Arch Environ Contam Toxicol (2011) 60:462–470
DOI 10.1007/s00244-010-9549-7

Effects of Cypermethrin (Pyrethroid Insecticide) on the Valve Activity Behavior, Byssal Thread Formation, and Survival in Air of the Marine Mussel *Mytilus galloprovincialis*

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Received: 25 January 2010 / Accepted: 10 May 2010 / Published online: 28 May 2010

Abstract Cypermethrin is a pyrethroid pesticide widely used to combat agricultural pests. However, little information is available about its toxicity in aquatic macroinvertebrates in the context of the Moroccan coastline. In this study, a suite of physiological and behavioral biomarkers for water quality surveillance using mussels are used to characterize the toxicity of Cypermethrin. In these tests, sublethal effects on valve activity behavior, byssal thread formation, and survival in air of the mussel *Mytilus galloprovincialis* were assessed in short-term 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. The lowest effect concentration was determined as 100 μg/l Cypermethrin. Prolonged closures of mussels were also observed for the exposures to 400 and 800 μg/l. The delay to the first detection of the pesticide was concentration dependent. The capacity to produce new byssus threads in a Cypermethrin exposure was inhibited at all concentrations and especially at high concentrations. Survival in air was not affected even at 800 μg/l Cypermethrin.

Synthetic pyrethroids represent the third largest class of chemical insecticides after organophosphates and chloronicotinyl insecticides, with a share of 15% of the global foliar and soil insecticide markets (Wirtz et al. 2009). These compounds have gained popularity over other insecticides due to their high effectiveness against target species, relatively low mammalian toxicity, and rapid biodegradability (Davies 1985). Cypermethrin, the α-cyano-3-phenoxybenzyl 1-*cis*,*trans*-3-(2,2-dichlorovinyl)-2,2 dimethylcyclopropane carboxylate, is the most widely used pyrethroid pesticide to control many pests, including moths, pests of cotton, fruit, and vegetable crops. It interacts with the Na^+ channels in nerve cells through which sodium enters the cell in order to transmit a nerve signal. These channels can remain open for up to seconds, compared to the normal period of a few milliseconds, after a signal has been transmitted. The effect is that of long-lasting trains of repetitive impulses in sense organs that can eventually lead to death of an organism (WHO 1989). Cypermethrin like other pyrethroids have a short half-life in the water column (generally less than 2 days) and rapidly adsorb to suspended particulate material and sediments, which greatly reduces their bioavailability to water column organisms (Hooper et al. 2003). However, the potential exists for benthic organisms like *Mytilus galloprovincialis* to be exposed by direct contact or ingestion of contaminated suspended matter and sediment particles. Moreover, several studies have shown that aquatic invertebrates and fish are extremely sensitive to Cypermethrin and pyrethroids in general (Köprücü and Aydın 2004; Mian and Mulla 1992; Saha and Kaviraj 2008; Srivastav et al. 1997; Svobodova et al. 2003; Tidou et al. 1992). In order to evaluate these effects, biomarkers that measure sublethal effects and changes on the biochemical, cellular, physiological, and behavioral levels have been used as effective tools of ecological risk assessment in marine environment monitoring (Livingstone et al. 2000).

Among behavioral markers, the valve activity behavioral test, which estimates the mussel's ability to close its shell as an alarm signal in response to a contaminant, is widely recognized as an integrative sublethal measure of physiological rate functions (Markich 2003). It was proposed as a reliable

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toxicity end point for assessing water quality (Baldwin and Kramer 1994; Borcherding 2006; Kramer 2009; Kramer and Foekema 2000). This test has been used in field studies as a biological early warning system (Borcherding 2006; de Zwart et al. 1995; Kramer and Foekema 2000) as well as in laboratory studies (Borcherding and Wolf 2001; Curtis et al. 2000; Markich et al. 2000; Tran et al. 2004).

Byssal threads production is considered a physiological biomarker that assesses the effect of toxicant on byssogenesis. It is based on the ability of mussels to reattach themselves by producing fresh byssus threads (Clarke and McMahon 1996; Rajagopal et al. 2002). By doing so, the mussels exhibit increased foot activity (Lee et al. 1990) and it was reported that frequent extension of the foot increases the exposure of the soft body parts to the external environment (Rajagopal et al. 2005).

The survival in air or "stress on stress" response (SOS) is considered as a possible index of general stress at the organismal level that might be used as a monitoring tool for the assessment of contaminated coastal areas (Viarengo et al. 1995). The test measures the time that mussels can survive when removed from water. Although removal from water for several days is unlikely to occur in the natural environment, the method has been used as a way of testing the relative fitness of mussels in a number of studies (de Zwaan et al. 1995; Nesto et al. 2004; Thomas et al. 1999b; Veldhuizen-Tsoerkan et al. 1991; Viarengo et al. 1995).

In Morocco, the coastline is economically important and ecologically very sensitive. However, it has been subjected to increased pesticide contamination, mainly from agricultural practices in the adjacent agricultural areas that can threaten their sustainability. Consequently, the need for consistent methods of environmental assessment of this important source of pollution is particularly required. So far, Moroccan research on the indigenous bivalve species *M. galloprovincialis* (Lamarck, 1819) and *Perna perna* (Linné, 1758) essentially focused on the biomonitoring of metals contamination of the coastal areas (Banaoui et al. 2004; Chafik et al. 2001; Cheggour et al. 1999; Maanan 2007, 2008). A few other studies assessed the changes in biochemical markers such as acetylcholinesterase (Najimi et al. 1997) and glutathione *S*-transferases (Kaaya et al. 1999) as a response to field pollution. However, data on the toxic effects of organic contaminants are still lacking. Moreover, no study had addressed the physiological or behavioral effects of these contaminants.

The purpose of the present work is to assess the sublethal toxicity of the pyrethroid insecticide Cypermethrin on the mussel *M. galloprovincialis* as an indicator of effects on the Moroccan coastal fauna. It is also aimed at selecting the most sensitive and relevant test in the biomonitoring of these effects.

Methods and Materials

Collection of Animals and Acclimation

Mussels *M. galloprovincialis* (average shell length = 45 ± 5 mm) were collected once a month at Cap Beddouza, Morocco (32°33′, 582N–09°15′, 813W) between January and March 2007 and transported to the laboratory within 3 h. The choice of Beddouza as a reference area was supported by previous chemical biomonitoring data (Banaoui et al. 2004; Cheggour et al. 1999; Maanan 2007).

The mussels attached to beach rocks were collected manually from the (infra) littoral zone by gently cutting their byssus threads using a pair of scissors. A volume of seawater of 1.2 m^3 was collected at the same time of mussels sampling to be used as acclimation and test medium. In the laboratory, byssus threads were cut without damaging the byssal trunk and cleaned from epibionte fauna. Then one valve of each individual was cemented with a nontoxic glue, Araldite (Ciba Polymers, UK), to a glass support, the other being free. In preparation of the valve activity experiments, a metallic piece (1 cm^2) was glued to the free valve as a part of the valve sensing system (Fig. 1). The mussels were then placed for acclimation in a glass tank containing 100 l aerated, unfiltered seawater for a minimum 48 h with a 12-h light:dark regime. The seawater was renewed twice a day (8:00 a.m. and 8:00 p.m.) and the temperature was maintained as close as possible to the sampling site temperature with variation in the range of 1°C. A preliminary study showed that this acclimation procedure was sufficient for the animals to adapt to indoor conditions and to attach by new byssus threads to the support, which is the required condition for the survival in air and valve activity tests. Under these conditions, mussels that did not attach themselves to the glass supports by new byssus threads were considered to be in poor condition and were removed.

Nine parameters were analyzed in water. Temperature, pH, and total salinity (Model 1116000-ORION) were measured in situ. Dissolved oxygen was measured according to the protocol of Winkler adapted to seawater. Suspended particulate matter (SPM) was estimated by differential weighing before and after filtration (Whatman 0.7 μm) in a determined volume of water. Phosphate, chloride, nitrate, and sulfate analyses were performed according to the methodology of Aminot and Chaussepied (1983). The means for water parameters were as follows: 18.7 ± 0.7°C, 35.4 ± 1.3‰ salinity, 8.3 ± 0.1 pH, 7.9 ± 0.2 mg/l dissolved oxygen, 16.9 ± 2.1 mg/l SPM, 247.2 ± 3.2 μg/l phosphate, 21.3 ± 0.9 g/l chloride, 128 ± 24 μg/l nitrate, and 1.9 ± 0.2 g/l sulfate.

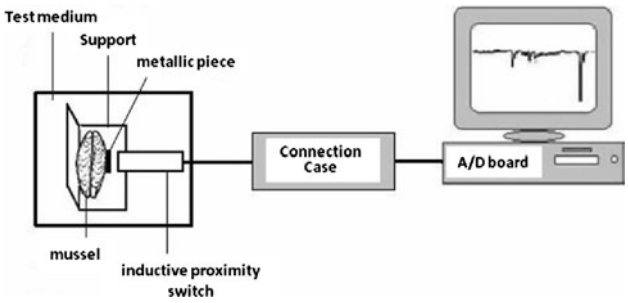

Fig. 1 Experimental setup of valve activity measurement

Valve Activity Test

Technique

Valve activity was monitored using an inductive proximity switch (Contrinex), which allows a continuous monitoring of the valve movements without disturbing the behavior of the mussels. The technique, which displays all of the activity figures from full closure to wide opening of the shell valves, is based on the modification in the inductance of a proximity switch placed in front of a metallic piece carried by the free-moving valve. The distance between the switch and the metallic piece was set at 4 mm to maximize the signal (Fig. 1), which is proportional to the valve opening amplitude (the full opening range of the mussels was ~4 mm). The signal is amplified and transferred via an A/D converter (Fastlab; Eurosmart) to a computer and saved. A rate of one reading per second was used to accurately measure the most subtle valve movement patterns. The relative position of the shell valves (between closed and open) was readily displayed graphically on the screen for direct observation. The constructed device could support eight individuals in each experiment.

Test Procedure

The toxicity tests were carried out on eight mussels × three replicates × six concentrations (both control and exposed mussels). The test medium (10 l) was continuously aerated during experimental assays.

Test concentrations (50, 100, 200, 400, and 800 µg/l) were prepared from a stock solution (1 g/l) of Cypermethrin, obtained from United Phosphorus Ltd. under the commercial name of Cyrux 25 EC (25% active ingredient). This range of concentrations was based on preliminary screening tests on the valve activity response to the toxicant.

The experimental procedure consisted of recording the valve activity over a period of 270 min. Prior to contamination, a 30-min recording was made to allow a maximum opening of the mussel that is used as the reference state for the behavioral response. In the beginning of the test, the minimum valve position (fully closed) was assessed by gently tapping the mussels with a fine brush (~5 s) to make them close their shell. Within 5–10 min, the mussels would reopen, allowing the maximum valve position (fully open) to be determined in the course of the following 30 min. Then the test concentration was added in a single step to the seawater and mixed by a magnetic stirrer. Because of the inherent individual variability in valve movements, each animal was its own control in all experiments.

Data Analysis and Toxicity Criteria

The acquired data files were transformed into Excel format and used to provide a graphical presentation of the valve activity and to determine the maximum (full opening) and minimum (closure) values. All valve positions less than 50% of the full opening value were interpreted as corresponding to stress activity (Ait Fdil et al. 2006). In regard to the changes in the behavioral figures between normal and stress activities, two toxicity criteria were set to evaluate the mussel response to increasing concentrations of the pesticide. The first was the reduction in the time of normal valve opening and the second was the increase in the frequency of valve adductions. An appropriate Excel-based program was developed to compute data and calculate the percentage of the normal opening time and the number of valve adductions at each interval of time.

For the reduction in the time of normal valve opening, the valve reactions were summed for 10-min intervals, starting from the beginning of the exposure to 4 h (0–10, 10–20, 20–30, 30–40, 40–50, 50–60, 110–120, 230–240 min), to differentiate between fast and delayed responses. The effects on normal opening were evaluated by computing the time required to have 15, 50, and 85% of mussels responding. The overall sensitivity of the mussels as related to the time of exposure was estimated by a dose-response model that calculates the effective concentrations to have the same rates of responding animals ($EC_{15\%}$, $EC_{50\%}$, and $EC_{85\%}$). For the frequency of valve adductions, the valve reactions were calculated as a sum of events in progressive periods of time (0–10, 0–20, 0–30, 0–40, 0–50, 0–60, 0–120, 0–240 min).

Survival in Air Test

Mussels were exposed for 24 h to Cypermethrin (10 individuals × 6 concentrations including control × 3 replicates). At the end of this period, control and exposed mussels were subjected to anoxia by air exposure at constant temperature (18 ± 1°C) in humidified chambers. Survival was assessed twice a day. Death symptoms were considered to be open valves and absence of muscular activity (Thomas et al. 1999b; Veldhuizen-Tsoerkan et al.

1991; Viarengo et al. 1995). Death counts were recorded until 100% mortality was reached.

Byssus Threads Production

The number of byssus threads produced by the mussels was determined following the method described by Van Winkle (1970), Rajagopal et al. (1995), and Masilamoni et al. (1997). At the arrival to the laboratory, a total of 180 mussels were selected for the experiment. They were then glued one by one on the right side of their body to a glass support and kept in 10-l glass beakers with static seawater from the reference area (control) and at five different nominal concentrations of Cypermethrin (10 individuals × 6 concentrations including control × 3 replicates). The number of byssus threads produced was counted after 24 h by microscopical observations using a hand lens and expressed as number of threads per mussel per day.

Data Analysis

Mean values for the valve activity and byssus threads production tests were tested for significance compared to control groups using the Mann–Whitney test ($\alpha = 0.05$). These analyses were carried out using the STATISTICA software (Microsoft). For the survival in air test, the median survival time (LT_{50}) with an associated 95% confidence interval was calculated for each collection site group using a trimmed Spearman–Karber method ($\alpha = 0.05$) (Hamiliton et al. 1977). Differences in survival between exposed and control groups were tested for significance with nonoverlap of 95% confidence intervals.

Results

Valve Activity Test

Normal Activity

A previous study of the valve movements of *M. galloprovincialis* showed that under normal conditions the mussel is widely open and continuously filtering (98.51 ± 0.19% of the time; Ait Fdil et al. 2006). Some occasional and rare adductions were also observed (2.66 ± 0.35 h^{-1}), whereas no rhythm of activity that would be related to the daily or the seasonal cycle was observed.

Stress Activity Related to Cypermethrin

Effects of Cypermethrin on time of normal opening When exposed to Cypermethrin, mussels react by reducing their valve opening and displaying increased intermittent valve adductions. Depending on the pesticide concentration and time of exposure, the displayed behavioral figures are rapid and repetitive adductions, intermittent and short closures (less than 1 min), and the prolonged closing of valves (several minutes). Full and continuous closure is the ultimate response of the mussel to an intense stress as for 400 and 800 µg/l Cypermethrin.

The activity recordings showed that Cypermethrin induced a profound impairment of the valve opening either by reducing the extent of valve gape and by the appearance of closing periods compared to normal behavior. This effect is concentration dependent, as shown by the gradual response of the mussels to the toxic stress. The time of exposure was also decisive in the appearance of the stress activity as shown by the percentage of bivalves that reacted in the range of 0–10, 10–20, 20–30, 50–60, 110–120, and 230–240 min (Fig. 2). For example, after a 10-min exposure period, the percentage of closure increased from ~6% at 50 µg/l to ~19% at 800 µg/l. After a 240-min exposure period, the percentage of closure increased from ~13% at 50 µg/l to ~85% at 800 µg/l. Moreover, the percentage of response was strongly related to the time of exposure, increasing noticeably by time. At a Cypermethrin concentration of 200 µg/l, the percentage of reacting bivalves increased from 14 to 52% as related to increasing time exposure from 10 to 240 min. The results show that, in general, the effect of the time is important at concentrations higher than 100 µg/l.

The relationship between valve closure response and time is presented in Table 1. It expresses the time (T in minutes) required to obtain the bivalve response to a nominal Cypermethrin concentration. T varies from 15 to 85%. For example, $T_{50\%}$ (time for 50% of bivalves to react to a single concentration) was 238 min for the detection of 100 µg/l, whereas it was just 51 min for 800 µg/l.

Effects of time on the detection occurrence of Cypermethrin The percentage of valve responses as a function of the Cypermethrin concentrations (0, 50, 100, 200, 400, and 800 µg/l) is presented in Fig. 3. It shows that the delay of first detection decreased with Cypermethrin concentration and the sensitivity was strongly dependent on the time of exposure. In general, a minimum of 30 mins was required to induce the first valve closure. No difference was observed between mussels for higher concentrations of Cypermethrin, especially for 400 and 800 µg/l.

The EC values (effective concentration that induce valve closure reactions) for each interval of exposure are presented in Table 2. The estimated values showed that the EC decreased with increasing time of exposure. So, at 10 min, $EC_{15\%}$ = 494.15 µg/l ($CI_{95\%}$ = 270.43–307.34 µg/l), whereas it was only 36.42 µg/l ($CI_{95\%}$ = 0.97–84.40 µg/l) after 240 min, which represents a 14-fold increase in sensitivity.

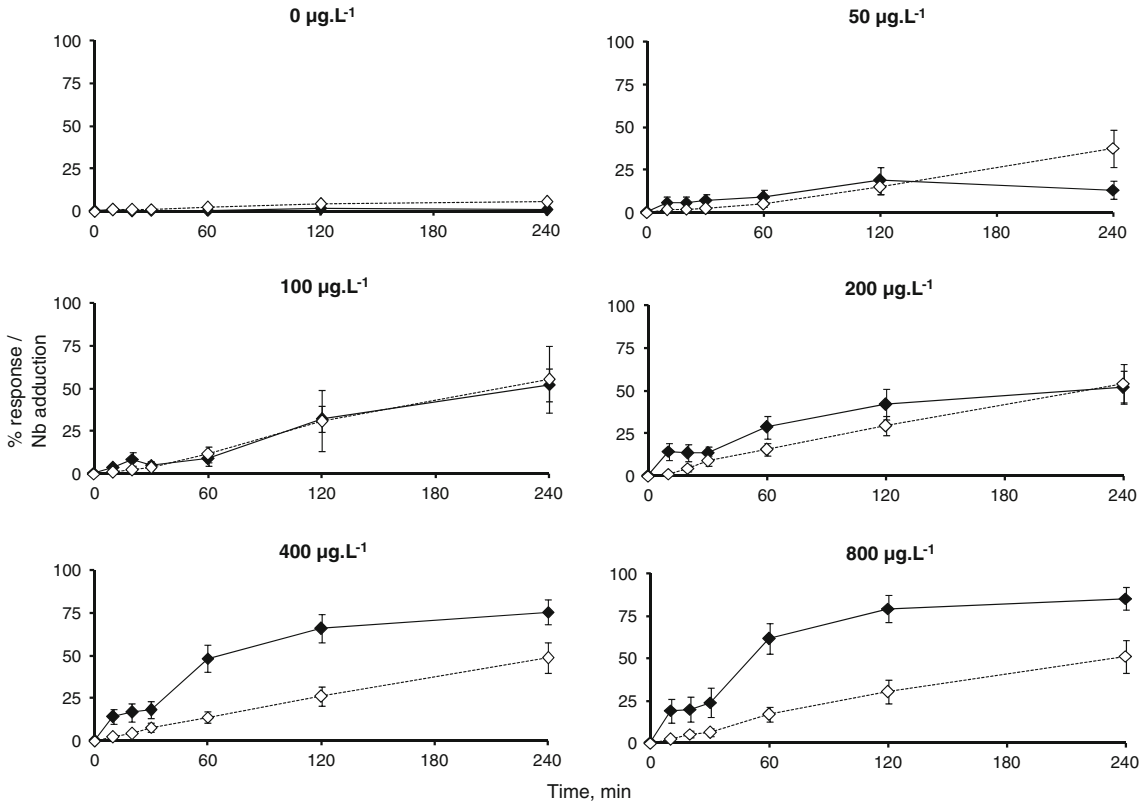

Fig. 2 Response curves over time by dose of the percentage of valve closure response and the frequency of valve adduction (Cypermethrin concentrations: 0, 50, 100, 200, 400, and 800 µg/l). Each value is a mean ($n = 24$, 8 mussels × 3 replicates) with standard error (*black squares* for percentage of valve response data and *white squares* for valve adduction frequency data)

Effects on valve adduction frequency Results show that exposure to Cypermethrin greatly changed the pattern of valve adduction, as a significant increase in their number was observed with all concentrations (Figs. 2 and 3). However, no particular dose-response relationship was obtained with increasing pesticide concentrations. There was first an increase in the frequency of valve adduction and then a decrease was observed at high exposure levels. This was due to the occurrence of complete and lasting closures.

Table 1 Response time for 15, 50, and 85% of total valve closure response ($T_{15\%}$, $T_{50\%}$, and $T_{85\%}$ in min) of *M. galloprovincialis* ($n = 24$) exposed to various Cypermethrin concentrations ranging from 50 to 800 µg/l, computed by a probit method

[Cyp] µg l^{-1}	$T_{15\%}$ $CI_{95\%}$	$T_{50\%}$	$T_{85\%}$
50	215.56	–	–
	91.587–2910.17	–	–
100	74.032	237.78	763.73
	50.603–93.98	191.646–327.56	497.189–1666.56
200	20.977	228.76	2494.88
	12.25–30.10	158.234–397.60	1121.77–9563.89
400	16.48	77.99	368.94
	11.68–21.37	64.99–95.10	268.11–570.46
800	12.75	50.97	203.65
	3.56–22.73	30.81–83.99	114.65–721.20

$CI_{95\%}$ confidence interval

Survival in Air Test

"Stress on stress" response measured as a mussel's ability to survive in air exposed to Cypermethrin is shown in Fig. 4. Results of the 24-h Cypermethrin toxicity test showed that Cypermethrin was nontoxic to *M. galloprovincialis* at the concentrations tested. There was 100% survival across all concentrations and control and the response was not exposure concentration dependent, even for the elevated concentrations.

Byssus Threads Production

The total mean number of byssal threads newly secreted by *Mytilus galloprovincialis* exposed to Cypermethrin is reported in Fig. 5. After 24 h, control mussels have secreted an average number of 10 new byssal threads. The difference between control and experimental populations

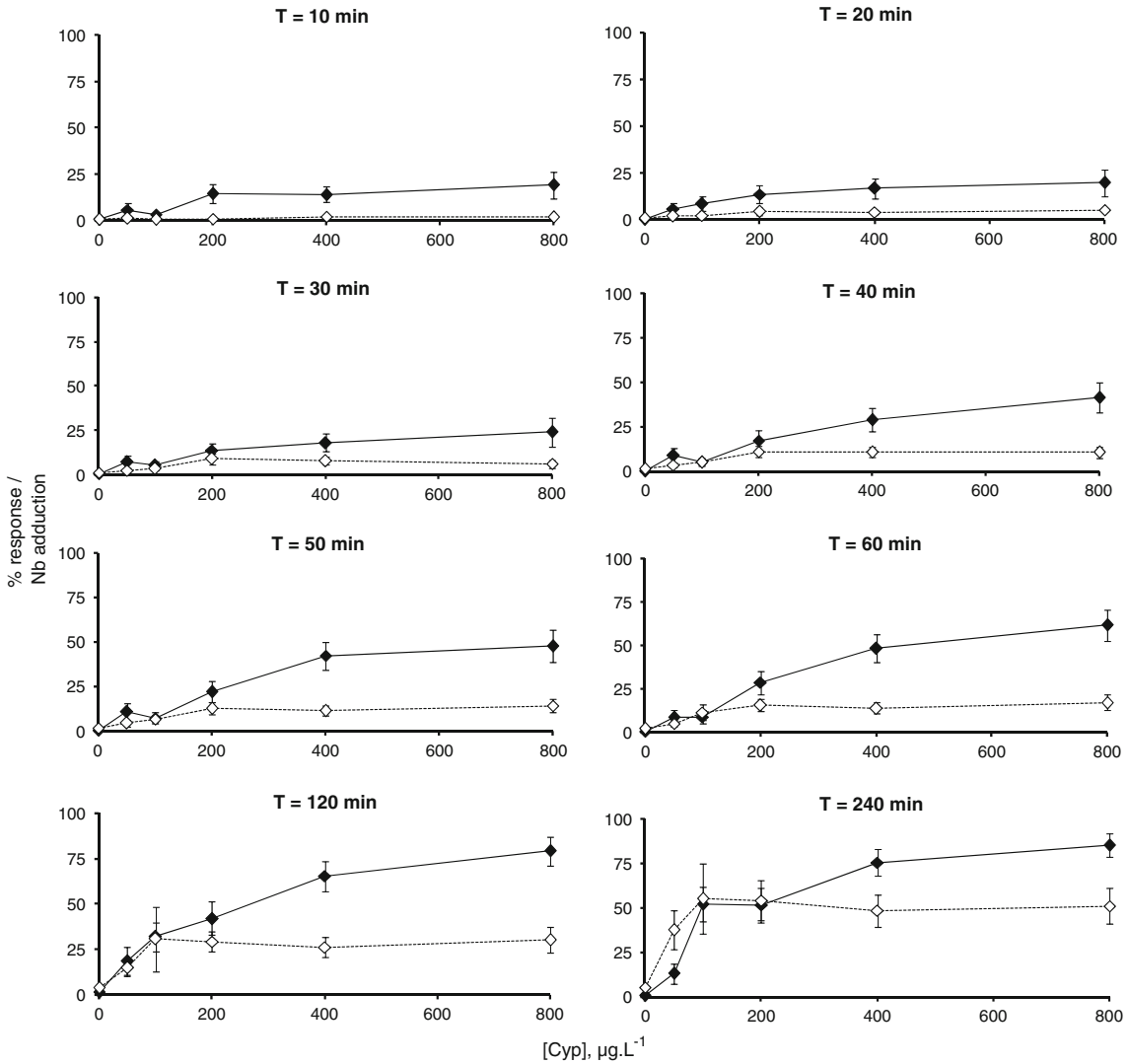

Fig. 3 Dose-response curves of the percentage of valve closure response and valve adduction frequency as a function of Cypermethrin concentration, at different interval times 0–10, 10–20, 20–30, 30–40, 40–50, 50–60, 110–120, and 230–240 min). Each value is a mean ($n = 24$, 8 mussels × 3 replicates) with standard error (*black squares* for percentage of valve response data and *white squares* for valve adduction frequency data)

were significant ($p < 0.05$) from 100 µg/l of Cypermethrin. Thus, the mussels exposed to Cypermethrin show a decrease of 84, 80, 72, 40, and 39% respectively for 50, 100, 200, 400, and 800 µg/l Cypermethrin when compared to control mussels.

Discussion

Cypermethrin reduced the valve closure in a time-dependent manner. With prolonged time of exposure, the effect of Cypermethrin became more evident and the increase in the concentration reduced the time to the first significant effect occurrence. The effects of Cypermethrin observed in the present study are in agreement with results previously reported on *Mytilus edulis* with the same chemical (Gowland et al. 2002). These authors showed that 25 and 92% of mussels were closed respectively after 1 h of exposure to 100 and 1000 µg/l. However, they determined the state of shell closure by simple binary observations (open/closed), which could neither allow one to continuously monitor all of the activity figures nor to follow the pattern of the detection of Cypermethrin by time. The increase in the valve closure at high Cypermethrin concentrations showed similarities with the response shown by the freshwater mussel *Anodonta cygnea* to the pyrethroid Deltamethrin, which inhibits filtration activity (Kontreczky et al. 1997). Donkin et al. (1997) studied the effects of Permethrin (Type I, pyrethroid) and

Table 2 Effect concentrations $EC_{15\%}$, $EC_{50\%}$, and $EC_{85\%}$ (μg/l) causing 15, 50, and 85% of total valve closure response of *M. galloprovincialis* ($n = 24$) exposed to various Cypermethrin concentrations ranging from 50 to 800 μg/l at various times of exposition, computed by a probit method

Time exposure (min)	$EC_{15\%}$ $CI_{95\%}$	$EC_{50\%}$	$EC_{85\%}$
10	494.15	–	–
	270.43–1307.34	–	–
20	364.45	–	–
	179.76–954.07	–	–
30	326.07	–	–
	184.63–573.67	–	–
60	111.22	491.67	2173.54
	77.83–143.67	399.58–635.82	1458.45–3957.59
120	43.64	229.82	1210.33
	27.17–60.68	188.24–282.05	856.04–1996.83
240	36.42	161.07	712.23
	0.97–84.40	57.48–334.24	340.89–1311.35

$CI_{95\%}$ confidence interval

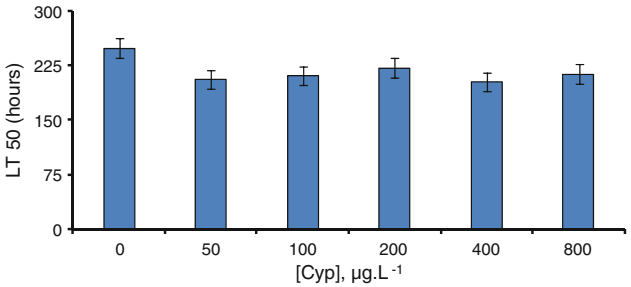

Fig. 4 Median survival time "in air" (LT_{50}) of *M. galloprovincialis* after exposure in water to different concentrations of Cypermethrin. *Error bars* indicate 95% confidence intervals as calculated by trimmed Spearman–Karber analysis. Each value is a mean ($n = 30$, 10 mussels × 3 replicates) with standard error

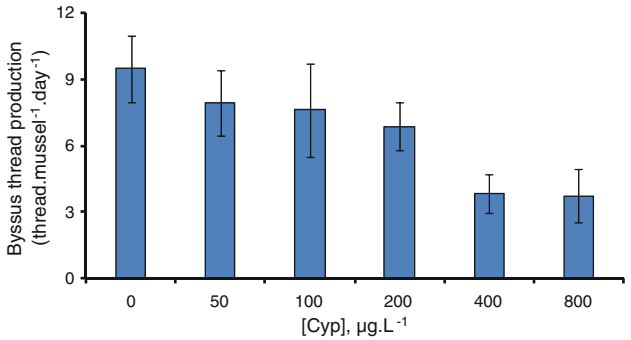

Fig. 5 Byssus threads production of *M. galloprovincialis* at different concentrations of Cypermethrin. Each value is a mean ($n = 30$, 10 mussels × 3 replicates) with standard error

Flucythrinate (Type II pyrethroid and a close structural analogue of Cypermethrin) on the filter feeding of *M. edulis* and reported inconsistent results for the two chemicals. Whereas 400 μg/l of Permethrin reduced the feeding rate by 67% after 7 days of exposure, the experiment carried out with Flucythrinate at the same concentration and exposure time showed no effect in the feeding efficiency. The authors concluded that pyrethroids had a very low toxicity toward mussel feeding activity.

How can Cypermethrin induce valve closure of mussels? The exact mechanism of action has yet to be elucidated. However, its mechanism of impact on insects might provide some useful information. Cypermethrin exerts its neurotoxic effect through the voltage-dependent sodium channel of the neuronal membrane as the target site (Soderlund and Bloomquist 1989) and integral protein ATPase in the neuronal membrane (Kakko et al. 2003). This channel would be kept open by Cypermethrin, causing membrane depolarization, repetitive discharges, and synaptic disturbances, leading to hyperexcitation in the target organism. This mechanism of action might be the starting point for understanding the pattern of the inhibitory effect of Cypermethrin on valve closure. Moreover, the exposure of *M. edulis* to high doses of Cypermethrin (Gowland et al. 2002), had no effect at all on the cell viability of *M. edulis* hemocytes as measured using the Neutral Red retention assay. This suggests that Cypermethrin has no cytotoxicity in the short term and supports the hypothesis of an effect on the voltage-dependent sodium channel.

Survival in air is a particularly sensitive measure of stress in mussels (de Zwaan et al. 1995; Nesto et al. 2004; Thomas et al. 1999b; Viarengo et al. 1995). Several studies have demonstrated that pollution-exposed mussels died in air much earlier than unexposed mussels. However, in our experiment, this index was not influenced when the mussels were exposed to sublethal doses of Cypermethrin. Variations were very small, and even in high concentrations, there was no correlation between the concentrations and the effects. Similarly, Gowland et al. (2002) studied the effect of Cypermethrin on the aerial survival of *M. edulis* and found that exposures to high doses of (even at 1000 μg/l) for 16 days showed insignificant differences.

Byssal thread production was applied to measure the metabolic activity and physiological status of mussels (Thomas et al. 1999a). It is a function of soft tissue mass (Clarke 1999), suggesting that the lower production might be a consequence of the amount of energy reserves in the mussel and that the inability to produce byssal attachments would affect the survival of mussels. Byssal attachment requires opening of the bivalve shell and extension of the muscular foot (Young 1985). This would automatically expose the soft tissues of the experimental mussels to any toxic chemicals present in the water (Rajagopal et al. 2005). The first line of defense against a toxic substance in the ambient water is valve closure. However, the tendency of mussels to reattach by producing fresh byssus threads

overrides this defense mechanism, which, in turn, exacerbates the exposure of their soft body parts to the toxic compound used and this might be the reason for their decreased byssogenic activity when exposed to Cypermethrin. In this study, the capacity of *M. galloprovincialis* to produce new byssus threads in a Cypermethrin exposure was impaired at all concentrations and especially at high concentrations. Our results are in accordance with work conducted on *M. edulis* with a range of pesticides, which caused a reduction in byssal attachment at high concentrations (Roberts 1975). For example, Endosulfan (organochlorine) caused 50% reduction in byssal attachment at 0.45 mg/l after 24 h, whereas Carbaryl (carbamate) had the same effect at 30 mg/l. However, Trichlorphon (organophosphate) did not affect byssal attachment at concentrations up to 30 mg/l.

Compared to species of other taxa, the sensitivity of the *M. galloprovincialis* is less than that of the fish (Köprücü and Aydın 2004; Svobodova et al. 2003), zooplankton communities (Tidou et al. 1992), and some aquatic arthropods (Saha and Kaviraj 2008; Srivastav et al. 1997). However, mussels are by their habitat in the frontline in any contamination received from the land, such as agricultural runoff, and then would be the first to deal with high concentrations before any dilution in the open sea.

As a conclusion, the valve movement and byssal thread production assays appear to be good tools for evaluating the effect of Cypermethrin on *M. galloprovincialis*. The stress on stress assay, although very simple and costless, seems to lack sensitivity toward this pesticide. Data are lacking about the Cypermethrin contamination levels in the coastal waters in Morocco, but it is likely that the concentrations used in this study are higher that those to be routinely expected in the environment. However, the two assays that were adapted here to a native and economically important species may yield useful information on subletal effects of Cypermethrin and other pesticides for regulatory purposes. They might be used as part of a battery of rapid and easy tests that are still needed in toxicity assessment in Morocco.

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