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Bifenthrine & Hymexazol Toxic Effects on *Helix aspersa* and the Evaluation of the Protective Effect of Orange Essential Oils

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ABSTRACT

The aim of this work is to study of the toxicity induced by two types of pesticides (bifenthrin (BF) and hymexazole (HY)) at the hepatopancreatic level in *Helix aspersa*, which is a bioaccumulator organism and bioindicators in environmental pollution, as well as the evaluation of the protective effect of orange essential oils (OEO).

This is an experimental study conducted in the laboratory on 40 Helix Aspersa snails that have been divided into four groups (10 snails each): (i) a control group, (ii) a group injected by the OEO (0.151 UL /g), (iii) a group injected by the combination BF (0.83 mg /ml) and HY (90 ug /g) and (IV) a group injected by the OEO, BF and HY mixture. The treatment was stopped after 90 days. Five oxidative stress markers (MDA, GSH, GST, GPX and CAT) as well as mitochondrial respiration have been measured. In this study, the results show that the BF and HY mixture induces an increase in MDA and GSH levels as well as an increase in the enzymatic activity of the GST, Catalase, and GPX at the hepatopancreatic level. We have also shown that these two pesticides cause a decrease in the level of the mitochondrial respiration. In addition, this results show that OEO supplementation restored most of oxidative stress markers to levels close to those of control group. Our study suggests that OEO can be used as an effective antioxidant to alleviate the of oxidative stress intensity induced by the BF and HY mixture.

Keywords :-Bifenthrine, Hymexazol, essential oil, orange, Helix Aspersa, toxicity, protective effects, oxidant stress, mixtures.

Introduction

Phytosanitary products include several classes, the three main ones of which are: fungicides, herbicides and insecticides. These three classes alonerepresent almost all of the world market

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(97%) [1]. In Algeria, the use of insecticides, fertilizers, detergents and other phytosanitary products is increasingly spreading with the development of agriculture, but also in the context of control of harmful vectors. This use of toxic chemicals is likely to cause serious soil pollution and water bodies and threatens the health of the population [2].

Bifenthrin is a Pyrethroid used against a range of agricultural pests [3]. BF comes in the form of a beige waxy product. It is practically insoluble in water but soluble in most organic solvents and remains stable at 25 °C and 50 °C for two years [4]. Its properties such as low solubility in water and photo-stability make it an effective insecticide and acaricide against a wide range of leaf pests in agriculture [5, 6]. Compared to other Pyrethroids, the toxic effects of bifenthrine are more important. Indeed, some studies describethe alteration of biochemical, hematological and histopathological parameters because of its toxicity [7,8].

Hymexazole is the active substance of a systemic seed fungicide marketed in Algeria under the name of Tachigazol. It belongs to the chemical family of Triazines, [9]. Hymexazol comes in the form of a liquid soluble in methanol, acetone, ethyl acetate, hexane, toluene and dichloromethane [10].

The data on the ecotoxicity of the BF and the HY are rare. To carry out toxicological studies, it is necessary to have indicators of environmental disturbance through organisms or a set of organisms that are used as sentinels by studying the physiological, biochemical and ecological changes that affect them. The snail *(Helix aspersa)* constitutes a biological model widely used in toxicological studies [11, 12, 13, 14, 15]. Invertebrates in the structure of communities and the functioning of terrestrial and aquatic ecosystems justify the interest of extending ecotoxicological research and procedures to these animal groups [12].

The toxicity induced by the two pesticides, at the hepatopancreatic level in *Helix asparsa* causes significantoxidative stress and changes in the activity of antioxidant enzymes. In order to reduce the harmful effects of pesticides, several chemicals with antioxidant properties have been proposed. Orange essential oils (*Citrus sinensis*) are widely used by pharmaceutical industries and phytotherapy and are known for their antioxidant properties which can be grouped into non-volatile and volatile fractions [16].

The first objective of this study is to assess the toxic effects of exposure to the mixture of these two pesticides (BF+HY) on certain biomarkers of oxidative stress at the hepatopancreatic level.

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The second objective is to estimate the possible protective effect of essential oils of the orange peel to reduce the toxic effects of these substances on the snail.

Material and methods

Plant material

The oranges were bought at the Tébessa commune market during the month of December 2020. Mature and healthy fruits were selected. The identification of the species *Citrus Sinensis (Thomson Navel)* was made by Ms. Hioun (botanist working at the University of Tébessa) on the basis of descriptive criteria (presence of seeds, navel, skin, color). The orange peel was cut into small pieces and dried in the shade for 15 days at room temperature.

OEO extraction

The extraction of the essential oils of the orange peel was carried out by hydrodistillationunisng the Clevenger-type apparatus. One hundred grams (100 g) of plant material was introduced with 1200 ml of distilled water in a balloon. After installing and closing the assembly, the balloon heating is set up with optimum heating adjustment to allow stability of the extraction at a constant and well controlled speed. The steam loaded with essential oil arrives in the condenser. The total duration of the extraction is estimated at 3 h. The essential oil is distinguished from hydrosol (aromatic water) by its difference in density and color. The OEO was separated and then recovered and kept in an amber bottle tightly closed and stored at 4 °C.

Animals

The biological material used in our research work is the *Helix aspersa* snail, commonly known as a little-gris, which is a gastropod mollusk, a terrestrial pulmonary pen belonging to the Helicidae family. The snails used (in the number of 40 snails) are adults with an average weight of 12 g (± 0.35g) collected in a site untreated by pesticides located in the Bekkaria region (Wilaya of Tébessa). They are then transferred to the laboratory where they are put in transparent plastic boxes with perforated cover containing water soaked in water to maintain humidity. They were fed on fresh eastern lettuce leaves. The boxes are cleaned regularly 3 times a week and the diet has been renewed daily. The snails were kept for an adaptation period of 15 days before the treatment, which was spread over a period of three months in optimal environmental conditions: Photoperiod of 18h of light/24h, temperature 20 ± 2 °C and hygrometry of 80 to 95 %.

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Chemical products

Bifenthrine: According to Nieradko(2015) [17], the bifenthrine insecticide nomenclature used in this study is [2-methylbifényl-3-ylmethyl (Z)-(IRS) -cis-3 (2-Chloro-3, 3, 3-Trifluroprop-1-Enyl)-2, 2-Dimethyl Cyclopropane Carboxylate] was purchased in a store selling agricultural equipment in the town of Tebessa and marketed under the name Tristar, 10% EC.

Hymexazol: [$C_4H_5NO_2$: 5-methyl-3 (2h) -isoxazolone] [9] used in this study was also purchased in a store selling agricultural equipment in the town of Tebessa and marketed Under the name Snake, 30% SL.

Experimental protocol

The experiments were carried out on 40 snails (*Helix aspersa*). Four (04) groups were created (10 snails each) as follows:

Group 1 (T): Control, the snails were injected with physiological water (20 µl/g/d).

Group 2 (OEO): The snails were injected by the OEO with a dose of 0.151µl/g/d body weight.

Group 3 (BF + HY): The snails were injected with HY (90 µg/g/d) + BF (0.83 mg/ml/g) of body weight.

Group 4 (OEO + BF + HY): The snails were injected with the mixture (OEO + BF + HY) with the same doses. OEO was administered 30 minutes before pesticides.

The injection was carried out using a micro-serringue over a period of 90 days.

After the treatment period, snails are kept on an empty stomach for 48 hours to empty their digestive system. They are then sacrificed by freezing at -20 °C and then dissected to remove hepatopancreas.

Dosage of glutathione and lipid peroxidation

• GSH

The dosage of glutathione carried out according to the method described in Weckbker and Cory (1988) [18]. The principle of this determination is based on the absorbance of 2nitro-5-mercapturical.

• MDA

The MDA can be detected by a colorimetric reaction with thiobarbitric acid (TBA). MDA detection after degradation of polyunsaturated fatty acids is 3 or 4 double peroxidated bonds. The dosage of MDA is carried out according to the method described in Esterbaer*et al.* (1992) [19].

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Dosage of antioxidant enzymes

- ✓ **GPX**

Enzymatic activity of glutathione peroxidase was measured by the procedure described in Flohe and Gunzler (1984) [20], using H_2O_2 as a substrate.The absorbance was recorded at 420 nm.

- ✓ **GST**

The mesurment of glutathione s Transferase (GST) activity was determined according to Habig*et al.* (1974) [21] It is on the basis of the conjugation reaction between the GST and a substrate, the CDNB (1chloro-2,4-Dinitrobenzene) as a glutathionic cofactor (GST), the conjugation leads to the synthesis of a new molecule : 1SGlutathionyl-2,4-Dinitrobenzene to measure the activity of the GST. The decrease of absorbance is recorded for three minutes by a spectrophotometer at a wavelength of 340 nm.

- ✓ **CAT**

The spectrophotometric dosage of the Catalase activity (CAT) was carried out according to the method of Cakmak and Horst (1991) [22]. The reduction in absorbance is recorded for 3 minutes using a spectrophotometer at a wavelength of 240 nm and a extinction coefficient = 39400 L • μm-1 • CM-1.

Mitochondria extraction

The extraction of mitochondria was made according to the method described by Kristal *et al.* (1991) [23]. It is based on the preparation of a homogenate followed by differential centrifugation. The cells were placed in an isolation pad containing 1 mm Tris-HCL PH 7.5, 1 mm EDTA PH 7.5, 70 mm Sacharose, 210 mm Mannitol, at +4 °C. Then, the homogenate was transferred to a centrifugal tube of several differential centrifugations at 4 °C. Finally, the mitochondrial base was washed twice and then suspended in 1 ml of insulation buffer to obtain the fresh mitochondrial suspension, a fraction of which will be used directly in the evaluation of mitochondrial breathing, the rest is kept at -80 °C for the rest of the dosages.

Mitochondrial Respiration test

Respiration has been estimated using an oxygraph (hansatech) using an oxygen electrode according to the method described by Rouabhi*et al.* (2006) [24] and Rouabhi*et al.* (2009) [25].

Statistical analysis

Statistical calculations were carried out using the minitab 18.1 statistical software and Excel 2007 software (Microsoft, Inc.). Our data were analysed statistically using one-way analysis of variance, followed by the Tukey test for the comparison between the different groups between them. The differences are considered significant as follows: significant when $P \leq 0.05$, highly significant when $P \leq 0.01$, very highly significant when $P \leq 0.001$.

Results

Orange peel yield in essential oils

Orange peel essential oil yield recorded in this study is 1.05%.

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GSH and MDA assessment

We have observed a very highly significant increase ($p \leq 0.001$) of the MDA rate in the lot treated by the HY+BF compared to witnesses (1.36 ± 0.06.µm /mg), while in the lottreated by the HY+BF combined with OEO we have noted a very highly significant decrease in the rate of MDA ($p \leq 0.001$) (0.62 ± 0.06 µm/mg) against (0.99 ± 0.09 µm/mg) among witnesses (fig. 01).

Treatment of snails with the HY+BF for 90 days leads to a very highly significant increase ($p \leq 0.001$) of the GSH content at the hepatopancreatic level (3.83 ± 4.52) x10-5µm/mg and decrease very highly significant after administration of OEO the snails compared to the control group (fig. 02).

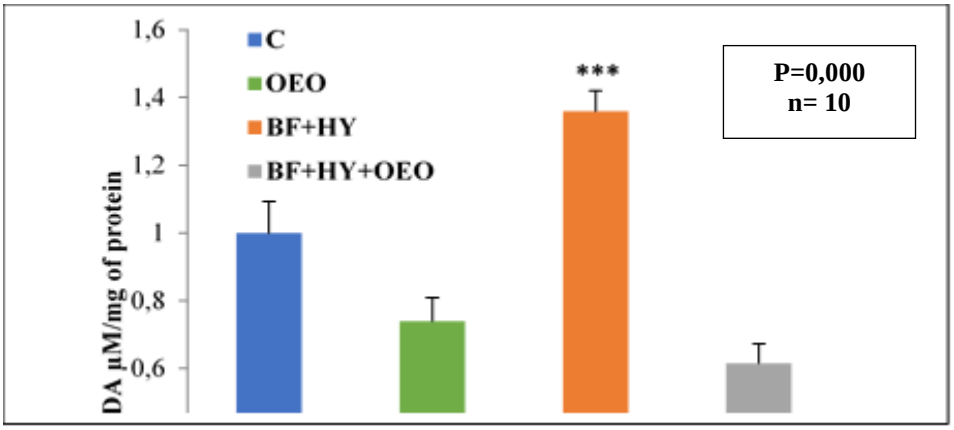

Fig 01. Variation of MDA rate (µM/mg prot) inhepatopancreasof *Helixaspersa* after 90 days of treatment (P<0.001).

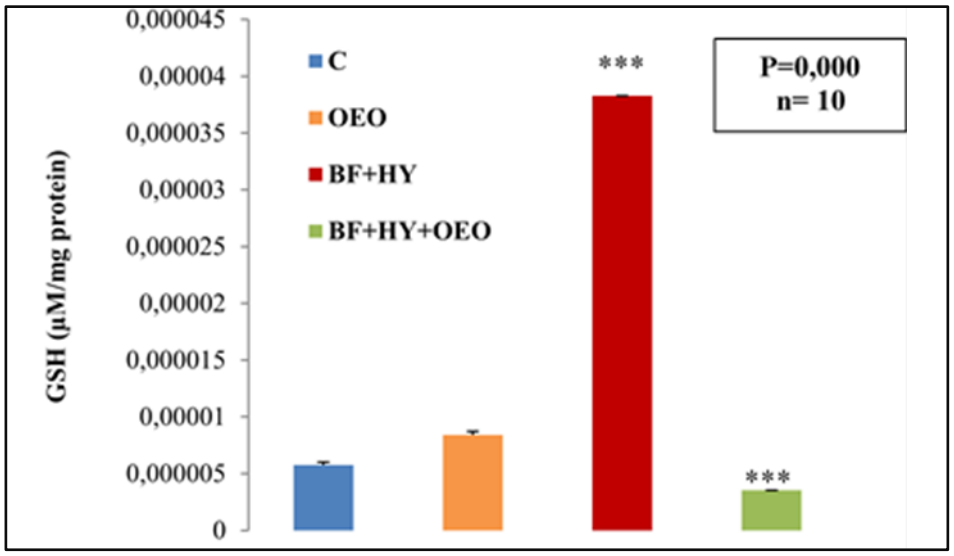

Fig 02. Variation of GSH content (µM/mg prot) in hepatopancreas of *Helix aspersa* after 90 days of treatment (P<0.001).

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Evaluation of the enzymatic activity of GPX, GST and CAT

The treatment with HY+BF has led to an increase in the enzymatic activity of the GST, CAT and significant decrease in GPX compared to the control. However, the group treated with a combination of OEO and HY+BF shows a decrease in GST and CAT and an increase in GPX respectively, in comparison with the control group (figs 03, 04 and 05).

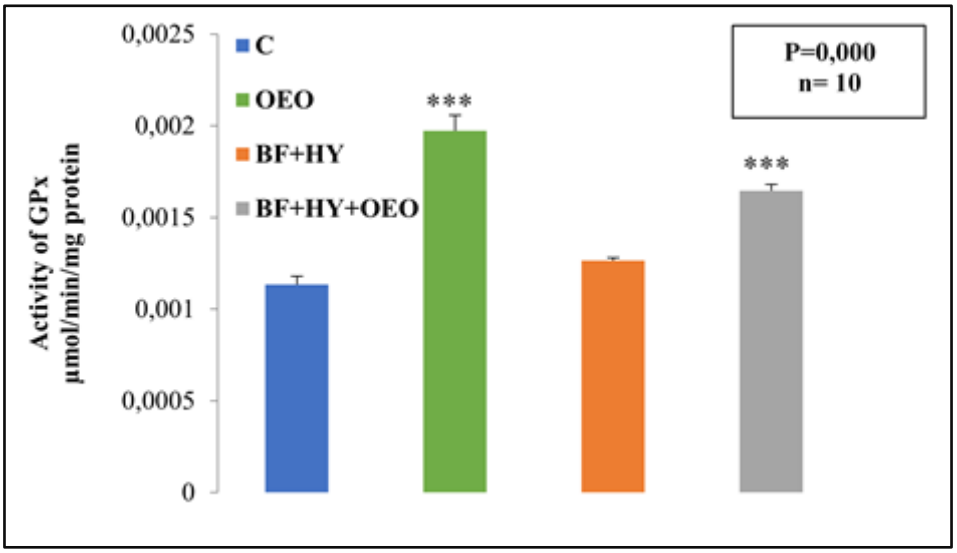

Fig 03. Variation of GPx activity (µM/min/mg) in hepatopancreas of *Helix aspersa* after 90 days of treatment (P<0.001).

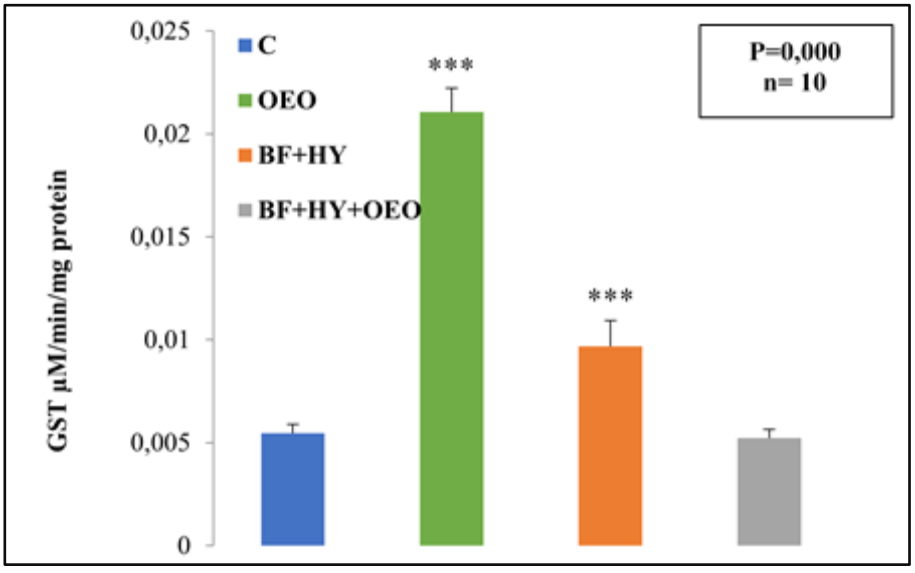

Fig. 04. Variation of GST activity (µM/min/mgprot) in hepatopancreas of *Helix aspersa* after 90 days of treatment (P<0.001).

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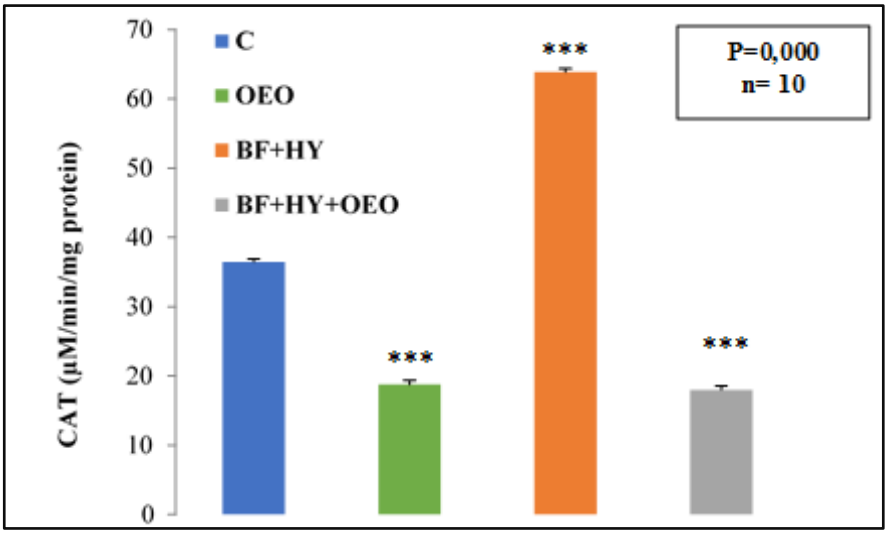

Fig. 05. Variation of CAT activity (µM/min/mgprot) in hepatopancreas of *Helix aspersa* after 90 days of treatment (P<0.001).

Mitochondrial respiration assessment

Our results illustrate a decrease in oxygen consumption at the mitochondrial level for all groups of snails compared with the control group. We have noted a slowdown in respiratory metabolism in particular in the snails treated by mixture of BF + HY.

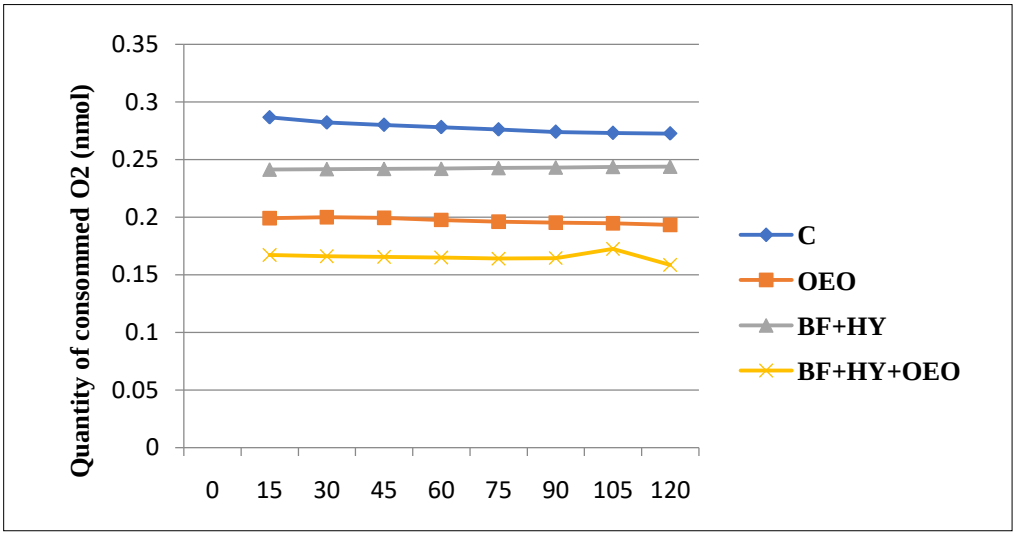

Fig 6. Effect of mixture (BF + HY) on mitochondrial respiration (nmol/sec) after 90 days of treatment .

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Discussion

Pesticides enter the body of the snail through various ways (oral, dermal or by injection) and cross several barriers before reaching the hepatopancreas which is an organ for detoxifying xenobiotics. Hepatopancreas, an important site of metabolism, accumulation, biotransformation, and the excretion of many xenobiotics, is one of the most studied organs [26].

In this research we were interested on the one hand on the hepatotoxicity of the mixture bifenthrin + hymexazol and on the other hand on the hepatoprotective properties of orange essential oils after chronic exposure in accordance with previous studies that have studied the toxicity of pesticide mixtures on a soil pollution bioindicator*Helix aspersa* [2, 15].

Currently, existing data are insufficient to conclude on the effect of pesticide mixture (hymexazol+bifenthrin) on gastropods on the one hand because of the lack of such studies in the literature and on the other hand because some studies have shown that the effect of a mixture does not necessarily reflect the effects of individual substances [27]. The possible interactions between the components of a mixture are therefore quite complex and make the prediction of the global effect very difficult [28]. The comparison of our results with those of the literature will be done, if possible, with the work carried out on pesticides of the same family and on other biological models close to snails.

The biomarkers of oxidative stress measured in this study are the rate of GSH and MDA and the activity level of CAT, GST and GPx, which are the most important parameters that can be useful in the evaluation of the hepatotoxic effects of BF and HY mixture.

A very significant increase in GSH content is recorded in our study after exposure to BF + HY mixtures at the hepatopancreatic level. Similar results have been observed by Salama *et al.* (2005) [29], in the same kind of gastropod after exposure to methomyl and chlorpyrifos, also the activity of this enzyme is in accordance with the results indicated by Zeriri (2013) [30], in earthworms treated by methomyl in comparison with the control group.

The rate of malondialdeydeis often used as an indicator of lipid peroxidation of membranes. Indeed, according to Bebianno*et al.* (2005) [31] and Al-Mutairi and Craik (2007) [32]. The increase in the rate of malondialdehyde indicates a lipid peroxidation. ThePampanin*et al.* (2005) [33] latter is followed by changes and a degradation of membrane structures, there follows a loss of receptors and enzymes of the membrane. The MDA level increases in

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proportion to the concentrations of two pesticides (BF + HY). This result is in accordance with the results of other studies that have highlighted an increase in lipid peroxidation after treatment with endosulfan and chlorpyriphos in rats [34].

The increase in the rate of MDA could be due to the generation of toxic free oxygen radicals. Bouaricha [35] studied the toxicity of biopesticide based on benzoate emamectin on the *Helix Aspersa* terrestrial snail. He found a dose dependent of MDA in the hepatopancreas. The same resultas are reported by Bourbia (2012) [15] who showed that snails exposed to a chronic dose of mixtures BF + HY increased the level of GP_X and GST and CAT in a very highly significant way compared to the controls.

A gradual increase in the rate of GP_X has been observed in the *Helix pomatia* treated by the CU (100 μM), this would be due to the primary defense mechanism against the reactive species of the "ERO" oxygen which catalyzes the conversion of peroxide H_2O_2 hydrogen in H_2O in hepatopancreas cells [36].

Sbartai*et al.* (2009) [37] and Benbouzide (2012) [38] have also shown an inhibition of the respiration of paramecies in the presence of bifenazate and phosphoramidate. The results of these two studies are consistent with ours. In fact, we have highlighted an inhibition of the respiratory metabolism of the *Helix Asperca* snails particularly in the mixtures BF + HY. These results suggest the release of free radicals at the mitochondrial level. The current results indicate that compounds:bifenthrine and hymexazole are very effective in creating this poisoning and influencing snail.

In this study, the hepato-protector effect of OEO was corrected for all enzymatic parameters (MDA, GSH, GST, CAT, GP_X). Some recent publications have reported that certain essential oils are more effective than some synthetic antioxidants [39]. Various experimental studies have shown the existence of an important relationship between the essential oils of the orange peel and the decrease in the oxidation of low -density lipoproteins in the blood [40]. The antioxidant effects of essential oils and plant extracts are mainly due to the presence of hydroxyl groups in their chemical structure [41].

Conclusion

In this work, we are interested in the toxic effect of BF + HY mixtures and the protective effect of OEO on a cellular and subcellular level. Their mixtures exercise toxic action in *Helix*

aspersa adults, resulting in an increase in the activities of the GST, GP_X and CAT as well as the rates of GSH and MDA. These changes are probably linked to an increase in the release of reactive oxygen species. In addition, we have shown that the OEO can effectively protect from cell death induced by the BF + HY mixture in the hepatopancreatic tissue of *Helix aspersa.*

Compliance with Ethical Standards:

This study has no funding from any institution.

Conflict of Interest:

There is no conflict of interest.

References

[1] Louat, F, Etude des effets liés à l'exposition aux insecticides chez un insecte modèle Drosophilamelanogaster. Sciences agricoles,Universitéd'Orléans, Français, **2013**. pp 224, p1.

[2] Zouaghi, M. F., Berrebbah, H., Boudoucha, I., Rekaik, I , Evaluation of the toxicity of a mixture insecticides used on a biological model: the snail *helix aspersa ,StudiaUniversitatis "VasileGoldiş", SeriaŞtiinţeleVieţii Vol. 30, issue 2 .,* ***2020****, pp. 69 – 75, p1.*

[3] Frances,S.P , Evaluation of bifenthrin and permethrin as barrier treatments for militsxcarytents against mosquitoes in queens land. AUSTRALIA, Journal of the American Mosquito Control Association 23(2).,**2007**.208-212,p208.

[4] Beausoleil, C ., Bonnard, N ., Falcy, M ., Jargot, D, Bifenthrine. Fiche toxicologique 274 ,Paris , INRS édition. Aturereview. Équiterre. Canada., **2009**, pp 68.

[5] Dar, M. A., Raina, R., Mir, A. H., Verma, P. K., Pankaj, N. K., & Sultana, M, Protective Role of L-ascorbic Acid in Oxidative Stress Induced by Repeated Oral Administration of Bifenthrin in Wistar Rats. Journal of Animal Research: v.6 n.1, **2016**, p. 39-42.

[6] Walker, M.H., andKeith, L.H, EPA's Pesticide Fact Sheet Database. Lewis Publishers, Chelsea.,**1992**. MI : 2-12.

[7] Shakoori, A.R.,Naveeda, T., and Sameem, M.A, Response of melathion-resistant and susceptible strains of *Triboliumcastaneum*(Herbst) to bifenthrin toxicity. *Pak. J. Zoology.*,**1994**.26**(**2): 169-178.

[8] Ahmed, S.,Saleem, M.A., and Khurram, R.K, Effect of cypermethrin (10 EC) and bifenthin (10 EC) on levels of acidand alkaline phosphatases in a strain of *triboliumcastaneum*(Herbst) (Coleoptera: tenebrionidae). *Pak. Entomol.,* **2004**.26: 47-51.

[9] Tahar, W ., Bordjiba,O ., Aimeur,N, Effet de l'hymexazole et de la prométhryne sur la qualité physico-chimique et biologique des sols agricoles. Rev. Sci. Technol., **2017**, Synthèse 35: 37-44 .

[10] European Food Safety Authority, Conclusion on the peer review of the pesticide risk assessment of the active substance hymexazol. *EFSA Journal*.,**2010**, 8:8. p 17.

[11] Little, E., Archeski, R., Flerov, B., Koslovskay, V, Bihavioural indicators of sublethal toxicity in rainbow trout. Environ. Contam. Toxicol.,**1990**,19 ,380-385.

[12] Gasmi S. 2018. Classic Labyrinth Test for Neurobehavioral Evaluation in Wistar Rats. Bioprotocol. https://bio-protocol.org/e3007.

[13] Youness, M, Impact de la formulation et du mélange de deux pesticides (mésotrione et tébuconazole) sur leur biodégradation et la croissance de microorganismes.,**2013**. N° d'Ordre : D. U. 2378, p321.

[14] Gasmi S et al. Preventive effects of *citrulluscolocynthis. L* plant extract on deltamethrin pesticide induced pneumotoxicity in wister rats. Journal of Microbiology, Biotechnology and Food Sciences.2022. 11(6).

[15] Bourbia-Ait-Hamlet, S, Évaluation de la toxicité de mixtures de pesticides sur un bioindicateur de la pollution des sols*Helix aspersa*.,**2012,**p 177.

[16] Mondello,L., Casilli, A., Tranchida, P. Q., Dugo, P., Dugo, G ,Comprehensive two-dimensional GC for the analysis of citrus essential oils.Flavour and Fragrance Journal.,**2005**. 20(2): 136-140pp.

[17] Nieradko-Iwanicka, B., Borzecki, A., &Jodlowska-Jedrych, B, Effect of subacute poisoning with bifenthrin on locomotor activity, memory retention, haematological, biochemical and histopathological parameters in mice. Journal of Physiology and Pharmacology.,**2015**,66, 1, 129-137 p 129.

[18] Weckbker, G., & Cory, J. G, Ribonucleotide reductase activity and growth of Glutathoine depleted mouse leukemia L1210 cells in vitro. Cancer Lett.,**1988**. 40, 257-264.

[19] Esterbaer, H., Gebicki, J., Puhl, H., &Jungens, G, The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Radic. Biol. Med.,**1992**. p13, pp 341.

[20] Flohe, L., Gunzler, W.A, Assays of glutathione peroxidase. Methods Enzymol ., **1984**, 105, 114¬-121.

[21] Habig, H., Pabst, M.J., Jokoby, W.B, Glutathione¬ S¬ transferase: the first enzymatic step in mercapturicacid formation. J. Biol. Chem., **1974**, 249, 7130-¬7139.

[22] Cakmak, I., Horst, W.J, Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max). Physiol. Plantarum., **1991**, 83, 463¬468.

[23] Kristal, B. S ., Park, B. K ., &Yu, B. P, 4-hydroxynonénal est un puissant inducteur de la transition de perméabilité mitochondriale. J. Biol. Chem., **1996**,271, 6033-6038.

[24] Gasmi S, Benaicha B, Rouabhi R, Kebieche M. Hematotoxicity Resulting from Chemotherapy in Patients with Breast Cancer in eastern Algeria. Annals of the Romanian Society for Cell Biology, 2021. 25(6), 20308–20319.

[25] Gasmi, S. Neurotransmission dysfunction by mixture of pesticides and preventive effects of quercetin on brain, hippocampus and striatum in rats. Toxicol. Environ. Health Sci. **2020.**12, 203–212.

[26] Odendaal, J.P., Reinecke, A.J, Quantifying histopathological alterations in the hepatopancreas of the woodlouse Porcelliolaevis (Isopoda) as a biomarker of cadmium exposure. Ecotoxicology and Environmental Safety, **2003**. 56(2): 319-325.

[27] Padhi, B.K., Pelletier, G., Williams, A., Berndt-Weis, L., Yauk, C., Bowers, W.J., and Chu, I, Gene expression profiling in rat cerebellum following in utero and lactational exposure to mixtures of methylmercury, polychlorinated biphenyls and organochlorine pesticides. Toxicological Letters.,**2008**. 176(2): 93-103.

[28] Lodovici, M., Aiolli, S., Monserrat, C., Dolara, P., Medica, A., Di, Simplicio, P, Effect of a mixture of 15 commonly used pesticides on DNA levels of 8-hydroxy-2-deoxyguanosine and

xenobiotic metabolizing enzymes in rat liver. Journal of Environmental Pathology Toxicology and Oncology.,**1994**. 13(3): 163-168.

[29] Salama, A.K., Osman, K.A., Saber, N.A., Soliman, S.A, Oxidative stress induced by different pesticides in the land snail, Helix aspersa. Pakistan Journal of Biological Sciences.,**2005**. 8: 92-96.

[30] Zeriri, I, Toxicité potentielle d'un insecticide sur un invertébré de la famille des coelomates.,**2013**.pp94,p57.

[31]Bebianno, M.J., Company, R., Serafim, A., Cosson, R.P., Fiala-Medoni, A, Antioxidant systems and lipid peroxidation in Bathy-modiolusazoricus from Mid-Atlantic Ridge hydrothermal vent fields. Aquat. Toxicol, **2005.** 75, p.354–373.

[32] Al-Mutairi D. A., Craik J.D., Batinic-Haberle I., Benov L T, Induction of oxidative cell damage by photo - treatment with zinc meta N-methylpyridylporphyrin. Free radical research.,**2007**. 41, 89-96.

[33] Pampanin, D.M., Camus, L., Gomiero, A., Marangon, I., Volpato, E., Nasci, C, Susceptibility to oxidative stress of mussels (Mytilusgalloprovincialis) in the Venic Lagoon (Italy).Mar.Pollut. Bull, **2005.**50, 1548-15557.

[34] Chebab,S ., Belli, N ., Leghouchi, E .,Lahouel, M, Stress oxydatif induit par deux pesticides : l'endosulfan et le chlorpyriphos . Environnement, Risques & Santé – Vol. 8, n° 5, septembre-octobre., **2009**, p430.

[35] Bouaricha, H, Evaluation du stress oxydatif induit par le Proclaim : Essai comparatif sur deux modèles biologiques (*Helixaspersa* et *Parameciumsp*.). Thèse de doctorat, Université Badji Mokhtar, Annaba, **2013**.55p.

[36] Halliwell and Chirico S, Lipid peroxidation: its mechanism, measurement, and significance. The American journal of clinical nutrition. **1993.**57, p.715-724

[37] Sbartai I ; Berrebbah H., Rouabhi R., Sbartai H. and Djebar M.R., Behavior of Paramecium sp treated with bifenazate with special emphasis on respiratory metabolism, protein and generation time. American Eurasian J. Toxicol.,**2009**. Sci1,13-18.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 3270 - 3284
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[38] Benbouzib, H , Evaluation et étude de la toxicité d'une famille d'acaricide sur des protistes ciliés. Thèse de doctorat de l'universitéd'Annaba.,**2012**. p 87.

[39] Hussain, A. I., Anwar, F., and Shahid, M, a Chemical composition, and antioxidant and antimicrobial activities of essential oil of spearmint (Menthaspicata L) from Pakistan. Journal of Essential OilResearch.,**2010**. 22 (1) : 78-84.

[40] Hussain, A. I, Characterization and biological activities of essential oils of some species of Lamiaceae.Thèsedoctorat, universited'Agriculture Faisalabad, Pakistan.,**2009**, p 257.

[41] Gonzalez-molina, E., Dominguez-perles, R., Moreno, D.A., Garcia-viguera, Natural bioactive compounds of citrus limon for food and health. Journal of Pharmaceutical and Biomedical Analysis.,**2010**.51: 327-345 pp.