COMPARATIVE IMMUNOTOXICITY OF BEES, *APIS MELLIFERA* (HYMENOPTERA: APIDAE), EXPOSED TO NATURAL AND SYNTHETIC XENOBIOTICS

ORIGINAL ARTICLE

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ABSTRACT

The objective of this study was to compare the effects of natural and synthetic chemical pesticides, thereby examining the supposed selectivity of these natural compounds on *Apis mellifera* bees. The LC_{50} values used in the bioassays were obtained from the research by Souza *et al*. (2023) and are as follows: Karate® (13.4 µL/100 mL), Limonene compound (1,440 µL/100 mL), and Roundup® (712,290 µL/100 mL). However, as reported by these authors, a concentration of 250 µL/100 ml was used for Azamax®. These LC_{50} values, along with a concentration of 250 µL/100 mL of Azamax®, were employed in immunohistochemical analyzes using the TUNEL method and PCNA in the midgut of bees. Immunological assessments (nitric oxide, phenoloxidase, and oxidative stress markers TBARS and GSH) were also conducted on adult worker bees. The xenobiotic treatments did not reveal apoptosis or cell proliferation. Nonetheless, we observed epithelial degeneration, marked by the presence of vacuolated cells, suggesting a necrotic process. Except for the Limonene compound, all substances induced oxidative stress, leading to increased levels of TBARS. Although there were no differences in GSH levels, we observed alterations in the immune system of these insects, characterized by increased phenoloxidase activity and NO_2 levels. Based on the acquired results, it is possible to conclude that caution should be exercised when using chemical pesticides in agriculture, whether they are

of synthetic or natural origin, as they have the potential to cause irreversible histopathological and immunological damage. This study also underscores the importance of conducting more comprehensive investigations into the impact of natural products on the physiology of pollinator insects.

Key words: *Apis mellifera*, Xenobiotics, Immunohistochemistry, Oxidative stress, Phenoloxidase.

1. INTRODUCTION

Honey bees, *Apis mellifera* Linnaeus (Hymenoptera: Apidae), play a prominent role in ecosystems due to their involvement in pollination, a role they perform both spontaneously in natural environments and within anthropomorphized settings. They facilitate the transfer of reproductive cell particles, thus contributing to the genetic diversity of both wild and cultivated plant species (Klein, *et al*. 2007; Michener, 2007; Potts, *et al*. 2016; Eardley, *et al*. 2016). As arthropods of great significance, honey bees intrinsically contribute to the survival of numerous organisms on Earth (Potts *et al*., 2016; Eardley *et al*., 2016). Despite their environmental and economic importance (Giannini *et al*., 2015), recent years have seen a decline in bee populations and a reduction in species diversity on all continents (Zattara & Aizen, 2021).

Agricultural pesticides impact honey bee populations in several ways and are identified as one of the multifactorial causes contributing to the phenomenon known as Colony Collapse Disorder (CCD), which affects social bees of the genus *Apis* and native species (Graystock *et al*., 2013; Goulson *et al*., 2015). Neurotoxic insecticides, such as neonicotinoids and pyrethroids, for example, are not only lethal but also capable of inducing behavioral changes in worker bees (Decourtye *et al*., 2005; Gill & Raine, 2014). Additionally, the widely used herbicide glyphosate has been found to reduce the learning abilities of bees (Herbert *et al*., 2014).

From an immunological perspective, Gregorc & Ellis (2011) conducted tests on *A. mellifera* larvae using chemical pesticides—organophosphate, neonicotinoid, and the herbicide glyphosate. They observed an increase in apoptosis in the midgut, with respective percentages of 61%, 65%, and 69% of cell death in the treated larvae compared to 10% in the control group. This demonstrates that the pesticides exert an

effect at the cellular level. Apoptosis is the process of programmed cell death that occurs naturally in organisms to maintain cell homeostasis and serve as a defense mechanism (Ashe & Berry, 2003). However, depending on an individual's capacity, it can recover through the process of cell proliferation (Caccia *et al*., 2019).

Oxidative stress has the potential to initiate the process of cell death and occurs due to the balance between reactive oxygen or nitrogen species (ROS and RNS) and antioxidant compounds (Maulik *et al*., 1998; Barbosa *et al*., 2010). ROS and RNS are free radicals that, when in balance, play important roles in the immune system of insects. An example of this is nitric oxide, which participates in processes signaling the presence of entomopathogens and xenobiotics, and is part of the humoral defense mechanisms of these arthropods (Bartling *et al*., 2021). Phenoloxidase is another component of this defense, as this enzyme participates in the formation of melanin, which is involved in the sclerotization of the cuticle, wound healing, and the recognition of foreign agents (Negreiro *et al*., 2004).

In this context, over the last two decades, research on botanical biopesticides has significantly increased due to several advantages they offer, including greater selectivity towards non-target organisms (Isman, 2006; Isman, 2020). However, despite the extensive body of research, little attention has been given to the effects of these natural insecticides on insect physiology (Isman, 2020; Giannini *et al*., 2015). This is a cause for concern, as studies have revealed that essential oils and their isolated compounds have the potential to impact the life history of insects, affecting pests as well as natural enemies and leading to histopathological (Almehmadi, 2011; Oliveira *et al*., 2021), nutritional (Cruz *et al*., 2017; Oliveira *et al*., 2021), and immunological changes (Silva *et al*., 2020).

Thus, physiological tests are necessary to ensure the safety of these substances on bees in agricultural fields. Bee workers can potentially contaminate themselves, and consequently the entire colony, either during foraging or through residues in the water in the case of synthetic chemical pesticides (Mullin *et al*., 2010; Potts *et al*., 2010; Ruiz-Toledo & Sánchez-Guillén, 2014).

Given the context provided, this research aimed to compare the immune responses of *A. mellifera* bees when exposed to synthetic xenobiotics (pyrethroid insecticide - Karate® and glyphosate herbicide - Roundup®) and natural xenobiotics (biopesticide - Azamax® and Limonene compound) commonly used in agriculture.

2. MATERIAL AND METHODS

2.1 OBTAINING INSECTS

The honey bees used in this research were obtained from the experimental apiary of the Department of Animal Science at the Federal Rural University of Pernambuco (UFRPE). Workers bees were collected using plastic containers with perforated lids placed at the hive entrances. Subsequently, the insects were transported to the Laboratory of Insect Physiology (LAFI) and the Laboratory of Morphological Studies in Vertebrates and Invertebrates (LABEMOVI), both at UFRPE, where they were utilized in the bioassays.

2.2 CONCENTRATIONS USED IN BIOASSAYS

The Lethal Concentrations 50 (LC_{50}) used for immunohistochemical and immunological evaluations of the *A. mellifera* bee population from the UFRPE apiary were obtained from Souza *et al*. (2023). They reported LC_{50} values as follows: 13.4 µL/100 mL for the Karate® insecticide (pesticide + honey solution 1:1 + 1% Dimethylsulfoxide (DMSO)), 1,440 µL/100 mL for the Limonene compound (honey solution + DMSO), and 712,290 µL/100 mL for the Roundup® herbicide (honey + water + DMSO). Regarding the botanical insecticide Azamax®, a concentration of 250 µL/100 mL of the solution (honey + DMSO) was used. As demonstrated by Souza *et al*. (2023), this concentration represented the highest level of the product that resulted in mortality without repelling insects; higher concentrations repelled 100% of the sampled individuals, making it impossible to determine the LC_{50} for Azamax®. The control group was treated solely with the honey solution (1:1) + 1% DMSO. After 24 hours of exposure to the treatments, the surviving bees were used in the subsequent tests.

2.3 APIS MELLIFERA MIDGUT APOPTOSIS

For immunohistochemical analysis, including apoptosis and cell proliferation, midguts were embedded in paraffin, and 5 µm-thick sections were obtained using a Minot microtome (Leica, 2035). These sections were then placed in a water bath and collected on silanized slides. Images were captured and digitized using Leica LAS Image software (Junqueira & Junqueira, 1983; Solomon, 2009). To detect apoptosis via DNA fragmentation, silanized slides containing midgut sections underwent the TUNEL test (Terminal Deoxynucleotidyl Transferase Uracil Nick End Labeling) Costa (2022); Gavrieli (1992), following the Apoptag Plus kit protocol (Merck®). The cuts were initially deparaffinized and hydrated, followed by a 5-minute incubation in PBS (Phosphate Buffered Saline) at room temperature. Proteinase K was then applied to the slides for 15 minutes. After washing in distilled water, the slides were incubated in hydrogen peroxide for 5 minutes at room temperature. Sections were washed in PBS and incubated in an equilibration buffer for 60 minutes at 4°C. Subsequently, sections were incubated in TdT at 37°C for 1 hour in a humid chamber. The stop solution was applied for 10 minutes at room temperature, followed by washing in PBS and incubation in anti-digoxigenin. The slides were rinsed in PBS, and the sections were developed with diaminobenzidine chromogenic substrate (DAB, DakoCytomationTM) for approximately 20 minutes. They were then counterstained with hematoxylin for 20 to 30 seconds. Following this, the slides were washed in running water, dehydrated in increasing alcohol concentrations, and placed in xylene for mounting and observation under a light microscope.

2.4 CELL PROLIFERATION OF THE MIDGUT

To assess cell proliferation, the paraffin was removed from sections, which were then hydrated and subjected to antigen retrieval using citrate buffer (pH=6) in a water bath for 20 minutes at 100°C. After allowing them to rest for 20 minutes at room temperature, a 3% hydrogen peroxide solution was applied for 30 minutes. Next, the sections were rinsed in Tris buffer (Tris-(hydroxymethyl)-aminomethane) and exposed to PCNA antibody (nuclear antigen of cell proliferation) (Spring) at a 1:100 dilution for 1 hour at room temperature. Sections were washed again in Tris buffer and incubated

with histofine for 30 minutes. They were subsequently subjected to diaminobenzidine chromogen (DAB, DakoCytomation™) and counterstained with hematoxylin. The apoptotic index and cell proliferation were determined based on the percentage of positive cells, calculated from the count of at least 500 nuclei per treatment, subdivided into 10 randomly selected fields using the 40x objective (Losa *et al*., 2000; Wu *et al*., 2013).

2.5 PREPARATION OF THE MACERATE

An adult bee was macerated in 500µL of phosphate buffer (0.1 mM, pH 7.4) within a crucible using a porcelain pestle. The resulting macerate was then transferred to a 2 mL centrifuge tube and centrifuged for 1 minute at 1000 rpm to remove any fragments of the insects' exoskeleton. The supernatant was subsequently transferred to another 2 mL tube and kept refrigerated at -20°C until analysis. Each individual bee was treated as one repetition, and each treatment consisted of 10 repetitions conducted within 24 hours after application.

2.6 NITRIC OXIDE MEASUREMENT

The Griess reagent Green *et al*. (1981) was employed to assess the concentration of the nitrite ion (NO2). In a 50 µL aliquot of the macerate, 70 µL of 1% sulfanilamide in 5% phosphoric acid (H3PO4) was added. After incubation, an additional 50 µL of 0.1% naphthylethyleneamine dihydrochloride (NEED) was added to the 50 µL of the sample (macerate/sulfanilamide) in a microtiter plate (Faraldo *et al*. 2005). The absorbance reading was conducted at 562 nm using the Biochrom Anthos 2010 microplate reader (São Paulo, Brazil), with the ADAP program operating in endpoint mode. Data underwent ANOVA, and means were compared using the Tukey HSD test, with analysis conducted using SAS Institute software.

2.7 PHENOLOXIDASE ACTIVITY

The analysis for each treatment was conducted using 10 µL of the macerate, with each treatment consisting of ten replicates. Duplicates of 50 µL from this mixture were transferred to the microtiter plate. Subsequently, 50 microliters of L-DOPA (L-

dihydroxyphenylalanine) at a concentration of 4 g/L (Sigma-Aldrich, St. Louis, MO, USA) were added to each well to activate the enzyme. The absorbance readings were performed using a Biochrom Anthos 2010 microplate reader (São Paulo, Brazil) at 492 nm, with the ADAP program set to kinetic photometry mode. Enzyme activity was measured during the linear phase of the reaction at intervals of 60 seconds for 20 minutes, following the method described by (Faraldo *et al*., 2006). The data were subjected to the Kruskal-Wallis non-parametric test at a significance level of 5%, using SAS Institute software.

2.8 OXIDATIVE STRESS

To assess oxidative stress, lipid peroxidation was evaluated by measuring thiobarbituric acid (TBARS) reactive substances following the method of (Ohkawa *et al*. 1979). Ten adult insects from each treatment were homogenized in an ice bath using 1.15% KCl + 3 mM EDTA. Subsequently, the reaction medium was prepared by adding 0.3% thiobarbituric acid, 0.4% Sodium Dodecyl Sulfate (SDS), and 7.5% acetic acid (pH 3.5). The mixture was then heated at 95°C for one hour. After centrifugation, the supernatant's absorbance was measured at a wavelength of 535 nm. The data obtained were normalized by the protein concentration of the homogenate, as measured according to (Lowry *et al*., 1951). These data were analyzed using the Kruskal-Wallis non-parametric test at a significance level of 5%, using SAS Institute software. Reduced Glutathione (GSH) levels were determined by measuring non-protein sulfhydryl groups, following the methodology of (Sedlak & Lindsay, 1968). From the homogenate obtained for lipid peroxidation assessment, 80 to 160 mg of tissue were precipitated in a 5% TCA solution. Then, a volume of the supernatant was mixed with a reaction medium containing TRIS 4mM, EDTA 4mM, and DTNB 4mM at pH 8.9. The reaction was incubated at room temperature for 5 minutes, and the absorbance was measured at 412 nm. The result was adjusted for the protein concentration of the homogenate. Data regarding GSH levels assumed normality and homogeneity and were analyzed using ANOVA, with means compared using Tukey's test at a significance level of 5%, as determined by the SAS Institute.

3. RESULTS

3.1 *APIS MELLIFERA* MIDGUT APOPTOSIS

Regarding the immunohistochemical evaluation of the bees' midgut, no apoptotic nuclei were observed following exposure to synthetic (Roundup® and Karate®) and natural (Azamax® and Limonene compound) xenobiotics after 24 hours. However, the epithelial tissue exhibited cellular degeneration characterized by the presence of cytoplasmic vacuoles, suggesting a necrotic process. It is believed that this cell damage may have been a significant factor preventing these cells from secreting the peritrophic matrix, leading them to come into contact with the contents of the lumen and, consequently, the digestive enzymes (Figure 1).

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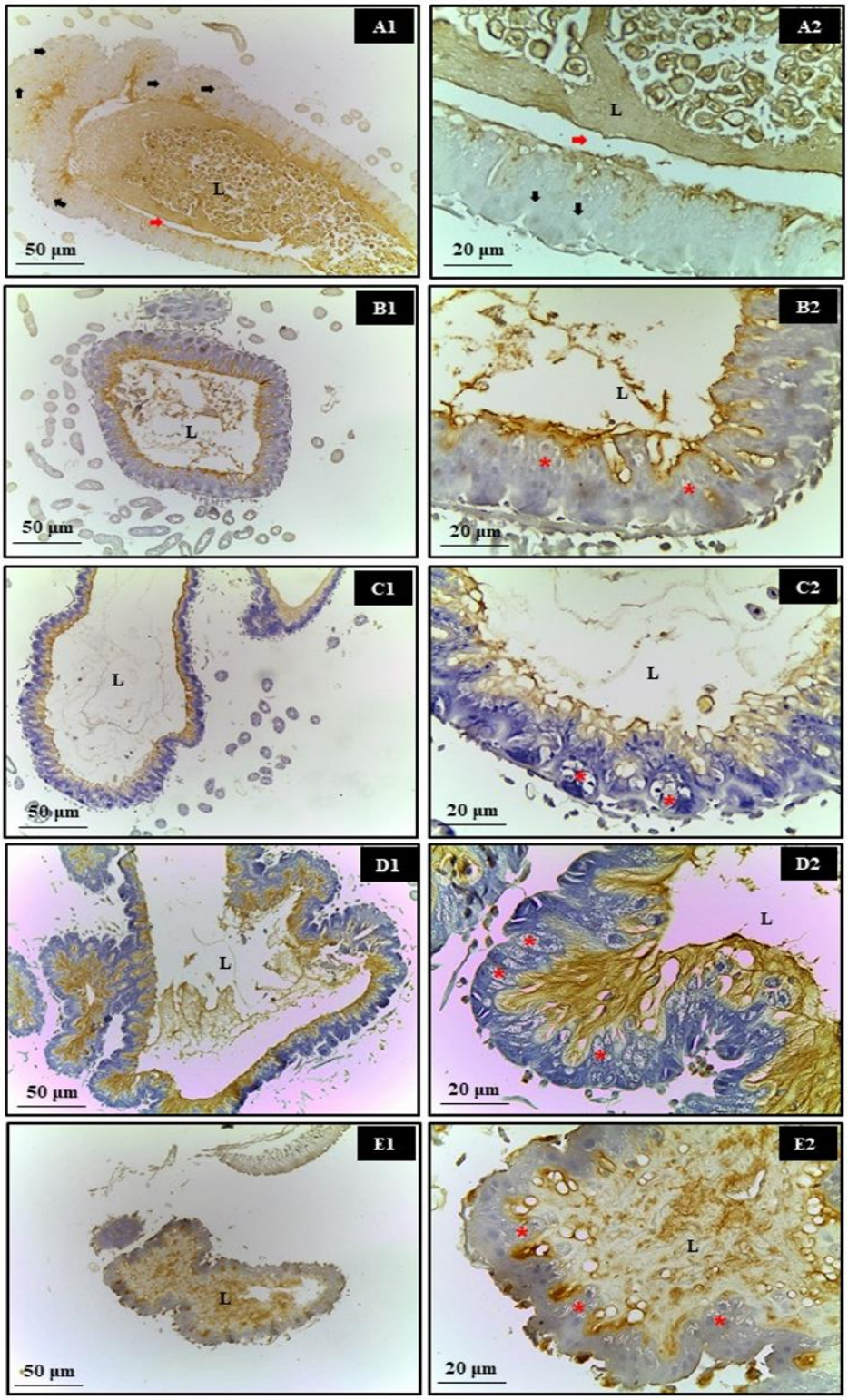
Figure 1. Evaluation of apoptosis in the midgut of *Apis mellifera* adults, using the Tunel test. In which, A1 and A2- Control, B1 and B2- Azamax®, C1 and C2- Limonene, D1 and D2- Roundup®, E1 and E2Karate®, L- Lumen, Black arrow- apoptotic nuclei, Red arrow- matrix peritrophic, Asterisk- Vacuolated cells



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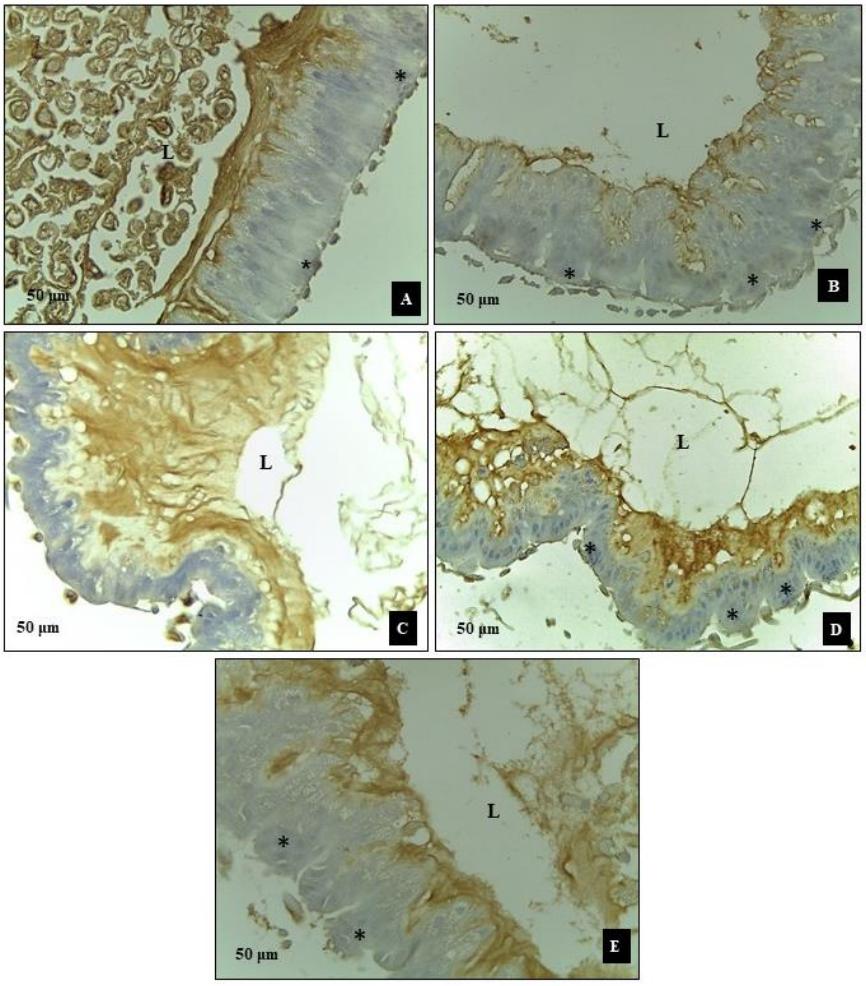
3.2 CELL PROLIFERATION OF THE MIDGUT

Epithelial cells did not demonstrate activation of regeneration mechanisms by the cell proliferation process in bees exposed to xenobiotics (Figure 2), with positive PCNA being identified only in the control group (Figure 2A). Epithelial cells did not exhibit signs of activation of regeneration mechanisms through the cell proliferation process in bees exposed to xenobiotics (Figure 2). Positive PCNA staining was only observed in the control group (Figure 2A).

Figure 2. Analysis of cell proliferation (PCNA). Cross section of the midgut of adult bees, *Apis melifera*. A- Control, B- Azamax®, C- Limonene, D- Roundup®, E- Karate®, L- Lumen, asterisk- regenerative cells



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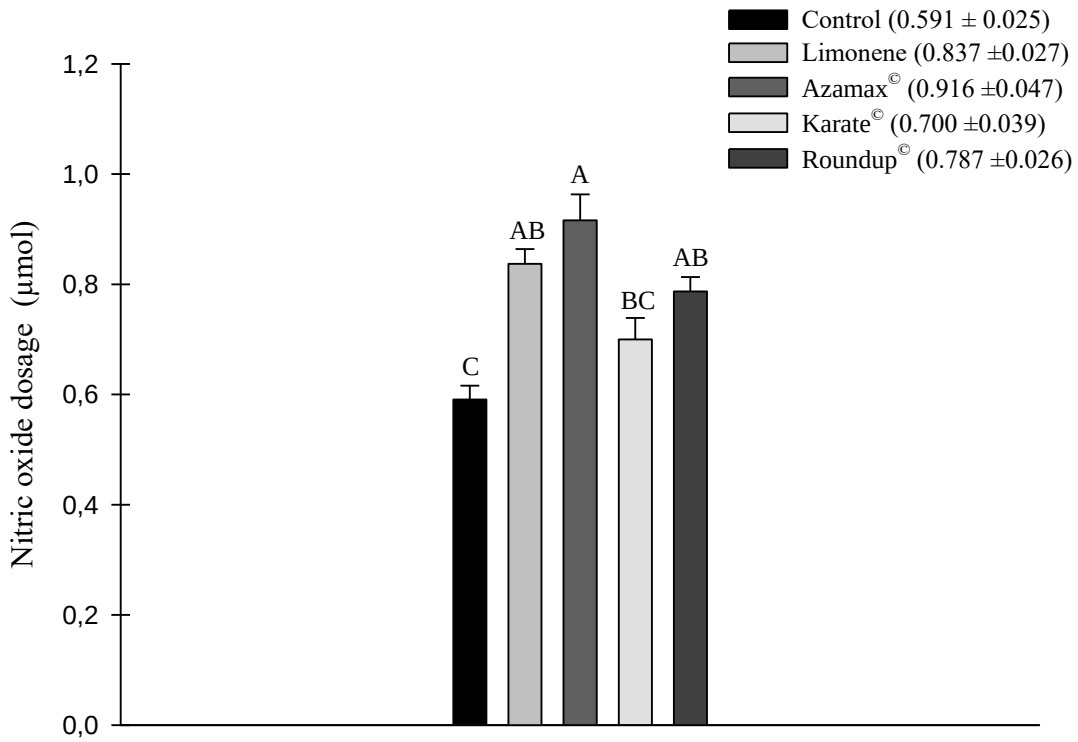
3.3 NITRIC OXIDE MEASUREMENT

After statistical analysis, it was observed that all the chemical products used, namely Karate® (0.700 ± 0.039 µM of NO_2), Limonene (0.837 ± 0.027 µM of NO_2), Azamax® (0.916 ± 0.047 µM of NO_2), and Roundup® (0.787 ± 0.026 µM of NO_2), increased the nitric oxide levels in bees. However, the most significant increase was observed in the Azamax® treatment. Limonene did not show a statistically significant difference compared to both Azamax® and Roundup®. Although bees fed with the honey+Karate® solution exhibited a response similar to the control (0.591 ± 0.025 µM of NO_2), it did not significantly differ from the treatments with Roundup® and Limonene, resulting in an increase in NO_2 levels in the samples (Figure 3).

Figure 3. Mean (+SE) of nitric oxide level dosage (µM of NO_2) in *Apis mellifera* bees submitted to LC_{50} of the compound Limonene, the insecticide Karate®, the herbicide Roundup®, and to concentration of 250 µL/100 mL of Azamax®. Columns with the same letter do not differ by the Tukey HSD test at 5% probability



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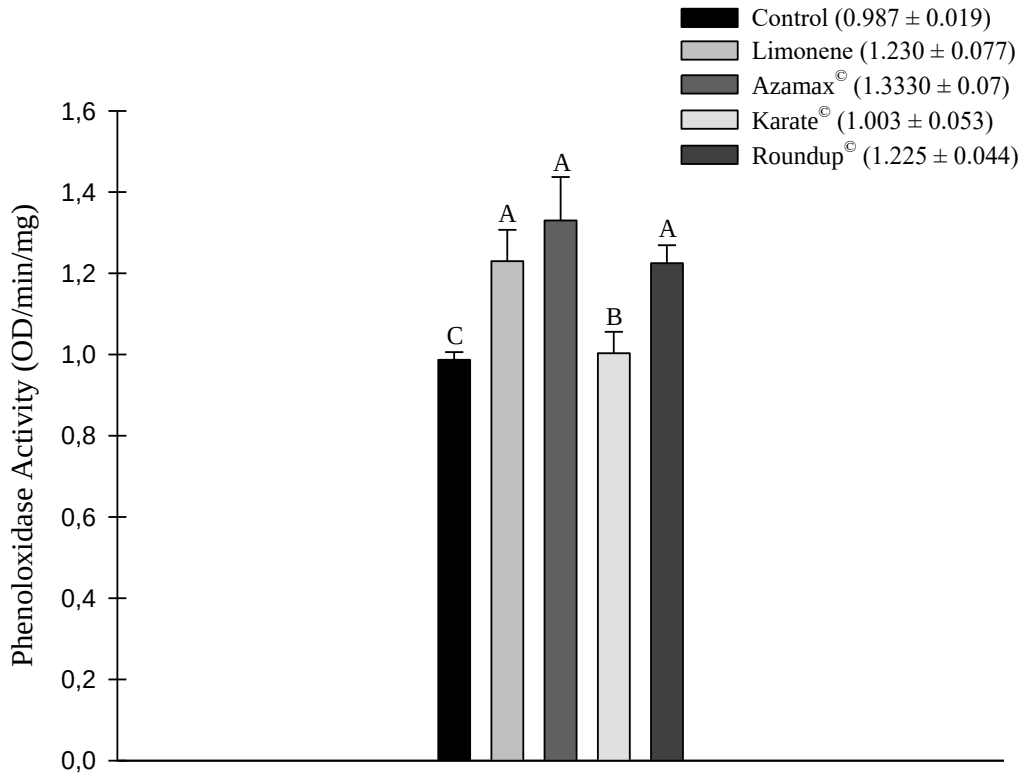
3.4 PHENOLOXIDASE ACTIVITY

All evaluated xenobiotics led to an increase in phenoloxidase enzyme activity after 24 hours of exposure. The most significant increases were observed in the treatments with the biopesticide Azamax® (1.333 ± 0.107 OD/min/mg), followed by the compound Limonene (1.230 ± 0.077 OD/min/mg) and the herbicide Roundup® (1.225 ± 0.044 OD/min/mg), which did not significantly differ from each other. Once again, the Karate® insecticide (1.003 ± 0.053 OD/min/mg) did not exhibit a statistically significant difference from the control group (0.987 ± 0.019 OD/min/mg) (Figure 4).

Figure 4. Phenoloxidase enzyme activity (OD/min/mg) in *Apis mellifera* bees subjected to LC_{50} of the compound Limonene, the insecticide Karate®, the herbicide Roundup®, and to concentration of 250 µL/100 mL of Azamax®. Columns with the same letter do not differ by the non-parametric Kruskal-Wallis test at 5% probability



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3.5 OXIDATIVE STRESS

All chemical agents tested, except for Limonene, demonstrated the ability to induce an increase in oxidative stress measured by TBARS, indicating cellular lipid peroxidation in the evaluated bee samples. The herbicide Roundup® (1.838 ± 0.205 nmol of MDA/mg/protein) was particularly noteworthy, being the product that induced the most significant oxidative stress in the cells, followed by Azamax® (1.280 ± 0.070 nmol of MDA/mg/protein) and Karate® (1.214 ± 0.055 nmol MDA/mg/protein). In contrast, the compound Limonene (0.914 ± 0.096 nmol of MDA/mg/protein) did not show a statistically significant difference in comparison to the control group (0.782 ± 0.109 nmol of MDA/mg/protein). Consequently, this substance did not promote this type of stress after 24 hours of exposure (Figure 5). Additionally, during this same evaluation period, there were no differences in the measurement levels of Glutathione-S-Transferase (GSH) (Figure 6).

Figure 5. Oxidative stress from the measurement of thiobarbituric acid (TBARS) as an indicator of lipid peroxidation (nmol of MDA/mg/protein) in *Apis mellifera* subjected to LC_{50} of the compound Limonene, the insecticide Karate®, the herbicide Roundup®, and to concentration of 250 µL/100 mL of Azamax®. Columns with the same letter do not differ by the non-parametric Kruskal-Wallis test at 5% probability

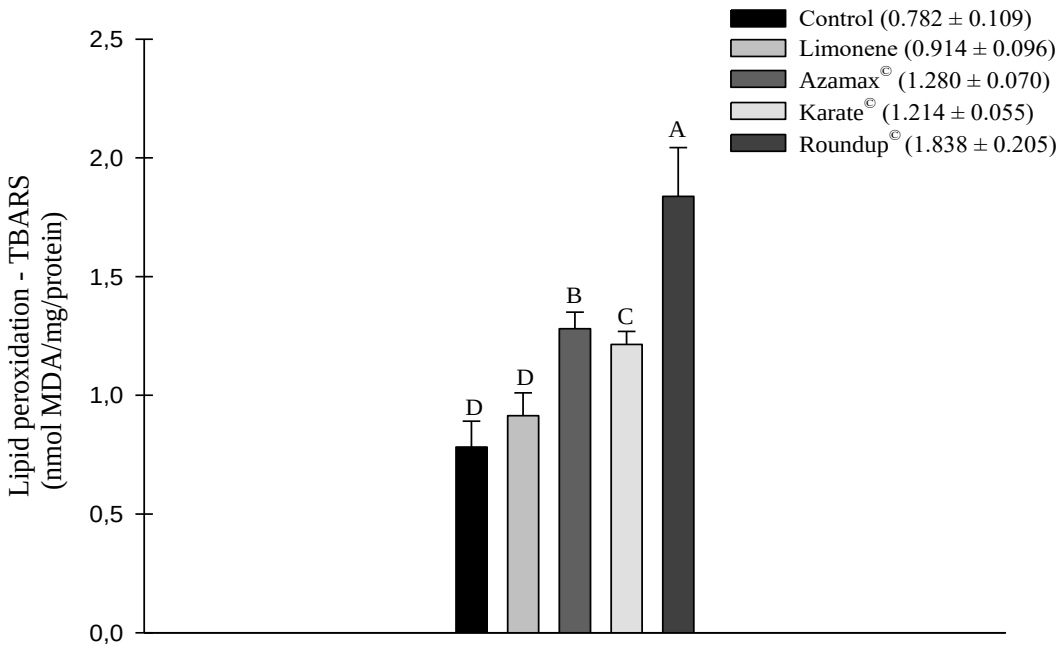

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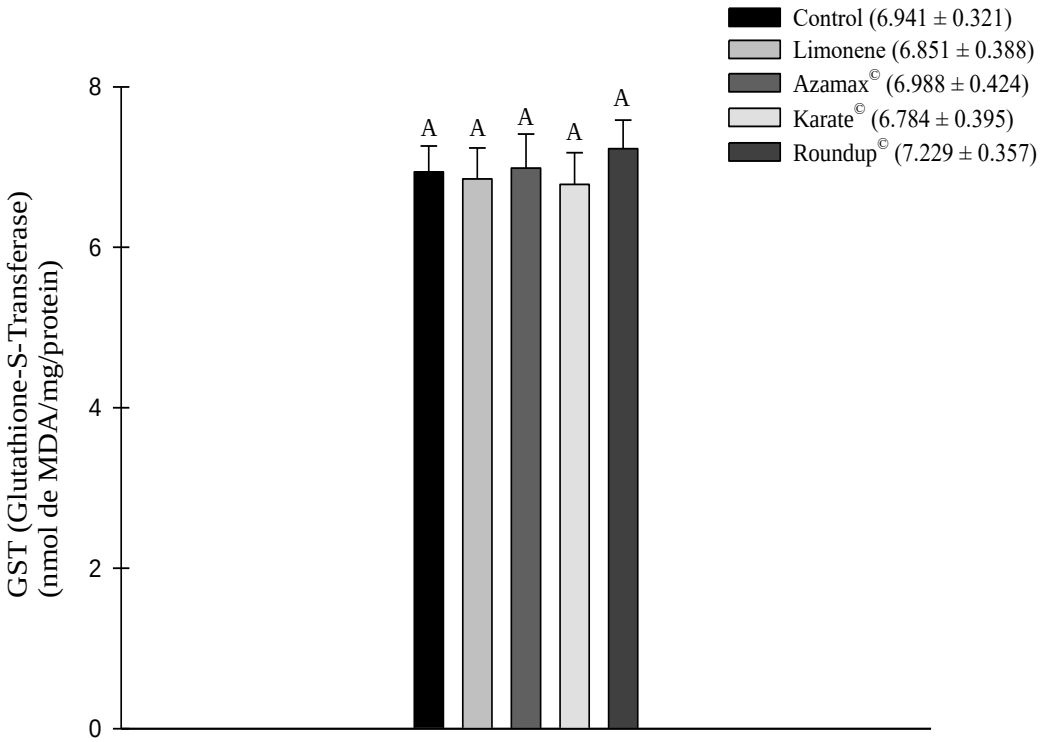
Figure 6. Oxidative stress from the measurement of Glutathione-S-Transferase (GSH) in *Apis mellifera* subjected to LC_{50} of the compound Limonene, the insecticide Karate®, the herbicide Roundup®, and to concentration of 250 µL/100 mL of Azamax®. Columns with the same letter do not differ by Tukey's test at 5% probability, for GSH data



Source: Authors, 2024.

4. DISCUSSION

The histopathological and immunological changes observed in treated bees suggest that the tested substances were highly harmful to these organisms and did not allow them sufficient time to activate regeneration mechanisms (Illa-Bochaca & Montuenga, 2006; Caccia *et al*., 2019). Considering that, in general, natural products are considered selective, this finding is significant. Natural compounds are primarily assessed for their acute toxicity to beneficial insects (Sabahi *et al*., 2022; Cappa *et al*., 2022). However, there is limited information on how these compounds affect bees at the cellular level, which has severe implications for their physiology and health.

Braga *et al*. (2020) also identified the absence of apoptosis in the midgut of 5th instar nymphs of the predatory bug *Podisus nigrispinus* (Dallas), which had been fed

Alabama argillacea (Hübner) caterpillars treated with a Lethal Dose (LD_{50}) of 2.50 and 2.82 mg/g insect, respectively, of the oils of *Mentha spicata* L. and *Melaleuca alternifolia* Cheel. However, in nymphs treated with *M. alternifolia*, histopathological changes such as columnar cell elongation and cell lysis, similar to the symptoms of necrosis, were observed. Necrosis is characterized by the dilation of cytoplasm, mitochondria, and endoplasmic reticulum, leading to cell rupture and, consequently, the extravasation of cellular content (Sun *et al*., 2021). Both oils consist solely of terpenes, a chemical class to which Limonene and azadirachtin belong. Therefore, epithelial tissue necrosis may be a mechanism of action of these compounds on insects.

Unlike the other analyzed substances, Limonene does not have the oxygen molecule in its composition, which is capable of triggering free radicals harmful to the body, making the individual susceptible to oxidative stress (Kumar *et al*., 2022). Furthermore, there is already a report that monoterpenes may have antioxidant properties (Yu *et al*., 2017). Thus, oxidative stress was not expected to be one of the ways in which Limonene acts on honeybees' physiology.

Limonene's mode of action is associated with the neuromuscular inhibition of acetylcholinesterase (AChE), leading to AChE accumulation, which generates neuronal excitation and insect death (García *et al*., 2005; Zarrad *et al*., 2017; Gadelhaq *et al*., 2023). Furthermore, in *A. mellifera*, effects on intestinal cells were observed, resulting from the necrotic lesions observed in the epithelium.

The oxidative stress observed in the other treatments indicates the occurrence of lipid peroxidation in honeybees, which, as a consequence, leads to alterations in the fundamental functions of the cell membrane (Barbosa *et al*., 2010). Studies have related the apoptotic process as one of the cellular responses to this stress (Li & Wogan, 2005; Kim & Lee, 2020). However, it may also be the cause of triggering epithelial necrosis in the tested *A. mellifera* population, with this effect being more pronounced in samples treated with glyphosate.

Indeed, studies on this herbicide have shown its impact on the survival and health of various bee species, whether larvae or adults, whether acutely or chronically exposed.

This exposure leads to changes in molecular, cellular, histological, and biological systems (Battisti *et al*., 2023). An important aspect reported by Liao *et al*. (2017) is that a solution containing water, sugar, and glyphosate (10 ppb) was found to be more attractive to bees when compared to a solution without the herbicide. This raises concerns about the potential contamination of these organisms through nectar in their natural environment.

The midgut constitutes one of the first defense barriers of insects against pathogenic organisms and xenobiotics (Schmid-Hempel, 2005). The impairment of this function is confirmed by the activation of the phenoloxidase cascade and alterations in NO_2 levels, indicating the involvement of humoral defense mechanisms in the bees' cells when exposed to synthetic and natural pesticides.

The low activity of the enzyme phenoloxidase and the reduced level of NO_2 in the tests with Karate®, was also observed by Silva *et al*. (2020) in bugs of the species *Podisus nigrispinus* (Dallas), submitted to another pyrethroid, deltamethrin, both under the evaluation time of 24 hours. However, after 72 hours of exposure, these authors found an increase in phenoloxidase activity, simultaneously with a rise in total protein levels in *P. nigrispinus*. Even so, the analysis of apoptosis by the Tunel test, in the case of the bee population sampled in this research, shows cellular necrosis. In addition, the insecticide Karate® and the other compounds studied here caused irreversible damage, as they could not be accompanied by cell repair, consequently leading to the death of the organism (Miller & Zachary, 2017).

Previous studies have also demonstrated the repellency of azadirachtin, the active ingredient in Azamax®, to other bee species (Zhao *et al*., 2022) and various insects (Ikeura *et al*., 2013; Andrade *et al*., 2013). In the case of *A. mellifera*, the repellent effect of Azamax® was observed at concentrations above 250 µL/100 mL. Therefore, there is potential for this insecticide to be used in Integrated Pest Management (IPM) programs to preserve the abundance of these pollinators in agroecosystems. This is because the mode of action of azadirachtin is associated with increased contamination through ingestion (Martinez & Van Emden, 2001), and in the field, the likelihood of contact with this substance decreases. However, due to Azamax®'s strong deleterious

effects on the digestive and immune systems of bees, it is crucial to take care to ensure that its spraying does not coincide with the foraging time of *A. mellifera*.

The exposure of this *A. mellifera* population to the natural insecticide Azamax® and Limonene resulted in responses analogous to those induced by the synthetic pesticides Karate® and Roundup®. These responses included the necrosis of epithelial tissue and the impairment of membrane functions. Despite the supposed safety of Limonene and Azamax® for non-target organisms, they were the substances that most significantly increased nitric oxide levels and phenoloxidase enzyme activity in bees. However, Roundup® was the treatment that caused the most oxidative stress, and like Karate®, it has the aggravating factor of leaving residues in hive substrates. As a result, bees are also affected by chronic exposure to synthetic chemicals.

5. CONCLUSION

Although the natural insecticides tested here are considered safe for non-target organisms, this research has shown that caution is warranted regarding their use in agroecosystems. They were capable of causing deleterious effects on the immune system of bees comparable to synthetic pesticides. Therefore, more research is needed to understand how natural products interfere with the physiology of bees, in order to ensure that botanical insecticides used in agriculture are truly selective and a safe alternative to synthetic pesticides.

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CONFLICT OF INTERESTS

There is no conflict of interest on the part of the authors.

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