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Oxidative stress and histological changes caused by lambda-cyhalothrin on the snail *Helix aspersa*

Bouaricha Houda[1], Bouchaala Laid [2*],Issaad Ghozlène[1], Berrebbah Houria[1], Djebar Mohammed Réda[2]

[1]*Cell Toxicology Laboratory, Annaba University. BP. 12, Annaba, 23000 Algeria.*
[2]*Environmental Research Center (CRE), Annaba, Algeria.*
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Abstract

This study was interested in evaluating the toxicity of an insecticide based on lambda-cyhalothrin, newly approved and widely used in agriculture in Algeria, on an alternative biological model, the snail *Helix aspersa*.In order to achieve these objectives, we based ourselves on the evaluation of the degree of oxidative stress by monitoring the average weight of the snails, the level of total proteins and Glutathione-SH (GSH), as well as the enzymatic activities of Glutathione S-transferase (GST) and Catalase activity (CAT) at the level of the hepatopancreas. We performed a histopathological examination of the kidney and hepatopancreas.The first results show that lambda-cyhalothrin causes a dose-dependent decrease in the average weight of snails, an increase in the content of total proteins, and a decrease in the level of GSH , in parallel with the induction of GST activities and CAT. Histopathological examination confirmed the toxicity of lambda-cyhalothrin in Helix aspersa.

Keywords: Toxicity, *Helix aspersa*, Insecticide, Lambda-cyhalothrin, Agriculture, Algeria

1 Introduction

Agriculture has seen progress in recent years and this with the appearance of new insecticide molecules, supposed to be less harmful such as pyrethroids, these lipophilic molecules are applied as a replacement for organophosphates, because of their low volatility and their inactivation. metabolic rate (Barr et al., 2010), agricultural pollution can pose major problems by affecting the ecology of rivers and estuaries, reducing the quality of drinking water and the health of coastal ecosystems (Morgan et al., 2016).

*e-mail: *l.bouchaala@cre.dz*

Pyrethroids are analogs of natural pyrethrins, they were developed as a new generation of plant protection products, and they are divided into two groups, type 1 and 2, according to the absence (type 1) or the presence (type2) of a nitrile group. Various synthetic forms of pyrethroids are used for insect control, such as lambda-cyhalothrin and cypermethrin, which are type 2 molecules (Morgan et al., 2016).

Humans can be exposed to pyrethroids throughout several routes due to their wide range of use in recent years, a significant amount of pyrethroids have been detected in dust, food and residential environments (Wondji et al., 2007 ; Julien et al., 2008 ; Riederer et al., 2008 ; Melnyk et al., 2012 ; Starr et al., 2014).

Lambda cyhalothrin acts as a poison on the peripheral and central nervous system of the insect. In fact, the voltage-dependent sodium channel of the membrane of nerve cells is the main site of action of pyrethroids, these molecules inhibit the closure of sodium channels and therefore disrupts the normal functioning of the nervous system (He et al., 2008). This chemicalis toxic not only to insects, but also to mammals (Ansariet al., 2012), the toxicity of lambda-cyhalothrin in animals showed changes in brain function involving the dopaminergic, cholinergic and serotonergic systems (Hossainet al., 2004).

Poisoning by lambda-cyhalothrin causes damage to the immune system in animals (Bhoopendra and Nitesh, 2014) and to the endocrine system (Riederer et al., 2008) and even oxidative damage manifested by the formation of radical species (Kaneko et al., 2010).

For the mechanism of action of lambda-cyhalothrin, begins with the absorption of this molecule by the digestive, pulmonary and cutaneous tracts (Bossou et al., 2020), then it is distributed in several tissues (Anadónet al., 2006), several studies show that pyrethroids are rapidly metabolized and excreted by the body (Kaneko et al., 2010), the metabolic reactions described are mainly oxidation reactions, hydrolysis reactions (Ross et al., 2006), and finally, conjugation reactions (Yang et al., 2018).

Our study was conducted to evaluate the toxicity of lambda-cyhalothrin on a biological model the snail: Helix aspersa. The snail *Helix aspersa* was used as bioindicator/biomarker for environmental pollution due to its ability to accumulate pollutants, and widely distribution (reflecting the adaptation to climatic changes), as well its ease of rearing (Boucenna, 2016).

The objective of our work aims to evaluate the potential toxic effect of lambda cyhalothrin through the evaluation of oxidative stress based on monitoring the average weight of the snails, the total protein level and the GSH, as well as the GST and CAT enzymatic activities. Moreover, the possible tissue damage caused by this xenobiotic in the kidney and hepatopancreas was also investigated.

2 Materials and Methods

Biological material

Helix aspersa snail was collected from an unpolluted area "Seraidi". The Seraidi area is a mountainous region characterized by a dense vegetation cover and a very important biological diversity It is characterized by a low population density, which limits negative human activity on this virgin nature and makes it an area far from sources pollution. Several studies show that the Seridae region is an unpolluted area (Tlili et al, 2009). Snails with average weight of 10 + 0.35g were reared in transparent plastic boxes with a perforated lid and a wet sponge to maintain humidity, the following optimal environmental conditions were necessary: Photoperiod 18 h light/24 h, temperature 20+ 2°C, relative humidity 70-80% (Gomot-de Vaufleury and Bispo,

2000). The animals were fed wheat flour (Grara et al., 2015).

Chemical material

In the current study, the insecticide based on lambda cyhalothrin of the Pyrethroids family ws used. This product contains 50g/L of Lambda cyhalothrin, whose molecular formula is as follows: C23H19ClF3NO3, and the chemical structure is shown in figure 1 (Worthing and Hance, 1991; Cluzeau, 1993).

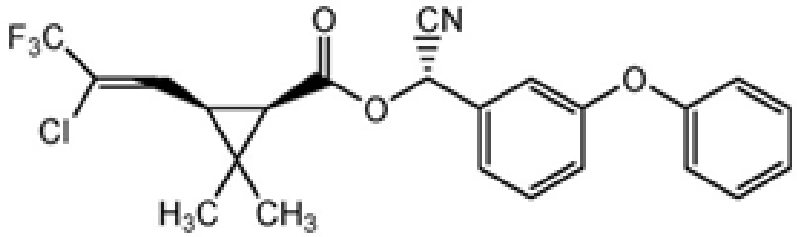

Figure 1. Structure chimique de lambda-cyhalothrine.

Treatment method

Snails were treated atop (Bouaricha, 2013), with increasing concentrations of the insecticide, 10/20 / and 40 µM /L, in three batches for each concentration, and five snails/batch.

Parameter measurement

First, the snails *Helix aspersa* were brought from the Seraidi area, weighed the snails, then the treatment started with lambda-cyhalothrin with concentrations of 10, 20 and 40 µM/L for 7 days, weighed the snails again, then the treatment continued with lambda-cyhalothrin (concentrations 10, 20 and 40 µM/L) until 21 days the snails were weighed again.

Determination of total protein

Proteins are quantified according to the method of Bradford (1976). Absorbances readings are taken at a wavelength of 595 nm with a visible spectrophotometer (JENWAY 6300). The calibration range is made from a standard protein, bovine serum albumin (BSA).

Determination of Glutathione (GSH)

The level of glutathione (GSH) is quantified according to the method of Weeckbeker and Cory (1988), whose principle is based on the colorimetric measurement of 2-nitro 5-mercapturic acid, resulting from the reduction of acid 5-5-dithiol-bis-2-nitrobénzoique (DTNB) by the thiol groups (-SH) of glutathione.

Absorbance readings are taken at a wavelength of 412 nm after standing for 5 minutes of rest for color stabilization against a blank where the 500 µl of the supernatant is replaced by 500 µl of distilled water.

Determination of glutathione S-transferase activity (GST)

The measurement of glutathione S-transferase activity (GST) is determined according to the method of Habig et al. (1974). It is based on the conjugation reaction between GST and a substrate, CDNB (1-chloro 2, 4 dinitrobenzenes) in the presence of cofactor glutathione (GSH).

Determination of the Catalase activity (CAT)

The measurement of Catalase activity (CAT) is determined according to the method of Regoli and Principato (1995), whose principle is based on the variation of the optical density following the dismutation of hydrogen peroxide (H2O2) at a wavelength of 240 nm.

Histopathological study

On samples of the hepatopancreas and the kidney, we carried histological sections in the laboratory of Anatomical-pathology of Ibn Rochd Hospital (Annaba), according to the technique described in Martoja and Martoja (1967).

Statistical analysis

Histograms of each measured factor were produced (Figure 2, 3,4, 5, 6). A student "t" test was used to test for significant differences in the mean values of each measured parameter.

3 Results

Effects of increasing concentrations of lambda-cyhalothrin on the average weight of snails

Figure 2 shows the evolution of the average weight of the snails in the presence of lambda-cyhalothrin, we note that after 7 days of treatment the average weight tends to decrease in a highly significant way ($p \leqslant 0.001$) for the concentrations of 20 and 40 µM/L, it is the same after 21 days of treatment.

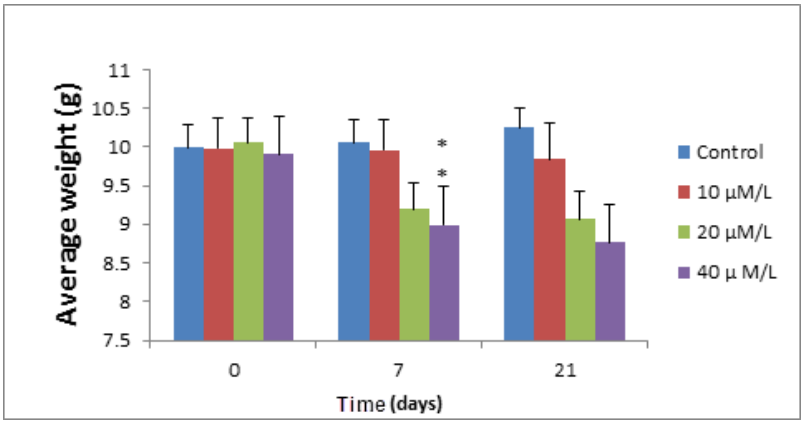

Figure 2. Evaluation of the average weight of snails treated with increasing concentrations of lambda-cyhalothrin.

Effects of increasing concentrations of lambda-cyhalothrin on total protein levels

Figure 3 shows a dose-dependent increase in the total protein level in snails treated with lambda-cyhalothrin. This increase in the protein pool is highly significant ($p \leqslant 0.001$) after 21 days of treatment of snails at the highest concentrations (20 and 40 µM/L).

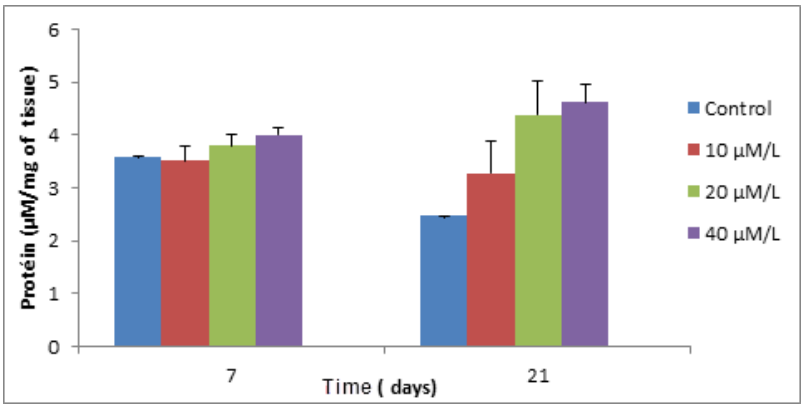

Figure 3. Changes in total protein content in the hepatopancreas of snails treated with increasing concentrations of lambda-cyhalothrin.

Effects of increasing concentrations of lambda-cyhalothrin on Glutathione-s-transferase activity

Figure 4 shows the GST activity in snails treated with lambda-cyhalothrin for 7 and 21 days, we notice a highly significant increase in GST activity, especially for snails treated for 21 days, at the highest concentration of insecticide ($p \leqslant 0.001$).

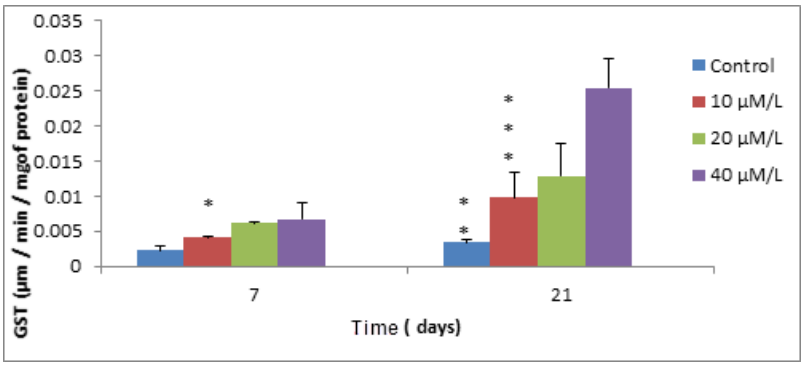

Figure 4. Changes in Glutathione-S-transferase activity in the hepatopancreas of snails treated with increasing concentrations of lambda-cyhalothrin.

Effects of increasing concentrations of lambda-cyhalothrin on Glutathione levels

Figure 5 represents the evolution of the GSH levels in lambda-cyhalothrin treated snails after 7 and 21 days, the results show a highly significant ($p \leqslant 0.001$) and dose-dependent decrease, especially for the snails treated with the highest concentration of 40 µM/L, for 21 days.

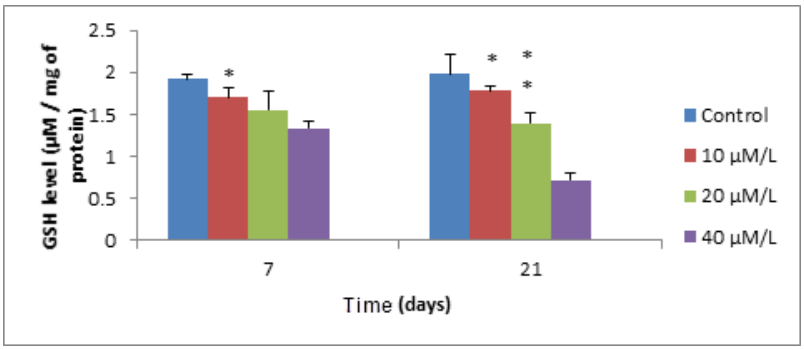

Figure 5. Changes in hepatopancreas Glutathione levels in snails treated with increasing concentrations of lambda-cyhalothrin.

Effects of increasing concentrations of lambda-cyhalothrin on Catalase activity

Figure 06 shows the variations of catalase activity in the hepatopancreas of snails after 7 and 21 days of treatment with lambda-cyhalothrin. We notice that the activity of this enzyme tends to increase in a very significant $p \leqslant 0.01$) and dose-dependent, particularly after 21 days of treatment.

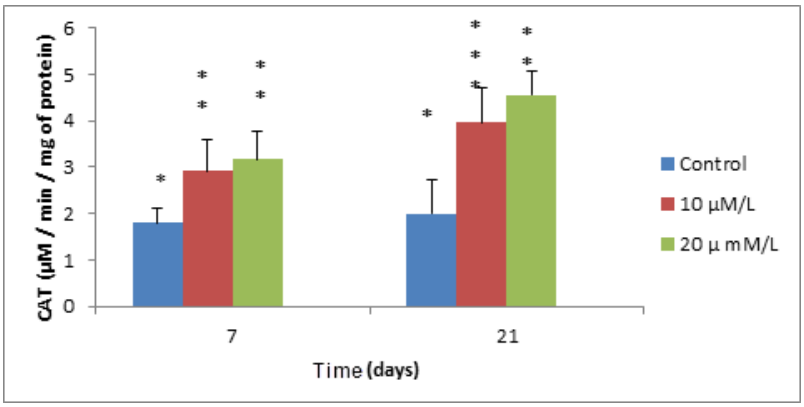

Figure 6. Changes in Catalase activity in the hepatopancreas of snails treated with increasing concentrations of lambda-cyhalothrin.

Histological aspect of the hepatopancreas after treatment with increasing concentrations of lambda-cyhalothrin

In snails the hepatopancreas occupies a large volume of the visceral mass, it is formed by two lobes, each connected to the mesentery by a hepatopancreatic duct.

The lobes of the hepatopancreatic are surrounded by connective tissue associated with some smooth muscle fibers, the whole constituting an envelope, they appear formed by juxtaposition of many tubules, the spaces that separate them being occupied by connective tissue within which circulates hemolymph. The lumen of the hepatopancreatic tubules is lined by a simple epithelium associating several cell types and is in continuity with that of the of small diameter ducts. The hepatopancreatic ducts result from the convergence of these small ducts (Heusser and Dupuy, 2011).

The wall of the hepatopancreatic tubules is constituted by a simple and high epithelium, the cells that compose it present divers' morphologies but fall into three main categories: Digestive or secretory cells (CD), producing digestive enzymes that are discharged into the lumen of the tubules and are responsible for the extracellular digestion of nutrients. Excretory cells (EC), which carry out the phagocytosis of food particles from the lumen of the tubules and are responsible for intracellular digestion and calcium cells (CC), which are basal and allow the renewal of other cell types (Zaldibar et al., 2008).

The histopathological examination of the hepatopancreas of snails treated with the lowest concentrations of Lambda-cyhalothrin reveals foci of necrosis and inflammatory infiltrates, while at the highest concentrations, a complete disorganization of the acini is observed, with the appearance of inflammatory infiltrates, as well as an enlargement of the basal lumen.

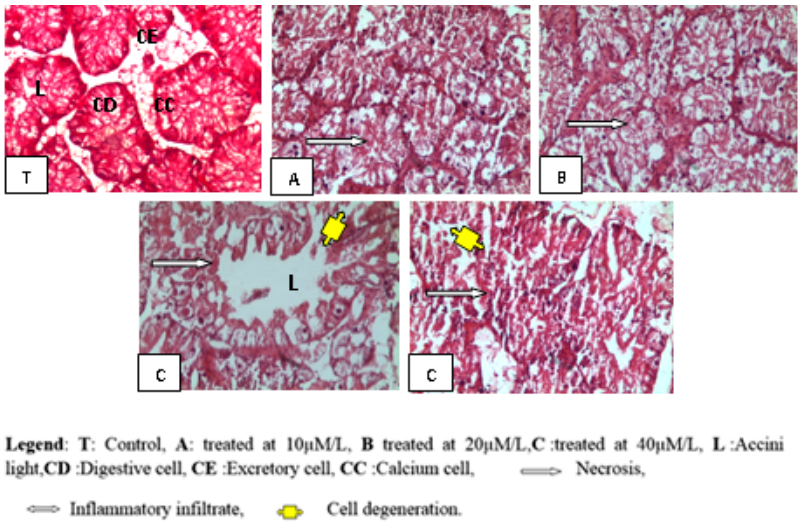

Figure 7. Appearance of the hepatopancreas after 21 days of treatment with increasing concentrations of Lambda-cyhalothrin (G × 40).

Histological aspect of the kidney after treatment with increasing concentrations of Lambda-Cyhalothrin

The kidney is located dorsally and posteriorly, on the surface of the visceral mass, It is connected on the one hand with the pericardial cavity by a reduced renal-pericardial orifice, and on the other hand with a urethra which takes care of the urine produced, the renal wall forms numerous and extensive folds, supported by connective axes within which hemolymphatic lacunae are present. These folds are lined with a simple prismatic epithelium with a brush border, formed by cells similar to each other, the nephrocytes, the ureter is on a part of its path adjoining the kidney. Its lumen is bordered by a more or less wrinkled wall, made up of a simple epithelium, cubic to the prismatic, surmounting a connective tunic rich in hemolymphatic lacunae (HeusseretDupuy, 2011).

The kidney consists of an excretory epithelium lined with renal lamellae, hollow connective lamellae, blood sinuses and smooth muscle fibers, made up of prismatic cells with a brush border. The epithelium contains a single type of excretory cells with a nucleus and a granular membrane, these excretory cells have rod-like (most numerous) and ciliated forms (Chabicovsky et al., 2003).

Histopathological examination of the kidneys of snails treated with the lowest concentrations of Lambda-cyhalothrin showed foci of necrosis in the nephrocytes, and the appearance of inflammatory infiltrates, while at the highest concentration, there was hypotrophy of the excretory cells accompanied by necrosis.

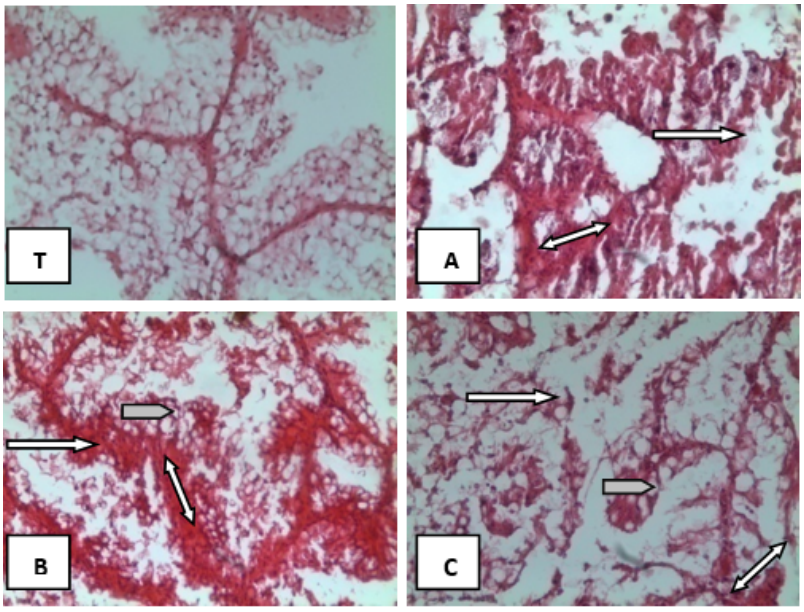

Figure 8. Renal tissue appearance after 21 days of treatment with increasing concentrations of Lambda-cyhalothrin (G × 40).

4 Discussion

Pesticides play an important role in agriculture by reducing yield losses and ensuring healthier and more regular harvests, which explains why modern agriculture and intensive cropping patterns are today heavily dependent on the plant protection industries (Tellier, 2006), These proven advantages are mainly associated with their growth regulation and weed and pest control properties.

In our work, we have chosen as a biological model the snail *Helix aspersa*, which plays a major role in many ecosystems, and is therefore increasingly used to assess the impact of contamination on its growth and physiology (Vaufleury and Kerhoas, 2000).

The bio-indicators of environmental pollution are sensitive to physicochemical variations in their environment, including temperature, electromagnetic frequencies and any form of urban pollution (Grara, et al., 2015), but also these species are sensitive to xenobiotics such as hydrocarbons, heavy metals or pesticides, gastropod mollusks are now the first sentinel organisms used in monitoring programs for chemical contamination of ecosystems (Régoli et al., 2006).

Firstly, we have highlighted a decrease in fresh weight of snails treated with the highest concentrations of Lambda-cyhalothrin, which is the first indication of potential toxicity, decrease in weight is probably due to the repulsion of food, and therefore a prolonged fasting, the same result was observed by Coeurdassier et al. (2001), who noted a growth inhibition of snails after exposure to dimethoate, in the same vein, the study by Farfar et al. (2018)to demonstrate a growth inhibition of the snail *Helix aspersa* treated with increasing concentrations of a copper-based fungicide. The studies of Laskowski and Hopkin (1995) and Coeurdassier et al. (2001), stipulate that in a polluted environment, the growth and development of animals are inhibited, this is confirmed by the studies of Boucenna (2016), Atailia (2017), and Khene (2020), which highlighted a loss of weight of the snail *Helix aspersa* after exposure to metal particles.

In this study we have highlighted an increase in the protein pool in Helix aspersa in the presence of the insecticide, our results are in agreement with those of Grara (2011), Boucenna (2016), and Khene (2020), who have recorded high total protein in *Helix aspersa* following its exposure to environmental pollutants of heavy metals, this phenomenon was described by Massaya et al. (2002) who have highlighted a significant increase in total protein levels in different biological models under chemical stress, high levels of total protein in the digestive glands were recorded following the treatment of the snail *Helix aspersa* with an insecticide (Radwan et al., 2010). El Gendy et al. (2009), evaluated the oxidative stress induced by a copper-based pesticide on *thiba pisana* snail, the study showed an increase in total protein levels in animals treated with the highest concentrations, this increase in protein pool suggests an induction of detoxification and metabolization enzymes synthesis, in the same vein, Zouaghi et al. (2020), demonstrated an increase in the protein content in *Helix aspersa* treated with a mixture of insecticides.

Studies by Franzellitti and Fabbri (2005) and Kourtidis et al. (2006), suggested that protein induction is due to the induction of HSps genes in mollusks exposed to environmental pollutants. When the capacities of the stress proteins for cellular detoxification is exceeded, xenobiotics will accumulate in the cells and their toxic effects become apparent, the cellular mechanisms that come into play allow to limit these effects by facilitating the excretion of contaminants and by loading the reactive products they generate.

Catalase activity is considered one of the most sensitive biomarkers to oxidative stress (Livingstone, 2001), an induction of the activity of this enzyme is probably due to the intensification of antioxidant activity in liver cells, the change in catalase activity is explained by cellular damage caused by exposure to contaminants (Arrighetti et al., 2018), the induction or inhibition of this enzyme testifies to the state of oxidative stress with excellence.

In this work, we have highlighted a dose-dependent increase in catalase activity in snails treated with Lambda-cyhalothrin, according to Belhaouchet et al. (2012) this increase would be due to the intensification of the activity antioxidant. Our results are in agreement with those of Zouaghi et al. (2020) who noted a dose-dependent induction of catalase activity in *Helix aspersa* treated with two insecticidal molecules from the neonicotinoid family. Also, the study by Khene et al. (2017), highlighted an induction of catalase activity in *Helix aspersa* following its exposure to TiO2microparticles, however the study by Besanci et al. (2019) shows a slight inhibition of catalase activity following exposure of *Helix aspersa* to iron oxide nanoparticles, as well as the study of Fernandez et al. (2020), which evaluated the toxicity of Lambda-cyhalothrin on the freshwater snails *Chilina parchappii*.

Glutathione is the major non-enzymatic antioxidant in animal cells, it is the most abundant sulfur-reducing compound in the intracellular compartment involved in metabolism, transport processes and in the protection of cells against the toxic effects of endogenous and exogenous compounds (Boucenna 2016; Dickinson et Forman, 2002), Glutathione S-transferases are enzymes of phase 2 metabolism capable of conjugating a molecule of reduced glutathione to the electrophilic center of a xenobiotic in order to make this one more soluble and therefore more easily excretable by the body, their expressions can be induced or inhibited by certain xenobiotics, which confer them great interest as potential biomarkers of pollution (Vidal, 2001).

In our work, we have highlighted an involvement of the GSH/GST system through an increase in GST activity and a decrease in GSH activity in snails treated with increasing concentrations of Lambda-cyhalothrin, in the same way, the study by Fernandez et al. (2020) to show an induction of GST activity.

The work of Farfar et al. (2018) indicates a decrease in the level of GSH in Helix aspersa treated with a copper-based fungicide. Exposure to terrestrial and aquatic pollutants can result

in very significant and irreversible tissue damage in the kidney and hepatopancreas of snails, these organs play a major role in the metabolism of toxicants (Frías-Espericueta et al., 2008).

The treatment of the snail *Helix aspersa* caused important tissue alterations in the hepatopancreas and kidney, such as the appearance of numerous foci of necrosis, deformation of the accini in the hepatopancreas, inflammatory infiltrates , hypertrophy of the cells, enlargement the basal lumen as well as deterioration of the membrane, our results are in the same direction as those of Fernandez et al. (2020), who have highlighted numerous tissue alterations following the exposure of *Helix aspersa* to an insecticide based on lambda-cyhalothrin, or the work of Zouaghi et al. (2020), which have demonstrated the appearance of foci of necrosis, cellular hypertrophy in the kidney and of hepatopancreas following the effect of insecticide family neonicotinoids, the work of Ait Hamlet et al. (2020), showed a degeneration of the digestive tubules, and advanced alteration and inflammation of the hepatopancreatic envelope and the presence of cellular debris, following the exposure of *Helix aspersa* to a xenobiotic of food additive-type.

5 Conclusion

This study allowed us to note an oxidative stress import through the evidence of a reduction of the fresh weight of the snails treated with the strongest concentrations of lambda-cyhalothrine, the evidence of an increase in the protein pool in *Helix aspersa* in the presence of the insecticide as well as the evidence of a dose-dependent increase in catalase activity in snails treated with lambda-cyhalothrin.

In addition, we noted the involvement of the GSH/GST system through an increase in GST activity and a decrease in GSH activity in snails treated with increasing concentrations of lambda-cyhalothrin.

Also, the treatment of *Helix aspersa* snail caused important tissue alterations in the hepatopancreas and kidney, such as the appearance of numerous foci of necrosis, deformation of the accini in the hepatopancreas, inflammatory infiltrates, cell hypertrophy, enlargement of the basal lumen and membrane deterioration.

Through these results, we can conclude that the use of an insecticide based on lambda-cyhalothrin, widely used in the agricultural field in Algeria, constitutes a threat to the living organisms because of its certain toxicity, which represents a source of pollution and the degradation of the environmental diversity and a threat for the public health.

Conflict of interests

The authors declare that they have no conflict of interest.

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References

Ait Hamlet, S., Bensoltane, S., & Berrebbah, H. (2020). Effets aigus de l'exposition à la tartrazine (E102) sur le comportement et l'histologie d'un modèle biologique l'escargot terrestre *Helix aspersa*. Nutr. Santé, 9, 43–51.

Anadón, A., Martínez, M., Martínez, M. A., Díaz, M. J., & Martínez-Larrañaga, M. R. (2006). Toxicokinetics of lambda-cyhalothrin in rats. Toxicology Letters, 165, 47–56.

Ansari, R.W., Shukla, R.K., Yadav, R.S., Seth, K., Pant, A.B., Singh, D., Agrawal, A.K., Islam, F., & Khanna, V.K. (2006). Involvement of dopaminergic and serotonergic systems in the neurobehavioral toxicity of lambda-cyhalothrin in developing rats. Toxicology Letters, 211, 1–9.

Arrighetti, F., Ambrosio, E., Astiz, M., Rodrigues Capítulo, A., & Lavarías, S. (2018). The differential response between histological and biochemical biomarkers in the apple snail *Pomacea canaliculata* (Gasteropoda: Amullariidae) exposed to cypermethrin. Aquatic Toxicology, 194, 140–151.

Atailia, A. (2017). Impact de la pollution industrielle (métauxlourds) sur le développement et la reproduction de l'escargotHelix aspersa :Effets sur les biomarqueurs. Thèse de doctorat. Université de Annaba, 194, 89p.

Barr, D. B., Olsson, A. O., Wong, L. Y., Udunka, S., Baker, S.E., Whitehead, R.D., Magsumbol, M.S., Williams, B.L., & Needham, L.L. (2010). Urinary concentrations of metabolites of pyrethroid insecticides in the general U.S. population: National Health and Nutrition Examination Survey 1999-2002. Environmental health perspectives, 118, 742–748.

Belhaouchet, N., Djebar, M.R., Meksem, L.,Grara, N., Zeriri, I.,& Berrebbah, H. (2012). Evaluation of the biomarkers of the oxidative stress induced by a biopesticide: The Spinosad on an alternate model: *Helix aspersa*. Journal of Applied Sciences Research, 8, 4199–4206.

Besanci, S., Bensoltane, S., & Djekoun, M. . (2019). Oxidative stress and histological changes induced by the nano-Fe2O3 in *Helix aspersa*. Scienctific study and reaserch, 20, 119–133.

Bhoopendra, K., & Nitesh, K. (2014). Immunotoxicity of lambda-Cyhalothrin in Wistar albino rats. International Journal of Toxicological and Pharmacological Research, 6, 47–56.

Bossou, A. F. A. D., Bogninou, G. S. R., AgbangnanDossa, C. P., Yedomonhan, H., Avlessi, F., & Sohounhloué, D. (2020). Volatile profiles and biological properties of cymbopogoncitratus, cymbopogongiganteus, cymbopogonshoenanthus, and their isolated compounds. Journal of Biomedical and Pharmaceutical Research, 9, 1–56.

Bouaricha , H. (2013). Evaluation du stress oxydatifinduit par le proclaim :Essaicomparatif sur deuxmodèlesbiologiques(*Helix aspersa* et paramecium sp.). Thèse de doctorat. Université de Annaba. Algérie, 131 p.

Boucenna, M. (2016). Etude de la toxicité des particulesmétalliquesd'origineindustrielle chez *Helix aspersa*.Evaluation nanotoxicologique du Fe2O3et Al2O3. Thèse de doctorat.Université de Annaba. Algerie, 193 p.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248–254.

Chabicovsky, M., Niederstätter, H., Thaler, R., Hödl, E., Parson, W., Rossmanith, W., & Dallinger, R. (2003). Localization and quantification of Cd- and Cu-specific metallothionein isoform mRNA in cells and organs of the terrestrial gastropod *Helix pomatia*. Toxicology and Applied Pharmacology, 190, 25–36.

Cluzeau, S. (1993). Index phytosanitaire.29ème éd.Paris. ACTA, 80p.

Coeurdassier, M.M., Saint-Denis, A., Gomot-de Vaufleury, D., Ribera, & Badot, P.M. (2001). The garden snail (*Helix aspersa*) as bioindicator of organophosphorus exposure: effects of dimethoate on survival, growth and acetylcholinesterases activity. Environmental Toxicology and Chemistry, 20, 1951–1957.

Dickinson, D.A., & Forman, H.J. (2002). Cellular glutathione and thiols metabolism. Biochemical pharmacology, 64, 1019–1126.

El Gendy, K.S., Radwan, M. A., & Gad, A.F. (2009). In vivo evaluation of oxidative stress biomarkers in the land snail, *Theba pisana* exposed to copper-based pesticides. Chemosphere, 77, 339–344.

Farfar, K., Khebbeb, M.E.H., & Djebar, M.R. (2018). Toxicity of a fungicide based on a copper oxychloride in the presence cadmium on snail (*Helix aspersa*) biomarkers. Journal of biodiversity and environmental sciences, 12, 39–47.

Fernandez san juan, M.R., Gortelezzi, A., Albornoz, G.B., Landro, S.M., Arrighetti, F., Najle, R., & Lavaria, S.M.L. (2020). Toxicity of pyrethrinoidcypermethrin on the freshwater snail *Chilina parachappii*: Lethal an sub lethal effects. Ecological and environmental safety, 196.

Franzellitti, S., & Fabbri, E. (2005). Differential HSP70 gene expression in the Mediterranean mussel exposed to various stressors. BiochemBiophys Res Commun., 336, 1157–1163.

Frías-Espericueta, M., Abad-Rosales, S., Aidée, C.N.V., Isidro Osuna, L., Páez-Osuna, F., Olvera, R.L., & Voltolina, D. (2008). Histological effects of a combination of heavy metals on Pacific white shrimp *Litopenaeus vannamei* juveniles. Aquatic Toxicology, 89, 152–157.

Gomot-de Vaufleury, A., & Bispo, A. (2000). Methods for Toxicity Assessment of Contaminated Soil by Oral or Dermal Uptake in Land Snails.1. Sublethal Effects on Growth. Environmental Science & Technology, 34, 1865–1870.

Grara, N. (2011). Evaluation de la toxicité de certainspolluantsindustriens sur un animal bioaccumulateur (gasteropode *Helix aspersa*) :Cas des métaux .Thèse de doctorat. Université de Annaba, 120p.

Grara, N., Bouloudenine, M., Khaldi, F., Zenir, Z., & Abdemadjid, S. (2015). Caractérisation Morphophysiologique de la Toxicité du ZnO (Nanoparticulemanufacturée) sur l'escargotl'Helixaspersa bio indicateur de pollution de l'environnement. J. Mater. Environ. Sci., 6, 2596–2603.

Habig, W.H., Pabst, M.J., & Jakoby, W.B. (1974). Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry, 249, 7130–7139.

He, L. M., Troiano, J., Wang, A., & Goh, K. (2008). Environmental Chemistry, Ecotoxicity, and Fate of Lambda-Cyhalothrin. Reviews of Environmental Contamination and Toxicology, -, 71–91.

Heusser, S. , & Dupuy, H.G. (2011). De la structure tissulaire à la réalisation des fonctions chez les gastéropodespulmonés (I) Elémentd'histologieet de physiologie des espècesHelix aspersaet *Helix pomatia*. Folia conchyliologica, 10, 16–25.

Hossain, M. M., Suzuki, T., Sato, I., Takewaki, T., Suzuki, K., & Kobayashi, H. (2004). The Modulatory Effect of Pyrethroids on Acetylcholine Release in the Hippocampus of Freely Moving Rats. NeuroToxicology, 25, 825–833.

Julien, R., Adamkiewicz, G., Levy, J.I., Bennett, D., Nishioka, M., & Spengler, J.D. (2008). Pesticide loadings of select organophosphate and pyrethroid pesticides in urban public housing. J Expo Sci Environ Epidemiol. Mar., 18, 167–174.

Kaneko, M., Shingai, H., Pohlman, J. W., & Naraoka, H. (2010). Chemical and isotopic signature of bulk organic matter and hydrocarbon biomarkers within mid-slope accretionary sediments of the northern Cascadia margin gas hydrate system. Marine Geology, 275, 166–177.

Khene, L. (2020). Toxicité des ETM (Micro et Nano particules)sur un organisme bio accumulateur /bio indicateur :Helix aspersa.Thèse de doctorat. Université de Annaba, 159p.

Khene, L., Berrebbah, H., Yahiyaoui, A., Bouarroudj, T., Zouainia, S., Kahli, H., & Bourayou,C. (2017). Biomarkers of oxidative stress, lipid peroxidation and ros production induced by TiO2 Microparticles on snails *Helix aspersa*. Studiauniversitatis, 27, 127–133.

Kourtidis, A., Drosopoulou, E., Nikolaidis, N., Hatzi, V.I., Chintiroglou, C.C., & Scouras, Z.G. (2006). Identification of several cytoplasmic HSP70 genes from the Mediterranean mussel (*Mytilus galloprovincialis*) and their long-term evolution in Mollusca and Metazoa. Journal of molecular evolution, 62, 446–459.

Laskowski, R., & Hopkin, P. (1995). Accumulation of Zn, Cu, Pb, and Cd in the garden snail (*Helix aspersa*): Implication for Predators. Environmental Pollution, 91, 289–297.

Livingstone, D.R. (2001). Contaminant-stimulated reactive oxygène species production and oxidative damage in aquatic organism. Mar pollut Bull., 42, 656–666.

Martoja, R., & Martoja, M. (1967). Initiation aux techniques de l'histologieanimale. Masson et Cie, 347p.

Massaya, M., Yoshinobu, H., Ai, Y., Maki, K., & Yasuo, O. (2002). Determination of cellular levels of nonprotein thiols in phytoplankton and their correlations with susceptibility to mercury. Journal of Phycology, 38, 983.

Melnyk, L.J., McCombs, M., Brown, G.G., Raymer, J., Nishioka, M., Buehler, S., Freeman, N.,& Michael, L.C. (2012). Community duplicate diet methodology: a new tool for estimating dietary exposures to pesticides. J Environ Monit., 14, 85–93.

Morgan, M., MacMillan, D.,& Zehr, D. (2016). Pyrethroid insecticides and their environmental degradates in repeated duplicate-diet solid food samples of 50 adults. J Expo Sci Environ Epidemiol, 28, 40–45.

Radwan, M.A., El-Gendy, K.S., & Gad, A.F. (2010). Biomarkers of oxidative stress in the land snail, *Theba pisana* for assessing ecotoxicological effects of urban metal pollution. Chemosphere, 79, 40–46.

Regoli, F., & Principato, G. (1995). Glutathione, glutathione-dependent and antioxidant enzymes in mussel *Mytilus galloprovincialis* exposed to metals under field and laboratory conditions: implication for the biomarkers. Aquatic Toxicology, 31, 143–164.

Riederer, A.M., Bartell, S.M., Barr, D.B., & Ryan, P.B. (2008). Diet and non-diet predictors of urinary 3-phenoxybenzoic acid in NHANES 1999-2002. Environ Health Perspect. , 116, 1015–1022.

Ross, M.K., Borazjani, A., Edwards, C.C., & Potter, P.M. (2006). Hydrolytic metabolism of pyrethroids by human and other mammalian carboxylesterases. BiochemPharmacol , 71, 657–669.

Starr, J.M., Graham, S.E., Ross, D.G., Tornero-Velez, R., Scollon, E.J., Devito, M.J., Crofton, K.M., Wolansky, M.J., & Hughes, M.F. (2014). Environmentally relevant mixing ratios in cumulative assessments: a study of the kinetics of pyrethroids and their ester cleavage metabolites in blood and brain; and the effect of a pyrethroid mixture on the motor activity of rats. Toxicology , 5, 15–24.

Tellier, S. (2006). Les pesticides en milieu agricole :état de la situation environnementale et initiatives prometteuses. Québec, ministère du Développement durable, de l'Environnementet des Parcs, 90 p.

Vaufleury, A. G., & Kerhoas, I. (2000). Effects of Cadmium on the Reproductive System of the Land Snail *Helix aspersa*. Bulletin of Environmental Contamination and Toxicology, 64, 434–442.

Vidal, M.L. (2001). Etude de marqueursbiochimiques de pollution chez le mollusque bivalves d'eaudouce *Corbicula fluminea* (Muller)- Purification et caraterisation des Glutathion S-Transferases. Thèse de doctorat. Université de Bordeaux, 267p.

Weeckbeker, G., & Cory, J.G. (1988). Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukameia L1210. Cells in vitro. , 40, 257–264.

Wondji, C.H., Morgan, J., Coetzee, M., Hunt, R., Steen, K., Black, W., Hemingway, J., & Ranson, H. (2007). Mapping a Quantitative Trait Locus (QTL) conferring Pyrethroid Resistance in the African Malaria Vector. BMC genomics , 8, 34.

Worthing, CR., & Hance, RJ. (1991). The pesticide manual. 9th ed. Old Woking, Surrey. British Crop Protection Council, 64, 203–204.

Yang, W., Chata, G., Zhang, Y., Peng, Y., Lu, J. E., Wang, N., & Chen, S. (2018). Graphene oxide-supported zinc cobalt oxides as effective cathode catalysts for microbial fuel cell: High catalytic activity and inhibition of biofilm formation. Nano Energy.

Zaldibar, B., Cancio, I.,& Marigómez, I. (2008). Epithelial cell renewal in the digestive gland and stomach of mussels: season, age and tidal regime related variations. Histology and Histopathology , 23, 281–290.

Zouaghi, M.F., Berrebbah, H., Boudoucha, I., Rekaik I. (2020). Evaluation of the toxicity of mixture insecticides used on a biologic