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EVALUATION OF THE TOXICITY OF A MIXTURE INSECTICIDES USED ON A BIOLOGICAL MODEL: THE SNAIL *HELIX ASPERSA*

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Abstract: In this study, we were interested in evaluating the impact of a mixture of two neonicotinoid insecticides and their effects on a bioaccumulative organism and bioindicator of environmental pollution, the snail *Helix aspersa*.
This is a subchronic toxicity study (21-day). Insecticide toxicity is determined in the snail *Helix aspersa* by a laboratory biotest on animals exposed to increasing concentrations of insecticide mixture (70 µg/g, 180 µg/g, 340 µg/g diet).
Our results show physiological disturbances in the weight and shell diameter of the treated snails, while metabolic changes indicate a disturbance in protein levels and a decrease in carbohydrate levels (dependent dose) in the hepatopancreas and kidney.
Our results also show the existence of an induction of catalase activity constituting one of the cellular defence mechanisms against the presence of insecticides in two target organs.

Keywords: insecticide, Helix aspersa, bioaccumulator, bioindicator, subchronic.

INTRODUCTION

To meet the food needs of a global population of 9 billion
by 2050, global agricultural production must increase by 70% and double in developing countries (Alexandratos et Bruinsma, 2012). In order to increase yields and regularize production; in this context, the use of plant protection products or pesticides is a powerful lever (Lichiheb et al., 2015).

In Algeria, the use of insecticides, fertilizers, detergents and other phytosanatary products is spreading more and more with the development of agriculture, but also in the context of actions to control harmful vectors.

This use of toxic chemicals on a national scale is likely to cause serious pollution to soils, water bodies and threatens the health of the population (Bouziani, 2007)

Studies show that neonicotinoids are active against a wide spectrum of economically important crop pests' .However; their broad spectrum leads to adverse effects on populations of honeybee, aquatic invertebrates and many bird species (Guégan and al., 2017; Elbert and al., 2008). The use of invertebrates for assessing ecosystem quality has a long history in aquatic and terrestrial environments (Eijsackers, 2010).Pulmonary terrestrial gasteropod molluscs are recognized as appropriate biological indicators due to their high capacity to accumulate different pollutants (Cortet et al., 1999).

Neonicotinoids bind to nicotinic receptors of acetylcholine (nAChR); activation of nAChRs often results in an increase in intracellular free Ca2, while their overactivation generates a blockage resulting in fatal paralysis (Aina et al., 2015). In addition, (Iwasa, 2004) confirms the paralysis and death of insects following the accumulation of acetylcholine in the central nervous system of the insect.

MATERIALS AND METHODES

The biological material used in our research work is the snail *Helix aspesra* called small gray, is a gasteropod mollusc, terrestrial pulmonary

We used in our experiment a mixture of two insecticides belonging to the neonicotinoid family; the choice was made according to a survey on the use of pesticides in the wilaya of Jijel. To support this survey, we approached farmers and sales outlets of plant protection products. This initiative has confirmed the frequent use of Imidacloprid and Acetamipride by farmers in the Jijel region, especially in the treatment of strawberry, the first insecticide with trade name FIDOR SUPER 70 Containing 70% Imidacloprid in powder form. The second insecticide with trade name ACEPLAN contains 20% of acetamipride

The animals were been treated under controlled conditions by adding increasing concentrations of pesticides in 20 g of feed (wheat flour). Each batch of four snails, a first control batch, the second, third and fourth batch of snails are intended for mixture treatments M1, M2, M3 respectively. A 21-day treatment was chosen by mixing two insecticides (Imidaclopride - Acetamipride), every two days the food is renewed when the boxes are cleaned. The mixtures were tested at the doses mentioned above (Tab.1).

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Table 1

Composition of the tested mixtures

Mixture	Designation	Acetamipride and Imidaclopride concentration
Mixture 1	**M1**	50 µg Imidaclopride + 20 µg acetamipride
Mixture 2	**M2**	140 µg Imidaclopride + 40 µg acetamipride
Mixture 3	**M3**	280 µg Imidaclopride + 60 µg acetamipride

Determination of total protein

According to the methode of Bradford 1976 ; which uses Commassie Brilliant Bleu G250 (BBC) as a reagent and a solution of Beef Serum Albumin (BSA) stock solution as a standard protein .The reading of the absorbances is obtained by a spectometer at a wavelength 595nm.

Determination of catalase (CAT) activity

The CAT activity was determined spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2 (Regoli et Principato, 1995). The result was expressed as µmol/ min/ mg of proteins.

Histological analysis

Our histological sections were performed at the pathology laboratory level of specialized hospital center Abdallah Nouaouria El Boui- Annaba.

RESULTS

Effect of insecticide treatment on physiological biomarkers in *Helix aspersa*

Evaluation of the weight of snails

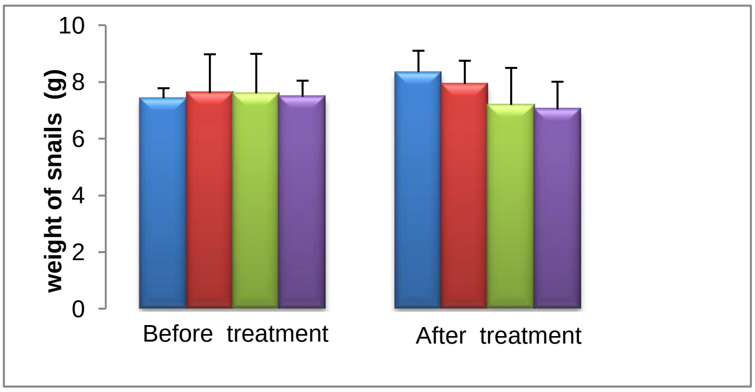

Fig. 1. Effect of the insecticide mixture on the evolution of weight of *Helix aspersa* snail.

Evaluation of the shell diameter of snails

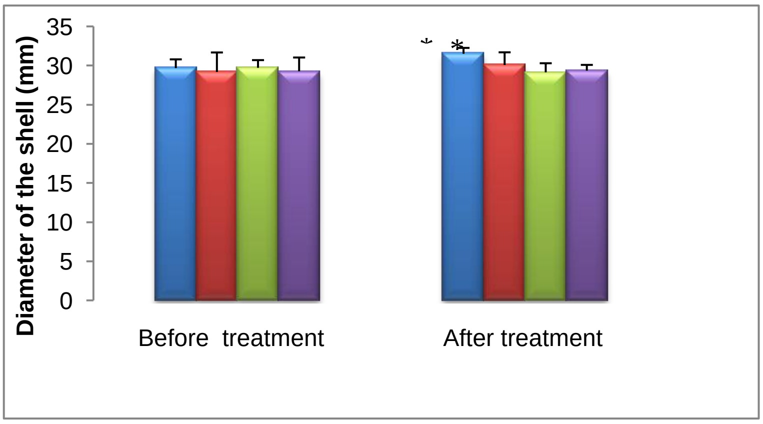

Fig. 2. Effect the insecticide mixture on the evolution of diameter of *Helix aspersa* snail.

Effects of insecticide treatment on biochemical and enzymatic parametres

Effects of insecticide treatment on total carbohydrate

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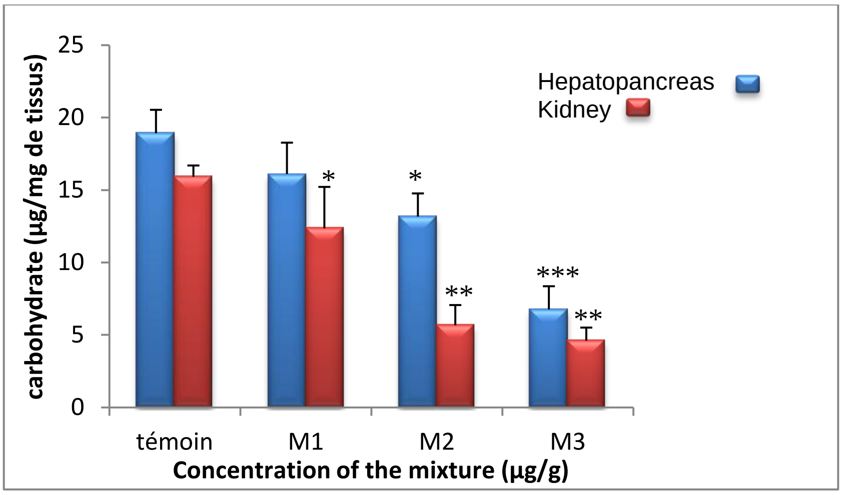

Fig. 3. Effect of the insecticide mixture on the total carbohydrate of *Helix aspersa* snail.

Effects of insecticide treatment on total protein

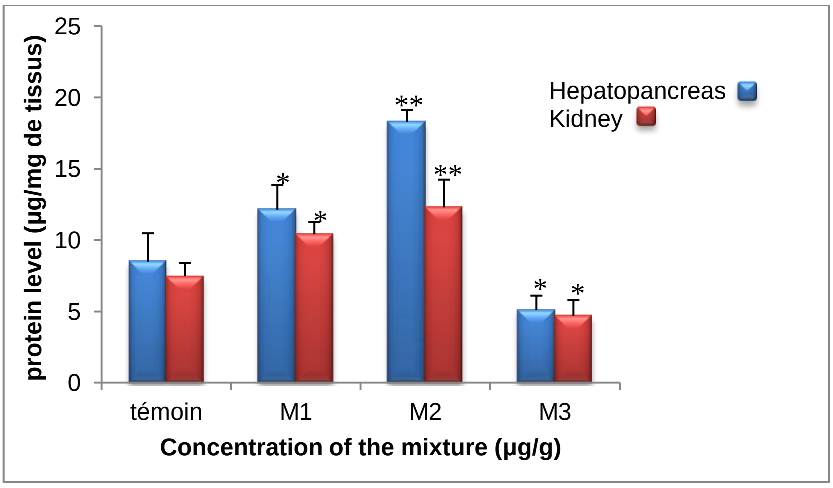

Fig. 4. Effect of the insecticide mixture on the total protein of *Helix aspersa* snail.

Effects of insecticide treatment on Catalase activity

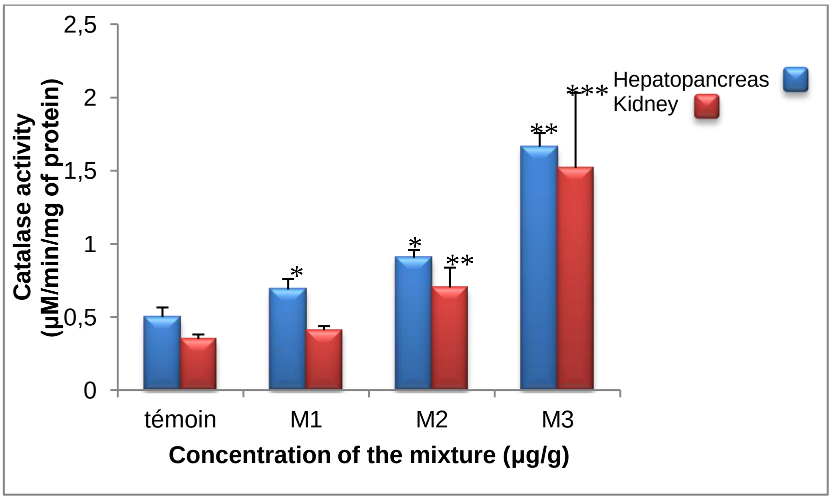

Fig. 5. Effect of the insecticide mixture on the activity Catalase of *Helix aspersa* snail.

Effects of treatment with insecticides on hepatopancreas and kidney snails *Helix aspersa* Histopathological study

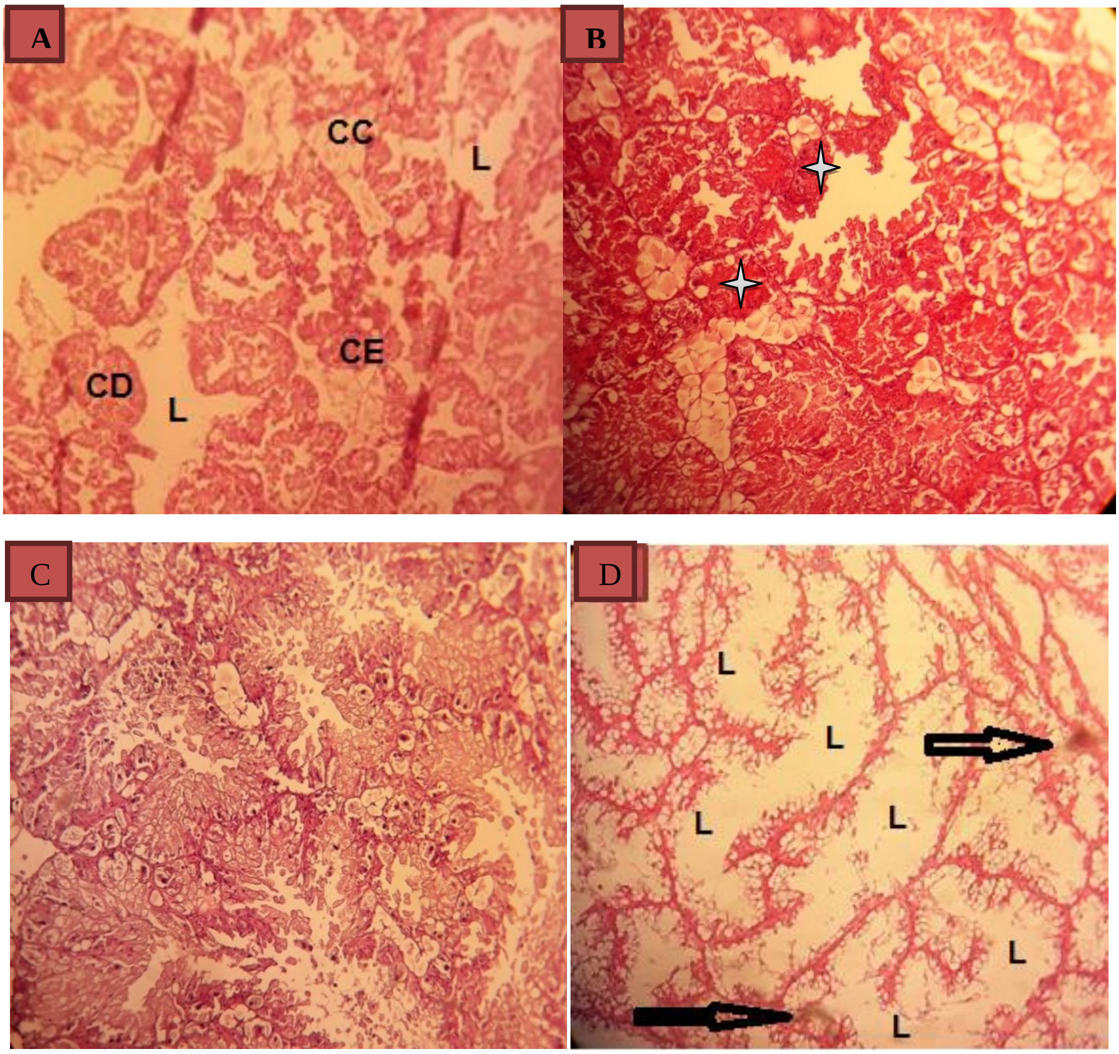

Fig. 6. Histological sections of hepatopancreas of snails control (A) and treated with different pesticide mixtures (B), (C), (D), (G×40).
Legend : L: Light of l'acini, CD: digestive cell, CE: excretory cell, CC: Calcium cell , ⇨ :cell necrosis, ✧ : cellular hypertrophy.

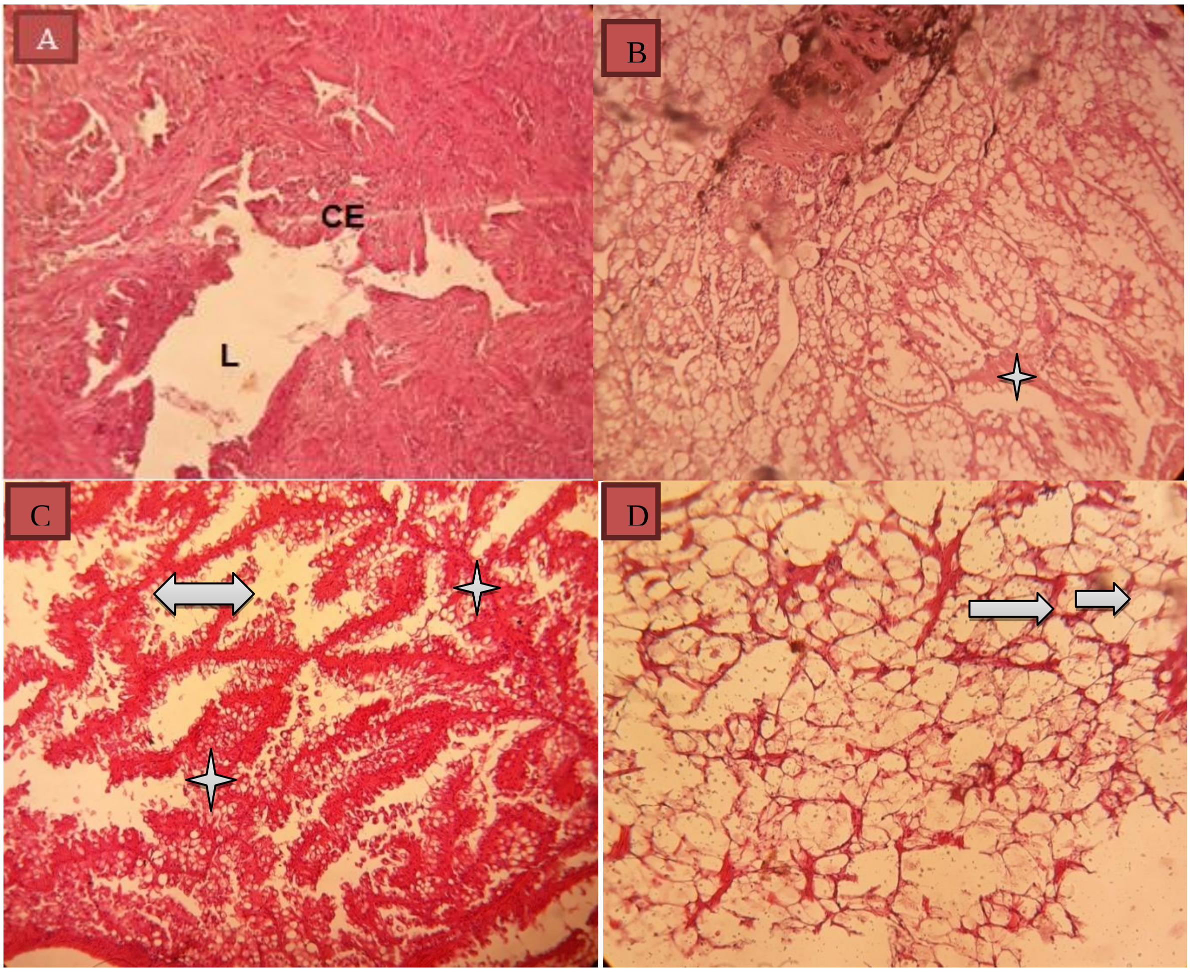

Fig. 7. Histological sections of kidney of snails control (A) and treated with different pesticide mixtures (B), (C), (D), (G×40).
Legend : L: light of l'acini, CE: excretory cell, ⇨ :cell Hyperplasia, cellular hypertrophy; ✧

DISCUSSION

Our results are in line with the work of Coeurdassier et al. (2001), which observed a dose-dependent decrease in the growth and survival of snails induced by dimethoate, this weight loss can be explained by the decrease in food consumption that we have observed particularly in animals treated by different concentrations. Thus (Grara et al., 2015) made similar findings in the presence of contamination by metal nanoparticles (ZnO) at increasing concentrations, for four weeks. They explained this loss of growth either by direct toxicity in conjunction with irreversible damage to certain enzymes or DNA, or indirectly causing disturbances to important physiological processes by competing with certain essential elements such as calcium (Ca).

Regarding the evolution of the diameter of the shell, we notice a decrease in the dose-dependent on the shell diameter of the snails treated with the mixtures from the M2. Our results are in line with that of (Smina, 2013), which showed a disturbance of snail shell diameters after exposure to thiamethoxam (neonicotinoid insecticide) and tefluthrin (pyrethroid insecticide), and those of (Grara et al., 2015) which showed a decrease in shell diameter in the presence of metal dust.

Carbohydrates are compounds of carbon, hydrogen and oxygen, in the body their main function is to store and provide energy (Brooker, 2000). Under stressful conditions, glucose levels are significantly reduced to ensure adequate energy intake (Carefoot et al., 1993). In our work, we noted that carbohydrate levels decrease in a dose-dependent manner after exposure of snails to the mixture of insecticides imidacloprid and acetamipride at the level of the two-targeted organs (Hepatopancreas and kidney).

On the other hand, (Padmaja et Rao, 1994), suggest that the depletion of glycogen levels in the tissues of the freshwater snail Bellamya dissimilis, exposed to pesticides (Endosulfan, Methyl-parathion, Quinalphos, Nuvan) may be due to the direct effects of the use of these compounds for energy production, or as a result of pesticide-induced hypoxia. For the same species exposed to hydrocarbons (Zouaghi et al. 2015), suggests a decrease in the total carbohydrate level when snails are exposed to hydrocarbons, to ensure a sufficient energy supply.

We looked at the effect of acetamipride and imidacloprid mixture on the evolution of the total protein levels in snails under controlled conditions.

We note a dose-dependent increase in total protein levels in the two target organs (hepatopancreas and kidney) for both mixtures M1 and M2 compared to the control, our results are in line with those of several authors which have shown a significant increase in total protein levels due to chemical stress because glutathione is an essential factor in the defence against oxidative stress (Zaafour et al., 2014; Redouane et al., 2004; Tadjine, 2007).

These results are consistent with those of (Masaya et al. 2002); (Peccini et al. 1994), which showed a significant increase in total protein levels as a result of chemical stress in different biological models (tadpoles, protists, rabbits).

This increase could be due to the induction of the synthesis of detoxification (gluthathion) and metabolizing enzymes under the effect of oxidative stress produced by the application of both insecticides.

In our work, catalase activity at the hepatopancreas and kidney tends to increase dose-dependentin in all batches compared to controls, these results are consistent with those of (El-wakil et Radwan., 1991), which showed a significant increase in catalase activity after exposure of gasteropod Eubania vermiculata to pesticides (Methomyl, Thiodicarb, Metaldehyde).

The increase in CAT activity has already been observed in other gasteropod species such as Theba pisana after exposure to copper-based pesticides (copper oxychloride, copper hydroxide) compared to the (el-Gendy et al. 2009).

Our histological study shows that snails' exposure to pesticide mixtures can cause very significant cytological and ultra-structural alterations in the hepatopancreas, which plays a crucial role in detoxifying pollutants (Frias Espericueta, and al., 2008). Indeed, the histological examination of the renal and hepatopancreatic epithelium reveals the appearance of inflammatory infiltraters Lymphoplasmocytics from the lowest concentration, this could be a first biological response due to the presence xenobiotics; these observations are consistent with the work of; (Chabicovsky et al. 2004), and (Russell et al. 1981), and (Tadjine 2007), we also found a deformation of acini and a destruction of digestive cells in a dose-dependent manner, leading to very significant necrosis of these cells at the highest concentrations.

Digestive cell necrosis appears to be a general response after exposure to pesticide mixtures in terrestrial gasteropods and is mainly related to the deterioration of the digestive process caused by the presence of metal particles (Zaldibar et al., 2008).

Histopathological responses to several organs (digestive gland, kidney, ovaries, etc.) of Helix aspersa exposed to increasing concentrations of pesticide mixtures in food are manifested by reactions that involve hyperplasia of epitheliums at the lowest concentrations tested and significant cellular necrosis that is accompanied by a proliferation of connective tissues (Chabicovsky et al., 2004; Radwan et al., 2008).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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