2013). In addition, the exposure of the freshwater mussel Unio gibbus to cypermethrin generates oxidative stress that can result from the increased production of reactive oxygen species (ROS) induced by CYP. The toxicity evaluation monitoring of CYP after 2 days of

exposure showed, in the gills, that hydrogen peroxide (H2O2) production increases with increasing concentrations (45% and 53% at 100 and 150 µg/L, respectively) and the same trend was observed in the digestive gland (72% and 73%, respectively). These results indicate that the sensitivity of the mussel is accentuated with the exposure concentration, reflecting the delicate nature of its organs. Catalase (CAT) is an antioxidant enzyme involved in cell defense against the toxic effects of hydrogen peroxide (H2O2) by catalyzing its decomposition into water (H2O) and oxygen (O2). CAT, a biomarker of oxidative stress, is sensitive to certain contaminants such as CYP (Uner et al., 2001; Pi et al., 2010). In the present study, exposure of the Unio gibbus mussel to CYP resulted in a significant increase in CAT activity after 2 days of exposure in a concentration-dependent manner. The induction of specific CAT activity, correlated with SOD, constitutes the first line of defense against oxidative stress. The increase in CAT activity in the mussel compared to controls suggests the elimination of H2O2 resulting from exposure to CYP. Numerous studies have shown changes in CAT activity during exposure to CYP. Shi et al. (2011) reported that CYP can induce a concentration-dependent increase in CAT activity in zebrafish. A dose-dependent increase in CAT activity was observed in rat tissues exposed to CYP (Nasuti et al., 2003). Comparable results were reported in the clam Ruditapes decussatus exposed to different concentrations of permethrin, highlighting a concentration-dependent induction of CAT activity (Sellami et al., 2014). Membrane lipid peroxidation is a chain reaction that occurs in three steps: (1) initiation, which involves the attack of a polyunsaturated fatty acid by a free radical, (2) propagation, which involves the peroxidation of neighboring phospholipids, and (3) the reaction ceases when a molecule traps the free radicals (Kanbur et al., 2008). This reaction leads to the formation of cytotoxic and mutagenic products such as hydroperoxides or malondialdehyde (MDA) (Alpha Jalloh et al., 2009). In the present study, the level of malondialdehyde (MDA) also increased in the gills and digestive gland. The MDA level was more pronounced when mussels were exposed to a high concentration of CYP. The remarkable induction observed in the targeted organs suggests that the freshwater mussel is under greater oxidative stress, likely due to the flow of radicals caused by high concentrations of CYP. Many studies have indicated that the level of lipid peroxidation is an important parameter evaluating the level of oxidative stress in living organisms when exposed to CYP (Kale et al., 1999; Viran et al., 2003). These results suggest that the production of ROS exceeded their decomposition by antioxidants, thus depleting trapping mechanisms and leaving cellular constituents (lipids and proteins) attacked by overproduction of reactive oxygen species. This finding is in correlation with previous studies that have shown that CYP is a ROS stimulator involved in cellular damage and decreased levels of GSH (Kanbur et al., 2008; Taju et al., 2014).Like most bivalve mollusks, the freshwater mussel Unio gibbus is a suspensivore organism : it consumes particles suspended in the water column that it filters through its gills. The study of filtration capacity is therefore essential to determine the amount of energy that the animal obtains from the environment in the form of particulate organic matter. Filtration is defined as the volume of water that is completely cleared of suspended particles per unit of time. This rate is different from the pumping rate, which is the total volume of water that passes through the gills. Like respiration, the study of filtration has been approached in the laboratory using different techniques. The freshwater mussel is a filter-feeding organism that feeds on plankton and organic waste, accumulating particulate matter (MES) and bacterial load directly from the water column. This mode of nutrition makes the mussel an interesting species as a biological filter that plays a significant role in regulating the density of microscopic algae (phytoplankton) in the aquatic phase. During filtration, the mussel retains particles by size and weight. It is at the level of the gills that they retain almost all particles with a diameter between 3 and 5 µm and about half of those with a diameter between 1 and 2 µm (Barnabé, 1989). The gills are like a sieve, with mesh made up of frontal lateral cilia covered in a layer of sticky mucus that traps particles smaller than the mesh size. As a result, the freshwater mussel can be a limiting factor in the growth of phytoplankton and microbiological communities in the water column. Therefore, the mussel plays an important role in reducing the density of suspended matter (Cranford et al., 2003) and contributes to the reduction of water turbidity through filtration. In this situation, mussel filtration promotes increased transparency and improved water quality. However, Jones et al., 2001 found that the introduction of mussels into areas affected by eutrophication can limit this phenomenon. Additionally, several environmental factors such as extreme temperature values and pollution can affect or impair the filtration capacity of bivalves (Paul et al., 2008). The study of the influence

of physical parameters on the evolution of nutritional behaviors of bivalves is a necessary step towards a better understanding of the functioning of aquatic ecosystems. In the present work, filtration capacity was chosen as an effect parameter as it has been described as a suitable sensitive criterion to improve the understanding of physiological responses to chemical stressor fluctuations in the freshwater bivalve Unio gibbus. In this study, monitoring the filtration capacity of freshwater mussels exposed to two concentrations of CYP (100 and 150 µg/L) shows that filtration capacity is time- and concentration-dependent. Thus, at 100 µg/L of CYP, the reduction in filtration begins after 4 hours of exposure and becomes minimal after 72 hours of exposure. This reduction begins after 2 hours of exposure and shows minimal values after 72 hours. In addition, an increase in mucus secretions in the environment was observed. This would be an immediate response of the mussel to exposure to a high concentration of CYP (150 µg/L). The decrease in filtration rate observed in this study can be explained by partial and temporary closure of the shells. Moreover, CYP can cause tissue irritation and stimulate the contraction of the adductor muscle, leading to temporary closure of the mussel's siphons. Our results indicate that the filtration rate is a sufficient sublethal criterion to assess the toxic effects of CYP on U. gibbus. The effects of cypermethrin observed in this study are consistent with previous results reported on Mytilus edulis with the same chemical (Gowland et al., 2002). These authors showed that 25% and 92% of mussels closed their valves after 1 hour of exposure to 100 and 1000 µg/L of CYP, respectively. The closure of the siphons at high concentrations of cypermethrin showed similarities with the response represented by the freshwater mussel Anodonta cygnea to deltamethrin, a contaminant that reduces filtration capacity (Kontreczky et al., 1997). Similar results have shown that cypermethrin reduces siphon closure in a concentration- and time-dependent manner in the marine mussel Mytilus galloprovincialis (Ait Ayad et al., 2011). These authors showed that the overall sensitivity of siphon closure is related to the duration of exposure. Cypermethrin exerts its neurotoxic effect through the voltage-dependent sodium channel of the neuronal membrane, which is the target site (Soderlund and Bloomquist, 1989), and the integral ATPase protein of the neuronal membrane (Kakko et al., 2003). This channel could be kept open under the action of cypermethrin, thus causing depolarization of the membrane, repetitive discharge, and synaptic disturbances leading to hyperexcitation of the target organism. This mechanism of action can be the starting point for understanding the inhibitory action of cypermethrin on siphon closure, which results in a decrease in the filtration capacity of mussels.

7. Conclusion

Our research indicates that exposure to synthetic pyrethroid CYP at non-lethal levels can disrupt the oxidant/antioxidant balance in the gills and digestive gland of the freshwater mussel U. gibbus. This disruption can cause oxidative stress and neurotoxicity. We observed an increase in the activity of antioxidant enzymes SOD and CAT, as well as levels of H2O2. Additionally, we found that the level of MDA, which measures lipid peroxidation, indicates the harmful effects of this insecticide. The study of the effects of cypermethrin on a physiological parameter, "filtration capacity" in U. gibbus, has demonstrated that this bivalve responds to cypermethrin contamination by reducing its exposure to the contaminant, by decreasing the time during which it leaves its valves open.These results suggest that measuring the levels of antioxidant parameters and physiological parameter in U. gibbus can be useful biomarkers for monitoring CYP exposure in North African streams.

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9. References

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