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The effect of diflubenzuron (Dimilin® 25 WP) on some non-target aquatic insect and crustacean species

Nassima Serradj[1], Fatiha Bendali-Saoudi[1*], Noureddine Soltani[1]

[1] Laboratory of Applied Animal Biology, University Badji Mokhtar of Annaba, 23000 Annaba, Algeria.

* Corresponding author: bendalisaoudif@gmail.com

Abstract: The study is aimed at evaluating, under laboratory conditions, the side effects of a commercial formulation of diflubenzuron (Dimilin® 25 WP), which is an insecticide considered a chitin synthesis inhibitor, on some non-targeted aquatic species. The effect of two lethal doses of Dimilin LC_{10} (3.9 ng/L) and LC_{50} (16 ng/L) previously determined on fourth instar larvae of *Culex pipiens* L. 1758 *w*ere tested on some non-target aquatic species, abundant in the Lake of Birds (Northeast Algeria), a site classified under Ramsar Convention. The tested species belonged to two classes of invertebrates (Insects and Crustaceans). The insects included four species of Heteroptera: *Corixa punctata* Illiger, 1807, *Notonecta glauca* Latreille, 1802, *Anisops sardea* Latreille, 1802 and *Plea minutissima* Leach, 1817, and one species of Coleoptera *Berosus signaticollis* Charpentier,1825. For benthic crustaceans *Daphnia magna* Straus, 1820 (Cladocera) was retained. The results showed significant mortality recorded in *C. punctata* and medium mortality for *B. signaticollis, A. sardea, N. glauca, P. minutissima and D. magna*. Three-way ANOVA indicated highly significant effects of species, dose, and time. According to the sensitivity to Dimilin, the pairwise comparison of Tukey's test indicates that the most sensitive species was *C. punctata* followed by *B. signaticollis* and the least sensitive was *N. glauca,* followed by *D. magna*; then *A. sardea* and finally *P. minutissima*.

Keywords: Cladocera, Coleoptera, *Daphnia magna*, Heteroptera, toxicity

Introduction

Insects are an integral part of the aquatic macroinvertebrate community. They play an important role in the cycling of nutrients within an ecosystem by transforming plant materials into animal tissue; moreover, they act as energy sources for other trophic levels (Albertoni & Palma-Silva 2010). Some groups among insects, such as mayflies, plecopterans and whipworms, serve as bioindicators of environmental impacts (Al-Shami *et al*. 2013, 2014, Wandscheer *et al*. 2017). Crustacean species have been known as good indicators of water quality in the global context of the eutrophication of aquatic habitats due to rapid urbanization and industrialization on the one hand, and the use of agrochemicals in agro-industrial activities on the other (Soro *et al*. 2020), because they have abundant populations and are distributed in various microhabitats. The group of aquatic invertebrates includes useful individuals, as well as individuals harmful to humans and animals (disease vectors); others are phytophagous (Callisto & Gonçalves 2005).

The control methods employed against these vectors are mostly chemical. The insecticides used belong to synthetic organophosphates, pyrethroids and carbamates (Becker *et al*. 2010, Hamaidia & Soltani 2014). Insecticides remain the main means of pest control despite their negative consequences for the environment. They cause, among other things, toxicity in the food chain and pollution of the surface and groundwater (Hénaut 2011). Some pesticides are applied directly in aquatic systems to reduce the numbers of mosquito larvae (larvicides), and thereby reduce the transmission of pathogens by mosquitoes to humans and animals (Lawler *et al*. 2017). 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.

Environmental imperatives have pushed research toward the use of natural pesticides (Tomé *et al.* 2013, Cepeda *et al*. 2014). These new products are insect growth disruptors. They include Chitin Synthesis Inhibitors (CSI), which interfere with cuticle formation (Soltani 1991, Soltani *et al.* 1993, Chebira *et al*. 2006, Berghiche *et al.* 2007, Sun *et al*. 2015).

In Algeria, diflubenzuron is widely used against pest insects in forestry. Moreover, diflubenzuron was previously found in *Penaeus kerathurus* shrimp to disturb the fine structure of the different cuticle layers (Morsli & Soltani 2003). Bioassays conducted under laboratory conditions have shown that CSI like diflubenzuron and triflumuron were found potent for mosquito control (Soltani *et al*. 1999, Soltani & Rehimi 2001). Many biochemical effects of diflubenzuron have also been reported on the metabolism of carbohydrates (Soltani 1990) and lipids (Khebbeb *et al*. 1997). Dimilin was reported to affect growth and glutathion activity in mosquitofish *Gambusia affinis* (Drardja-Beldi & Soltani 2003). More recently Novaluron, a CSI, was reported to affect moulting hormone, cuticle secretion and chitin contents in the shrimp *Palaemon adspersus* (Berghiche *et al* . 2018), while in the shrimp *Palaemon adspersus* the same product altered the biochemical composition of cuticle and induced oxidative stress (Lechekhab & Soltani 2018).

In this study, we analyzed the effect of diflubenzuron against a few non-target aquatic species, all considered good bioindicators. Two lethal doses of Dimilin LC_{10} (3.9 ng/L) and LC_{50} (16 ng/L) on *C. punctata; N. glauca; A. sardea; P. minutissima*; *B. signicollis* and *D. magna*, were tested.

Material and methods

Sampling area

Lake of Birds (36° 47'N 08° 7'E) has a more or less oval surface with a characteristic pond tail stretching to the northwest with shallow sloping shores. It covers a total area of 70 ha with a deposit of 20 cm of organic matter (Houhamdi & Samraoui 2002) (Fig. 1). The study site was chosen due to its ecological significance as a continental freshwater aquatic site, favorable for the development of Culicidae and a wide range of invertebrate animals, and also because it is geographically located in an ecosystem protected by Ramsar Convention and notable for its rich animal and floral biodiversity.

Biological models

The insect species tested are all considered good bioindicators. The aquatic Heteroptera is a heterogeneous taxonomic group of Hemiptera. They are a suborder of hemipterans characterized by the piercing – sucking rostrum type and partially sclerified front wings. The aquatic lifestyle provides them with a number of adaptations such as water repellent pads, natatory setae, respiratory siphons and aerial plastrons (Poisson 1957, Loncle 2020). The aquatic Coleoptera are the only holometabolous insects present in both imaginal and larval forms, characterized by the presence of a mouthpiece of the crusher type, and leathery forewings unfit for flight (Forge 1981). However, Cladocerans species are small aquatic crustaceans, with a much reduced number of segments, and thorax and abdomen fused. They move thanks to swimming movements, jerks of the well-developed antennas.

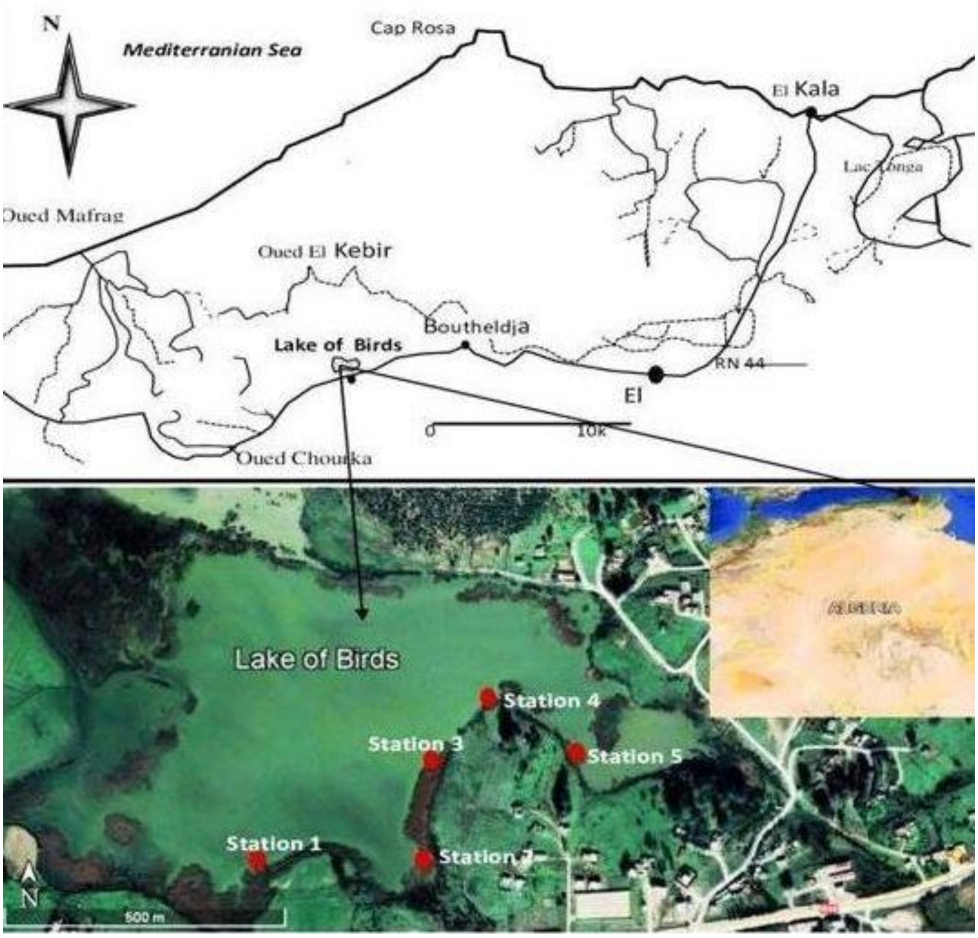

Fig. 1. Geographical location Lake of Birds and five sampling stations.

Insecticide and treatment

Dimilin® (Wettable Powder, 25% active ingredient, a.i.), a commercial formulation of diflubenzuron, is an insecticide belonging to benzoylphenyl urea derivatives. The two concentrations of Dimilin, previously determined on fourth stage larvae of *Culex pipiens* L. 1758 (Rehimi 2004), were added to bioassay vessels. The first concentration test corresponds to the LC_{10} (3.9 ng/L) and the second to the LC_{50} (16 ng/L). The two concentrations were tested on the six species present in abundance at the study site (*C. punctata*; *A. sardea*; *N. glauca*; *P. minutissima*; *B. signaticollis* and *D. magna*). The test was carried out in plastic boxes containing 250 ml of lake water and food (mosquito larvae) under laboratory conditions at a temperature of 24°C and 76% humidity. Each test consisted of three controls and three repetitions for each dose, tested on the six species; 20 individuals were exposed to these concentrations in each repetition of each species. Mortality was recorded 24 h, 48 h, and 72 h after treatment.

Data analysis

All statistical analyses were performed using R, version 4.0.1 (R Core Team 2020). The results are given as the means ± SD (standard deviation), variations regarding the percentage of mortality are plotted through the histogram. Two-way ANOVA was used to analyze the variation in mortality between the species for each exposure time. Three-way ANOVA was used to test the variance in individual deaths, according to species, dose and time. In box plots graphs and comparison of Tukey's HSD test between times and between studied species using the package 'ggplot2' (Wickham 2016) different lowercase letters indicate a significant difference between the levels of the studied factors. All the statistical analyses were conducted at α=0.05 as a significance level.

Results

The sensitivity to the insecticide was highly significant between species ($p < 0.001$), depending on the time of exposure.

Dimilin at lower dose (3.9 ng/L) showed a toxic effect on the six species. According to the mortality rate, the species are classified in descending order (Fig. 2). At LC_{10}-after 24 h of exposure, *B. signaticollis* was the most sensitive with 2.66±0.57% mortality, followed by *C. punctata* (2.33 ± 0.6%), *N. glauca* (2.33 ± 0.6%), *D. magna* (2.0 ± 0.57%), *P. minutissima* (1.33 ± 0.1 %) and *A. sardae* (0.66 ± 0.1%). After 48h of exposure, mortality was estimated at 7.33 ± 0.5% to *C. punctata*, followed by *N. glauca* (4.33 ± 0.6%), *B. signaticollis* (4.00 ± 0.15%), *A. sardae* (3.66 ± 1.73%), *D. magna* (2.33 ± 0.5%) then *P. minutissima* (2.33 ± 0.2%).

After 72h of exposure, *C. punctata* was still the most sensitive to Dimilin with 11.33 ± 1.8% mortality, followed by *N. glauca* (7.66 ± 1.2%), *B. signaticollis* (6.33 ± 0.57%), *A. sardae* (4.66 ± 0.2%), *P. minutissima* (4.33 ± 0.57%) and *D. magna* (4.0 ± 1%).

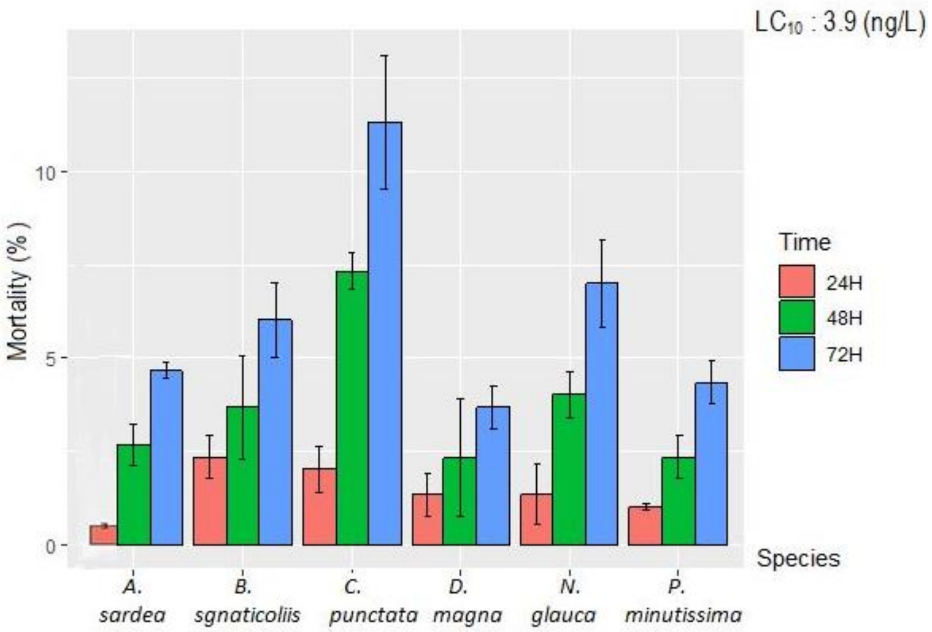

Fig. 2. Mortality rate (%) of the six species treated with Dimilin at LC_{10} during the three exposure times.

For the higher dose of Dimilin (16 ng/L) the results show a highly significant ($p < 0.001$) sensitivity depending on the species and time of exposure (Fig. 3). At $LC_{50,}$ after 24h of exposure, *C. punctata* was the most sensitive species with 9.0 ± 0.8% mortality followed by *B. signaticollis* (4.0 ± 0.2%), *D. magna* (3.0 ± 1%), *N. glauca* (3.0 ± 0.1%), *A. sardea* (2.66 ± 0.4 %) then *P. minutissima* (1.0 ± 0.6%). After 48h of exposure, mortality increased significantly for *C. punctata* (13.66 ± 1.2%), then *B. signaticollis* (7.66 ± 0.57 %), *N. glauca* (5.33 ± 1%) and *D. magna* (4.33 ± 0.57 %), *P. minutissima* (4.0 ± 1%) and *A. sardea* (3.66 ± 0.5 %). After 72h of exposure, *C. punctata* was the most sensitive with 17 ± 1.5% mortality, followed by *B. signaticollis* (12.33 ± 1.0%), *D. magna* (7.66 ± 1.15%), *N. glauca* (7.33 ± 1.25%), *P. minutissima* (6.0 ± 1.0%) and *A. sardea* (6.0 ± 1.0%).

The three-way analysis of variance showed that the toxicological effect of Dimilin was significantly dependent on species (F_{99} = 40.71; p = 0.000), dose (F_{99} = 55.36; p = 0.000) and exposure time (F_{99} = 85.50; p = 0.000).

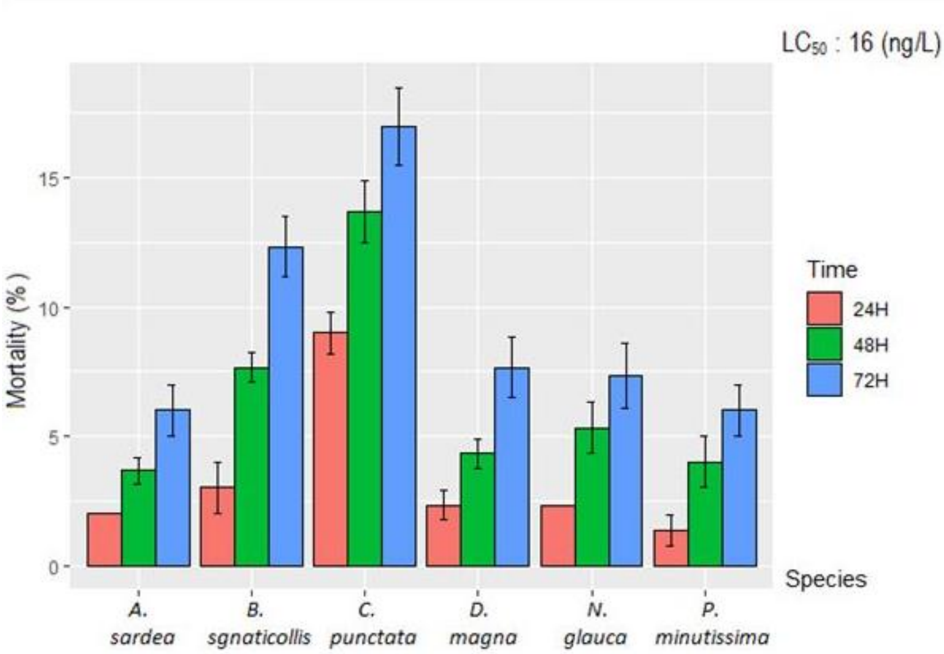

Fig. 3. Mortality rate (%) of the six species treated with Dimilin at LC $_{50}$ during the three exposure times.

The pairwise comparison of Tukey's test indicates that the box plots of the variation between time revealed the existence of highly significant differences, a single group for 24 h and 72 h, and 2 homogeneous groups for 48 h (Fig. 4).

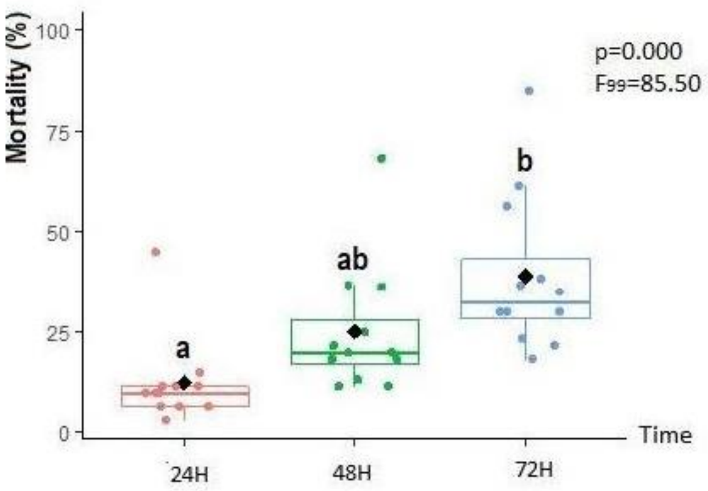

Fig. 4. Time variations regarding the percentage of mortality under the effect of Dimilin. The whisker boxes labeled with the same letter are not significantly different at p > 0.05 (Tukey's test). The center box boundaries show the interquartile range (IQR) with the first quartile (lower bound) and third quartile (upper bound).

While analyzing the box plots of the species factor, we observed significant differences in the studied species, with clear heterogeneity, two groups homogeneous for *B. signaticollis* and only one group for *A. sardea*, *C. punctata*, *N. glauca*, *P. minutissima* and *D. magna* (Fig. 5).

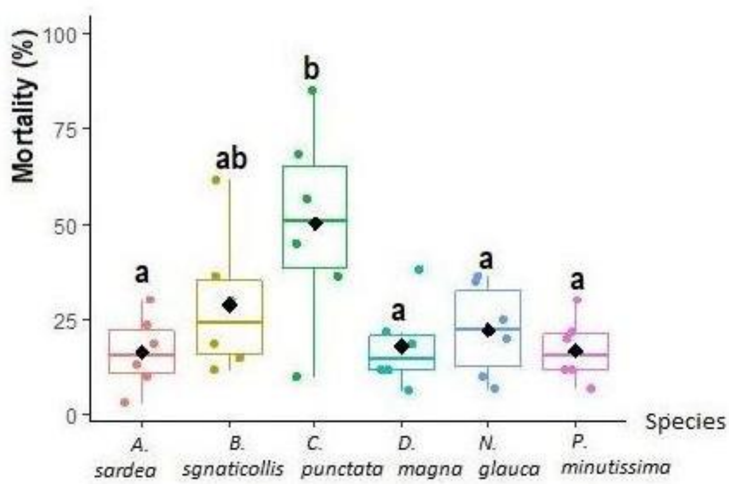

Fig. 5. Species variations regarding the percentage of mortality under the effect of Dimilin. The whisker boxes labeled with the same letter are not significantly different at $p > 0.05$ (Tukey's test). The center box boundaries show the interquartile range (IQR) with the first quartile (lower bound) and third quartile (upper bound).

Discussion

The data obtained from this experiment indicates that Dimilin has a negative effect on associated insect fauna. In our work, the treatment of six species of invertebrates resulted in variable sensitivity to Dimilin (LC_{10}: 3.9ng/L and LC_{50}: 16ng/L). The results show significant toxicity recorded in *C. punctata; B. signaticollis* and average toxicity for *N. glauca*; *D. magna*; *P. minutissima* and *A. sardea*.

The insecticides play a great role in the management of insect pests, however the concentration and indiscriminate use of treatment methods are criticized in view of their negative effect on some non-target aquatic insects., and adverse effects on soil characteristics (Khudhur & Sarmamy 2019). Benzoylphenyl urea derivatives interfere with the molting process by disrupting cuticular secretion via chitin synthesis (Morsli & Soltani 2003, Soltani *et al*. 2009, Berghiche *et al*. 2018). The detrimental effects of CSI are felt during various critical phases of insect development. It was also reported that Novaluron, another inhibitor of chitin synthesis, caused a decrease in the level of cuticular chitin of *P. adspersus* (Berghiche *et al*. 2016).

Diflubenzuron is an insecticide that has been widely used for the selective control of insect pests (Subrero *et al*. 2018). It was first discovered as a post-ingestion larvicide, but further studies determined that this insecticide could also prevent egg hatching after direct egg contact or after the female treatment method (Singh 2015). The sensitivity of *D. magna* to diflubenzuron could be related to inhibition of chitin synthesis and chitinase activity (Kota *et al*. 2022). Subrero *et al.* (2018) determined acute toxicity following exposure to the diflubenzuron (0.15; 0.015; 0.0015 mg/L) on egg hatching rate and motility inhibition of *D. magna*. Similarly, diflubenzuron showed high toxicity to *D. magna*, indicating that the use of this substance could lead to a high environmental risk for this species (Abe *et al*. 2014).

Dimilin in dose of 16 ng/L and 1 ng/L applied to the crustacean of type *P. adspersus* shrimp induced a significant decrease in the amounts of chitin at the treated doses after 96 h (Lechekhab & Soltani 2018). This product may have side effects on non-target arthropods such as shrimps, since a similar effect has been reported on another shrimp *Penaeus kerathurus* (Soltani *et al*. 2009). According to Macken *et al* (2015), diflubenzuron interferes with enzymes that contribute to the synthesis of chitin in crustacean species such as benthic copepods *Tisbe battagliai*. Dimilin was also found to be toxic to Cladocera and Copepoda crustacean zooplankton (Lahr *et al*. 2000). A study by Harðardóttir *et al* (2019) explained the mechanism of diflubenzuron (93.2 ng/L) action on *Lepeophtheirus salmonis* (Copepoda, Caligidae) and confirmed inhibition of chitin production. On the other hand, in their work Ferreira *et al* (2020) tested the use of diflubenzuron in following doses 250 mg/L, 750 mg/L and 1 g/L for *Buenoa* sp. (Heteroptera: Notonectidae). A strong relationship between the mortality and exposure time in all concentrations was observed. The obtained lethal concentrations of LC_{50} (2.77 x 10^{-3} g/L) and LC_{90} (0.86 g/L) for *Buenoa* sp. were below the recommended dose for mosquito control.

Our results show that Dimilin was found to exhibit significant toxicity against *C. punctata*, and average toxicity on the other studied species. In this context, there is a need for tools to monitor the toxicity risk of these pesticide to the environment, a negative effect on non-target insects and natural predators. The evaluation of the richness and the toxicology of the study site will allow us to carry out further studies concerning the use of mainly natural control products for the preservation of the environment and biological balance.

Conclusions

Chemical control has become a source of enormous environmental problems, and alternative methods are searched. The obtained results showed that the two tested doses had a lethal effect on the studied species over time. Our results show that Dimilin was found to exhibit significant toxicity against *C. punctata*, and average toxicity to the other studied species. The evaluation of the richness and the toxicology of the study site will allow us to carry out further studies concerning the use of mainly natural control products for the preservation of the environment, the development of new biomonitoring strategies.

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