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Imidacloprid exposure disrupts gene expression in *Apis mellifera*, potentially threatening pollination efficiency

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Abstract

Bees are indispensable pollinators for maintaining both ecosystems and agricultural productivity. However, their populations have been reduced, potentially attributed to habitat loss, climate change, heightened vulnerability to diseases and parasites, and the use of pesticides. Neonicotinoids, acting as agonists of nicotinic cholinergic receptors, have been associated with detrimental impacts on bees, including decreased survival rates. In this study, *Apis mellifera* L. in their foraging phase were exposed to lethal or sublethal doses of imidacloprid through ingestion or contact. After 1 and 4 hours, the bees were euthanised and stored in an ultra-freezer (−80 °C) for transcriptomic analysis. After 1 hour and 4 hours of all treatments, twenty-four genes were differentially expressed, potentially impacting foraging activity and thus pollination, promoting genetic dysregulation, and affecting genes associated with odorant-binding proteins (OBPs) and chemosensory proteins (CSPs). This suppression suggests compromised olfactory function and chemical communication, essential for foraging and pollination. Additionally, metabolic alterations were observed, influencing crucial enzymes involved in carbohydrate and protein digestion, potentially impacting bee performance. Imidacloprid exposure also disrupted the gene encoding a circadian clock-controlled protein, which regulates circadian rhythms essential for behaviours like orientation, navigation, and sleep, all crucial for colony survival. Evidence of an adaptive response to oxidative stress was observed, indicated by increased expression of genes related to hexamerin 70a and vitellogenin. Beyond threats to bee health, substantial economic and environmental impacts are evident, underscoring the urgent need for sustainable alternatives in pesticide management to ensure bee preservation and global food security.

Key words: honey bee, neonicotinoids, pesticides, pollination.

Introduction

The combined use of apiculture with agriculture presents an intriguing approach to enhance the productivity of both activities, owing to the pollination services provided by bees (Vidal, 2021). Pollination involves the transfer of pollen between the male and female parts of flowers, facilitating fertilization and reproduction, and can be achieved through wind and water. Pollination occurs naturally during bees' foraging for natural resources.

Animal pollination contributes to approximately 35% of global agricultural production and plays a vital role in maintaining natural ecosystem balance (Klein *et al*., 2007). Globally, the majority of crops are entomophilous, thus their yield depends on, or at least benefits from, pollination services. The primary categories of crops pollinated by insects are vegetables and fruits, followed by edible petroleum crops, stimulants, nuts, and spices (Khalifa *et al*., 2021; Gazzea *et al*., 2023). Consequently, pollination has significant economic value due to its enhancement of crop quality and quantity, with an estimated worth of €153 billion for global agriculture in 2005 (Gallai *et al*., 2009). The production of a ton of crops not dependent on insect pollination typically sells for an average of €151, compared to an average of €761 for pollinator-dependent crops (Gallai *et al*., 2009).

Bee pollination not only improves yield but also agricultural product quality, impacting various aspects such as appearance, nutrient content, and shelf life (Klatt *et al*., 2014). Bees are the most significant group of pollinators, visiting over 90% of the primary crops (Klein *et al*., 2007), and *Apis mellifera* L. is the most widely distributed agent globally (Aizen and Harder, 2009), promoting food production of plant origin for both humans and animals (Eilers *et al*., 2011). In many regions globally, agricultural pollination primarily relies on the managed *A. mellifera* species. These bees are kept in hives that are strategically relocated to agricultural areas during crop flowering periods (Winfree *et al*., 2011).

However, various factors negatively impact pollinator health, including climate change, habitat loss, pathogens, and parasites (Cox-Foster *et al*., 2007; Potts *et al*., 2010; Raymann *et al*., 2018). The decline in the global bee population poses a significant threat to pollination services, potentially leading to a decrease in fruit supply by 22.9%, vegetables by 16.3%, and nuts and seeds by 22.1% (Goulson *et al*., 2008). Additionally, the use of pesticides for crop protection has contributed to increased colony loss rates (Pisa *et al*., 2015; Sánchez-Bayo *et al*., 2016; Woodcock *et al*., 2017; Gao *et al*., 2020; Fent *et al*., 2020).

Pollinators might be exposed to lethal and sublethal concentrations of pesticides, negatively affecting various aspects of their behaviour (Guez *et al*., 2001), locomotion and motor activity (Williamson *et al*., 2013; Lunardi *et al*., 2017; Bovi *et al*., 2018), learning, memory (Williamson and Wright, 2013), cognitive development (Palmer *et al*., 2013), colony development (Elston *et al*., 2013), foraging, pollination success (Henry *et al*., 2012), reproductive physiology (Williams *et al*., 2015), and can disrupt a

wide range of genes related to metabolism, nutrition, olfactory activity and general health of the bees (de Castro Lippi *et al*., 2024; Lima *et al*., 2024).

Neonicotinoids, widely used insecticides, are a primary focus for investigating their potential association with high bee mortality rates (Blacquiere *et al*., 2012; Simon-Delso *et al*., 2014). These neurotoxins act selectively on insect nicotinic acetylcholine receptors, disrupting central nervous system function (Matsuda *et al*., 2001). Imidacloprid, a notable neonicotinoid, contains nitro, which is generally more toxic than cyano-containing compounds like acetamiprid and thiacloprid (Pisa *et al*., 2015). Furthermore, bees have shown an attraction to nectar containing imidacloprid, suggesting their inability to identify neonicotinoids to avoid them (Kessler *et al*., 2015).

Thus, this study aimed to evaluate, through transcriptomic analysis, the effect of imidacloprid (lethal and sublethal doses) on *A. mellifera* in the foraging phase, through ingestion and contact tests, aiming to investigate genes that may be related to pollination.

Materials and methods

The use of bees in this research was approved by the Ethics Committee on the Use of Animals (CEUA/FMVZ), registered under protocol number 0093/2020.

Preparation of the bees

The experiment was conducted using ten colonies of Africanized *A. mellifera* housed in a 5-frame nucleus beehive Langstroth-type. The beehives were standardized to include three brood frames and two frames for food (bee pollen and nectar), all of which contained naturally mated queens. Two frames containing a sealed brood were removed from each experimental colony, and then these frames were carefully wrapped in perforated tissue and returned to their respective hives until the emergence of the new bees (Camilli *et al*., 2022). Subsequently, approximately 1,000 newly emerged honey bees were individually marked on the thorax using a Uni posca pen (Mitsubishi Pencil, Tokyo, Japan). Following the marking process, the bees were reintroduced into their original colonies following the methodology previously used in our research (Astolfi *et al*., 2022).

Collection of the bees and exposure to pesticides in the laboratory

Twenty-one days after reintroduction, a total of 360 marked honey bees in the foraging phase were carefully collected using tweezers. Any bees displaying abnormal behaviours, such as sluggishness, deformities, or physical injuries caused by handling, were replaced with other marked bees of the same age. The bees were then anaesthetized at low temperature, and promptly placed in plastic Petri dishes (90 × 20 mm), which were perforated to ensure ventilation. Six Petri dishes per treatment were used, with each dish containing 5 bees, resulting in a total of 30 bees per treatment, kept inside an incubator (33 ± 1.0 °C and 75% humidity).

To analyse the acute effect of the neonicotinoid, 1-hour and 4-hour periods were selected for the analyses. Twelve treatments were assessed: Ingestion test: 1 hour of exposure to a sublethal dose, 1 hour of exposure to a lethal dose, 1 hour of the control treatment, 4 hours of exposure to a sublethal dose, 4 hours of exposure to a lethal dose, and 4 hours of the control treatment/ Contact test: 1 hour of exposure to a sublethal dose, 1 hour of exposure to a lethal dose, 1 hour of the control treatment, 4 hours of exposure to a sublethal dose, 4 hours of exposure to a lethal dose, and 4 hours of the control treatment.

The bees underwent a 3-hour fasting period to empty their crop for the ingestion test. A consumption of 50 µl per bee, corresponding to the average volume of the nectariferous vesicles, was used to calculate the imidacloprid concentration (Crane, 1990). The food was provided in plastic troughs, with 250 µl added per trough onto each plastic Petri dish containing 5 bees (50 µl per bee). Subsequently, the bees were provided with syrup (honey and water, 1:1) containing either a lethal dose (IG50) of 0.081 µg/bee (Bovi *et al*., 2018) or a sublethal dose LD50/100 (IGSUB) of 0.00081 µg/bee of imidacloprid (SIGMA® 37894). A control group of bees, collected in the same way but fed uncontaminated honey syrup, was maintained.

For the contact exposure, the fasting period was not applied. The bees were contaminated on the pronotum region with 2 µl of distilled water with the lethal dose (CONT50) of 0.063 µg/bee or a sublethal dose (CONTSUB) of 0.00063 µg/bee of imidacloprid (SIGMA® 37894) or just 2 µl of distilled water, for the control group (Bovi *et al*., 2018). After 1 and 4 hours of exposure, 2 alive bees were collected randomly from the Petri dishes, resulting in a total of 12 bees per treatment, for transcriptome analyses. These bees were immediately frozen at −80 °C in an ultra-freezer until processing (Alstolfi *et al*., 2022; de Castro Lippi *et al*., 2024).

RNA extraction, library preparation, and sequencing

Each honey bee head was dissected using stereoscopic microscopes, tweezers, and a sterilized scalpel to remove the compound eyes and antennae (Astolfi *et al*., 2022). The total RNA was extracted from the bee brain using TRIzol reagent (Invitrogen, USA). RNA quality and quantity were evaluated using a Qubit fluorometer (Invitrogen, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., USA). To assess the extent of RNA degradation a 1% agarose gel was used. For the construction of cDNA libraries, 200 ng of total RNA was utilized, following the manufacturer's instructions provided in the SureSelect Strand Specific RNA Library Preparation Kit (Agilent Technologies, Santa Clara, USA). Subsequently, the library products were subjected to sequencing on an Illumina Nextseq platform (Illumina, San Diego, USA) in a single-bore run with read lengths of 150 bp.

Sequencing data processing and statistical analysis

NCBI sequencing data is available at https://shorturl.at/hBPX4, BioProject code is PRJNA1017469.

The raw sequencing data was saved in a FASTQ file format, which contains comprehensive information about the read sequences and their corresponding quality

scores. To evaluate the sequencing quality of the raw reads, the FastQC program (Andrews *et al*., 2010) was employed. Quality control for sequencing data was conducted using the Trimmomatic software (Bolger *et al*., 2014). The following filters were applied: elimination of identified adapters, exclusion of bases with a quality score below 20 (Phred Score), discarding reads shorter than 40 base pairs, and trimming of the initial 10 base calls. Detailed information on the sequencing performance, number of mapped reads and mapping quality for each sample is available at NCBI with the following BioProject number: PRJNA1017469 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1017469).

The sequencing data was aligned using the Burrows-Wheeler Aligner (BWA) software, with the reference sequence Amel_HAv3.1 (NCBI accession number GCF_003254395). Using the GTF annotation from Amel_HAv3.1, the feature count matrix was generated using the HTSeq program (Anders *et al*., 2015). RStudio for the R language (R Development Core Team, 2013; RStudio, 2015) and the tidyverse ecosystem of packages were utilized for data processing, analysis, and plotting. The differential expression analysis was performed using the edgeR v3.38.4 package from the Bioconductor software project for the R language, employing a binomial generalized log-linear model. Genes with a count per million less than one in at least two samples were excluded. The counts were normalized using the TMM normalization. Multiple correction tests were conducted using the Benjamini-Hochberg false discovery rate (FDR) procedure. Differentially expressed genes (DEGs) were deemed significantly when the FDR-adjusted p-value was less than 0.05, and the absolute value of Log2 fold change was equal to or greater than 1.

Results

Twenty-four genes that were differentially expressed and may be related to possible impacts on foraging activity and, consequently, pollination are presented in table 1. Only the differentially expressed genes from each treatment were selected in the present work.

After 1 hour of sublethal dose exposure (IGSUB), there was an upregulation in the transcripts of genes circadian clock-controlled protein (LOC409602) and take-out-like carrier protein (JHBP-1). Within the same 1-hour period of IGSUB treatment, the transcripts of chemosensory protein genes 1 and 4 (CSP1 and CSP4) and odorant binding protein genes 1 and 2 (Obp1 and Obp2) were downregulated. Four hours after IGSUB treatment, transcripts of the circadian clock-controlled protein (LOC409602)

Table 1. Genes differentially expressed which may have impacts on pollination activity. IGSUB: ingestion treatment with a sublethal dose; IG50: ingestion treatment with a lethal dose; CONTSUB: contact treatment with a sublethal dose; CONT50: contact treatment with a lethal dose.

Treatment	Hour	Gene	Product	Expression	P-value
IGSUB	1	LOC409602	circadian clock-controlled protein	upregulation	8.11×10^{-9}
IGSUB	1	JHBP-1	take-out-like carrier protein	upregulation	1.92×10^{-5}
IGSUB	1	CSP1	chemosensory protein 1	downregulation	9.19×10^{-4}
IGSUB	1	Obp1	odorant binding protein 1	downregulation	1.61×10^{-3}
IGSUB	1	CSP4	chemosensory protein 4	downregulation	6.24×10^{-3}
IGSUB	1	Obp2	odorant binding protein 2	downregulation	1.10×10^{-9}
IGSUB	4	LOC409602	circadian clock-controlled protein	upregulation	3.60×10^{-5}
IGSUB	4	JHBP-1	take-out-like carrier protein	upregulation	5.69×10^{-5}
IG50	4	LOC409602	circadian clock-controlled protein	upregulation	8.22×10^{-9}
IG50	4	JHBP-1	take-out-like carrier protein	upregulation	2.61×10^{-6}
IG50	4	LOC406114	alpha-amylase	downregulation	6.03×10^{-4}
IG50	4	Obp1	odorant binding protein 1	downregulation	4.39×10^{-8}
IG50	4	Obp2	odorant binding protein 2	downregulation	1.68×10^{-13}
CONTSUB	1	Obp15	odorant binding protein 15	upregulation	1.29×10^{-5}
CONTSUB	1	LOC406114	alpha-amylase	downregulation	3.50×10^{-6}
CONTSUB	1	Hbg3	alpha-glucosidase	downregulation	1.19×10^{-4}
CONTSUB	1	LOC410149	carboxypeptidase Q	downregulation	1.14×10^{-5}
CONTSUB	4	Obp15	odorant binding protein 15	upregulation	6.35×10^{-6}
CONTSUB	4	Hex70a	hexamerin 70a	upregulation	1.19×10^{-5}
CONTSUB	4	Vg	vitellogenin	upregulation	2.27×10^{-6}
CONTSUB	4	LOC408603	glucose dehydrogenase [FAD, quinone]	upregulation	6.06×10^{-5}
CONT50	1	Obp15	odorant binding protein 15	upregulation	5.34×10^{-7}
CONT50	1	Obp6	odorant binding protein 6	downregulation	8.91×10^{-8}
CONT50	1	LOC410149	carboxypeptidase Q	downregulation	3.13×10^{-5}
CONT50	4	Obp15	odorant binding protein 15	upregulation	5.34×10^{-7}
CONT50	4	Hex70a	hexamerin 70a	upregulation	3.91×10^{-5}
CONT50	4	Vg	vitellogenin	upregulation	1.14×10^{-6}
CONT50	4	LOC408603	glucose dehydrogenase [FAD, quinone]	upregulation	1.16×10^{-4}

and take-out-like carrier protein (JHBP-1) were upregulated. Four hours after ingestion of the lethal dose treatment (IG50), there was an upregulation of the genes of circadian clock-controlled protein (LOC409602) and take-out-like carrier protein (JHBP-1), while there was a downregulation of the transcripts of alpha-amylase (LOC406114) and odorant binding protein genes 1 and 2 (Obp1 and Obp2) (figure 1).

In contact tests with imidacloprid, after 1 hour of sublethal dose exposure (CONTSUB), the odorant binding protein 15 gene transcript was upregulated. However, the transcripts of alpha-amylase (LOC406114), alpha-glucosidase (Hbg3), and carboxypeptidase Q (LOC410149) were downregulated. After 4 hours of CONTSUB treatment, the transcripts of odorant binding protein 15 (Obp15), hexamerin 70a (Hex70a), vitellogenin (Vg), and glucose dehydrogenase [FAD, quinone] (LOC408603) were upregulated (figure 2).

One hour after contact with the lethal dose of imidacloprid (CONT50), there was upregulation of the odorant binding protein 15 (Obp15) gene transcript, downregulation of the odorant binding protein 6 (Obp6), and carboxypeptidase Q (LOC410149). After 4 hours of CONT50 treatment, there was upregulation of transcripts of odorant binding protein 15 (Obp15), hexamerin 70a (Hex70a), vitellogenin (Vg), and glucose dehydrogenase [FAD, quinone] (LOC408603).

Discussion

The impact of environmental stressors, particularly pesticide exposure, on bee populations is a topic of increasing concern due to its potential ramifications on both bee health and agricultural ecosystems. Moreover, exposure to pesticides like imidacloprid induces alterations in metabolic genes, disrupting the expression of key enzymes involved in nutrient digestion (Uddin *et al*., 2022). These metabolic shifts may have adverse effects on bee health, impairing foraging efficiency, flight performance, and overall survival. While bees exhibit adaptive responses to oxidative stress induced by pesticide exposure, these responses may also lead to behavioural changes affecting foraging patterns and return rates (Christen *et al*., 2018).

Odorant-binding proteins (OBPs) and chemosensory proteins (CSPs) are essential for odour recognition and chemical communication (Sanchez-Gracia *et al*., 2009; Erban *et al*., 2017). Insects possess highly sensitive and sophisticated olfactory systems to detect and identify various odours in their environment, with olfactory tissues largely mediated by OBPs. These proteins, found in significant concentrations in insect lymph, enhance the sensitivity of the olfactory system by transporting odorants through the lymph to olfactory receptors. The binding interactions between odours and OBPs likely play a role in the initial phase of olfactory molecular recognition and

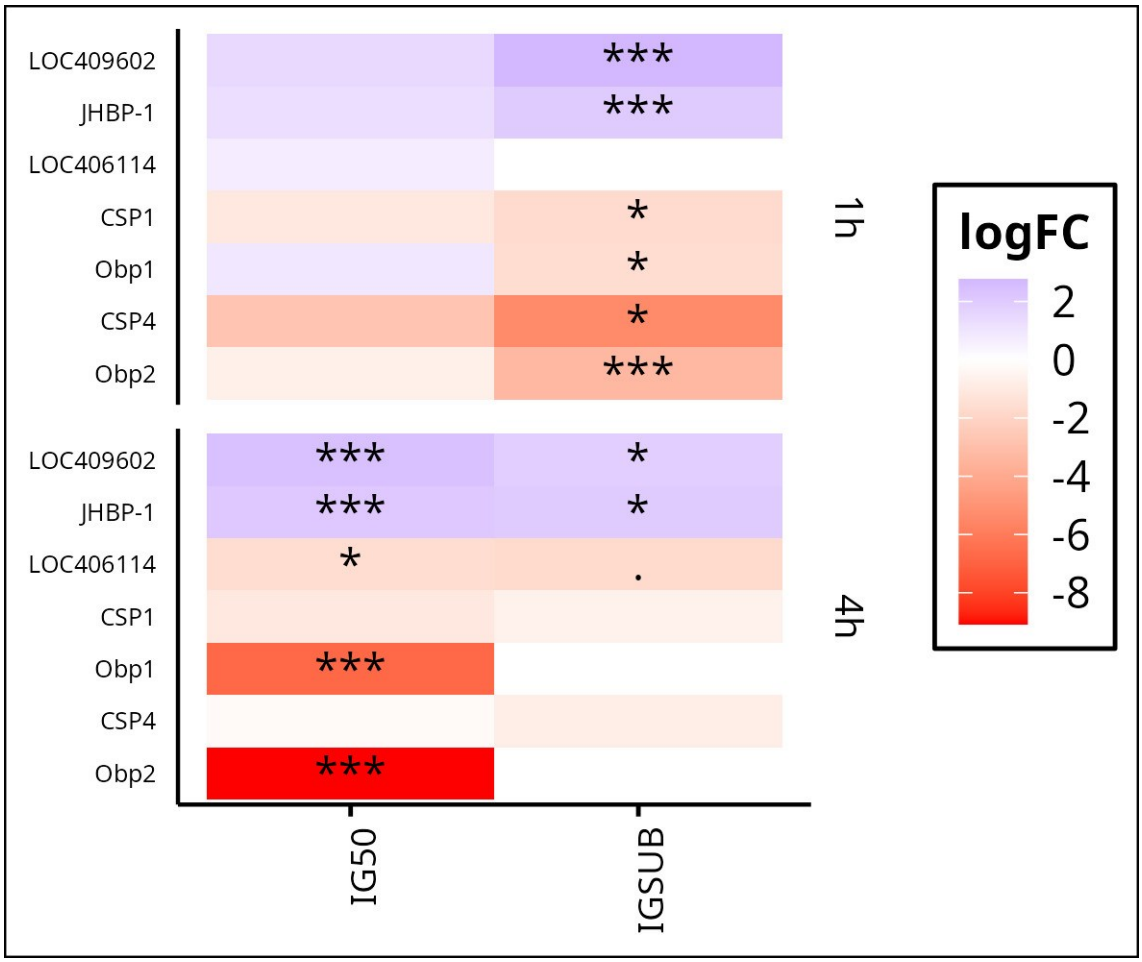

Figure 1. Cluster analysis of the differentially expressed genes after 1 and 4 hours of ingestion exposure to the used doses (lethal: LD50 and Sublethal: IGSUB). . < 0.1, * < 0.05, ** < 0.01, *** < 0.00.

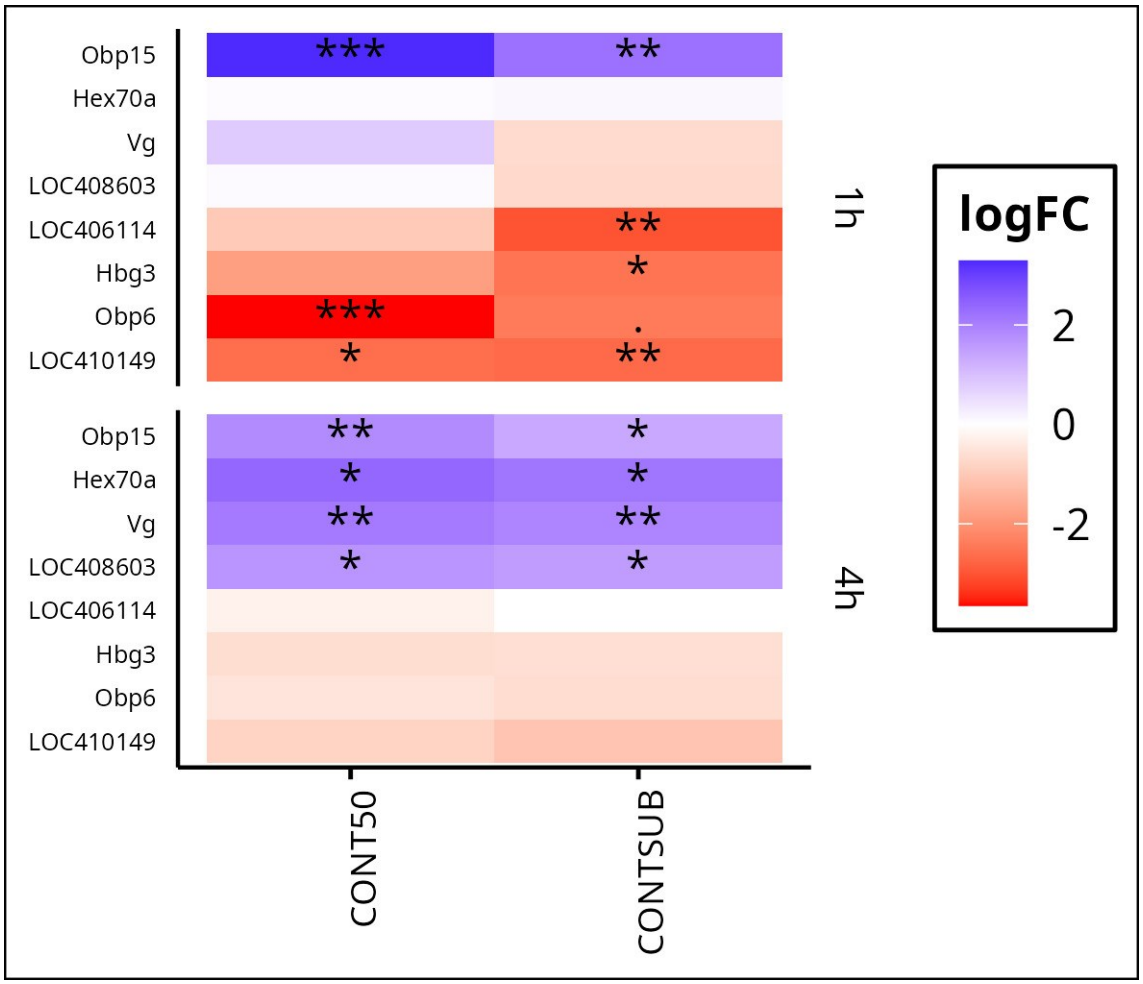

Figure 2. Cluster analysis of the differentially expressed genes after 1 and 4 hours of topic exposure to the used doses (lethal: CONT50 and Sublethal: CONTSUB). . < 0.1, * < 0.05, ** < 0.01, *** < 0.00.

signal transduction in insects (Leal *et al*., 2005). However, the current misuse of neonicotinoid pesticides in monoculture cultivation disrupts the binding of OBPs to odours, thus altering this crucial pathway involved in olfactory discrimination (Li *et al*., 2015).

The ingestion treatments caused a downregulation of OBPs genes and CSPs genes. The contact treatment, both CONTSUB and CONT50 doses resulted in upregulation of the Obp15 transcript gene after 1 and 4 hours, while exposure to the lethal dose led to downregulation of the Obp6 transcript gene after 1 hour. The aberrant regulation of OBP and CSP transcript genes suggests potential impairment in olfactory function and chemical communication among bees exposed to imidacloprid, consequently influencing their behaviours (Li *et al*., 2019), and potentially compromising foraging and pollination success.

In the treatment involving ingesting a sublethal and lethal dose of pesticide in food, the transcript of the vital enzyme alpha-amylase, crucial for nutritional status, was downregulated after 4 hours. Following a 1-hour exposure to CONTSUB, transcripts of enzymes such as carboxypeptidase, alpha-glucosidase, and alpha-amylase were downregulated. Alpha-amylase and alpha-glucosidase are primary enzymes involved in saccharide hydrolysis, influencing the degradation and digestion of carbohydrates (Uddin *et al*., 2022), whereas carboxypeptidases are key proteases engaged in protein hydrolysis. The resultant energy deficiency from reduced carbohydrate digestion can impact colony health, potentially interfering with the energy supply for the foraging activity. Worker bees heavily rely on carbohydrate metabolism products, as well as for thermogenesis performed by bees through wing flapping (Kunieda *et al*., 2006). Consequently, alterations in metabolic genes can have adverse effects on pollination by influencing bee physiology and performance, including foraging duration, flight performance, and survival (Kunieda *et al*., 2006; Tison *et al*., 2016; Christen *et al*., 2018).

Following topic exposure to both lethal and sublethal doses of imidacloprid for 4 hours, there was an upregulation in the transcription of the glucose dehydrogenase (FAD, quinone) gene. This particular enzyme plays a crucial role in carbohydrate metabolism and energy production, and its heightened activity could potentially lead to increased carbohydrate oxidation, consequently impacting metabolic pathways and overall organismal function, including brain function (Fent *et al*., 2020). Such disruptions have the potential to interfere negatively with foraging activities. Moreover, the transcript expression of the gene encoding the take-out-like carrier protein (JHBP-1) increased after 4 hours of ingestion at both lethal and sublethal doses of imidacloprid, and after 1 hour of ingestion at the sublethal dose. The JHBP-1 gene is

implicated in influencing the development of worker bees and their responses to varying food availabilities and resources (Fuller *et al*., 2015).

The circadian clocks of honey bees serve as fundamental regulators of behaviours crucial for their survival, including foraging orientation, navigation, memory consolidation for food sources, sleep patterns, and learning/memory processes (Moore, 2001). Neonicotinoids disrupt the intricate mechanisms controlling bee orientation and learning, both of which are tightly regulated by the bees' internal circadian clocks (Zhang and Nieh, 2015). Moreover, sleep patterns, under the influence of the biological clock, play a pivotal role in reinforcing navigational memory and facilitating social communication among foragers to relay information about food source locations (Klein *et al*., 2010). The disruption of bees' circadian rhythms and sleep induced by neonicotinoids, likely through neuronal stimulation, poses a significant threat to bees' navigation abilities, temporal memory consolidation, social communication, and consequently, their foraging and pollination activities (Bloch *et al*., 2017; Tackenberg *et al*., 2020). In the present study, following 1 hour of exposure to IGSUB and 4 hours to IG50 and IGSUB treatments, an upregulation in the transcripts of the gene encoding the circadian clock-controlled protein (LOC409602) was observed.

After 4 hours of exposure, both CONT50 and CONTSUB treatments through contact resulted in an upregulation of hexamerin 70a gene transcripts. Hexamerins are pivotal in the storage and utilization of amino acids during insect development, with hexamerin 110 and 70a being exclusively observed in the body fat of adult bees in a caste- and sex-specific manner, notably with foraging bees exhibiting the highest expression (Corona *et al*., 2007; Martins *et al*., 2010). The pesticide exposure might have induced heightened oxidative stress in bees, leading to increased exploration of stored amino acids and potentially compromising the well-being of bees responsible for foraging. In a study with *A. mellifera*, the ingestion of food contaminated with imidacloprid increased glutathione peroxidase and catalase activities, which are antioxidant enzymes, indicating the occurrence of oxidative stress (Balieira *et al*., 2018). Excessive reactive oxygen species (ROS) generation from pesticide exposure led to severe oxidative stress in bees, triggering endoplasmic reticulum stress-mediated apoptotic pathways (Kumar *et al*., 2022). The upregulation of endoplasmic reticulum stress indicates protein misfolding, a hallmark of neurodegenerative diseases caused by proteotoxic stress in insect brains, such as those of Drosophila (Rincon-Limas *et al*., 2012).

Furthermore, these treatments also induced an increase in *vitellogenin* transcript expression, known for its defensive properties against oxidative stress by scavenging free radicals to safeguard bees (Christen *et al*., 2016). Vitellogenin levels may also correlate with shifts in foraging behaviour, potentially influencing pollen search efforts (Amdam *et al*., 2012), and prompting bees to undertake longer and occasionally less efficient foraging flights. As a result, these alterations may lead to diminished return rates and reduced pollination success due to neurological changes induced by neonicotinoid pesticides (Friedrich *et al*., 2004; Schneider *et al*., 2012; Christen *et al*., 2016).

Conclusion

The findings of this research reveal significant impacts on genes related to the physiological and behavioural processes of honey bees exposed to imidacloprid, highlighting the complexity and scope of the impacts caused by neonicotinoids on *A. mellifera*. It is important to emphasize the need for more sustainable management strategies and consideration of potential adverse effects on biodiversity and pollination. In an economic context, the decrease in the effectiveness of pollination by *A. mellifera* can cause significant losses in agriculture. Many crops rely heavily on pollination to achieve optimal yields, and the reduction in food production can directly impact farmers and food security. Additionally, the need for compensatory measures, such as the introduction of alternative pollinators or manual pollination methods, may incur additional costs for producers.

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References

AIZEN M. A., HARDER L. D., 2009.- The global stock of domesticated honey bees is growing slower than agricultural demand for pollination.- *Current biology*, 19 (11): 915-918.

AMDAM G. V., FENNERN E., HAVUKAINEN H., 2012.- Vitellogenin in honey bee behavior and lifespan, pp. 17-29. In: *Honeybee neurobiology and behavior* (GALIZIA C., EISENHARDT D., GIURFA M., Eds).- Springer, Dordrecht, The Netherlands.

ANDERS S., PYL P. T., HUBER W., 2015.- HTSeq-a Python framework to work with high- through put sequencing data.- *Bioinformatics*, 31 (2): 166-169.

ANDREWS S., KRUEGER F., SEGONDS-PICHON A., BIGGINS L., KRUEGER C., WINGETT S., 2010.- *FastQC: a quality control tool for high throughput sequence data*.- Babraham Bioinformatics, Cambridge, UK.

ASTOLFI A., KADRI S. M., LIPPI I. C. C., MENDES D. D., ALONSO D. P., RIBOLLA P. E. M, ORSI R. O., 2022.- Field-relevant doses of fipronil affect gene expression in honey bees *Apis mellifera*.- *Apidologie*, 53 (6): 69.

BALIEIRA K. V. B., MAZZO M., BIZERRA P. F. V., GUIMARÃES A. R. D. J. S., NICODEMO D., MINGATTO F. E., 2018.- Imidacloprid-induced oxidative stress in honey bees and the antioxidant action of caffeine.- *Apidologie*, 49: 562-572.

BLACQUIERE T., SMAGGHE G., VAN GESTEL C. A., MOMMAERTS V., 2012.- Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment.- *Ecotoxicology*, 21: 973-992.

BLOCH G., BAR-SHAI N., CYTTER Y., GREEN R., 2017.- Time is honey: circadian clocks of bees and flowers and how their interactions may influence ecological communities.- *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372 (1734): 20160256.

BOLGER A. M., LOHSE M., USADEL B., 2014.- Trimmomatic: a flexible trimmer for Illumina sequence data.- *Bioinformatics*, 30 (15): 2114-2120.

BOVI T. S., ZALUSKI R., ORSI R. O., 2018.- Toxicity and motor changes in Africanized honey bees (*Apis mellifera* L.) exposed to fipronil and imidacloprid.- *Anais da Academia Brasileira de Ciências*, 90: 239-245.

CAMILLI M. P., KADRI S. M., ALVAREZ M. V. N., RIBOLLA P. E. M., ORSI R. O., 2022.- Zinc supplementation modifies brain tissue transcriptome of *Apis mellifera* honeybees.- *BMC Genomics*, 23: 282.

CHRISTEN V., MITTNER F., FENT K., 2016.- Molecular effects of neonicotinoids in honey bees (*Apis mellifera*).- *Environmental Science & Technology*, 50 (7): 4071-4081.

CHRISTEN V., SCHIRRMANN M., FREY J. E., FENT K., 2018.- Global transcriptomic effects of environmentally relevant concentrations of the neonicotinoids clothianidin, imidacloprid, and thiamethoxam in the brain of honey bees (*Apis mellifera*).- *Environmental Science & Technology*, 52 (13): 7534-7544.

CORONA M., VELARDE R. A., REMOLINA S., MORAN-LAUTER A., WANG Y., HUGHES K. A., ROBINSON G. E., 2007.- Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity.- *Proceedings of the National Academy of Sciences*, 104 (17): 7128-7133.

COX-FOSTER D. L., CONLAN S., HOLMES E. C., PALACIOS G., EVANS J. D., MORAN N. A., LIPKIN W. I., 2007.- A metagenomic survey of microbes in honey bee colony collapse disorder.- *Science*, 318 (5848), 283-287.

CRANE E., 1990.- *Bees and beekeeping: science, practice and world resources*.- Heinemann Newnes, Oxford, UK.

DE CASTRO LIPPI I. C., DA LUZ SCHEFFER J., DE LIMA Y. S., LUNARDI J. S., ASTOLFI A., KADRI S. M., DE OLIVEIRA ORSI R., 2024.- Intake of imidacloprid in lethal and sublethal doses alters gene expression in *Apis mellifera* bees.- *Science of The Total Environment*, 940: 173393.

EILERS E. J., KREMEN C., SMITH GREENLEAF S., GARBER A. K., KLEIN A.-M., 2011.- Contribution of pollinator-mediated crops to nutrients in the human food supply.- *PLoS ONE*, 6 (6): e21363.

ELSTON C., THOMPSON H. M., WALTERS K. F. A., 2013.- Sublethal effects of thiamethoxam, a neonicotinoid pesticide, and propiconazole, a DMI fungicide, on colony initiation in bumblebee (*Bombus terrestris*) micro-colonies.- *Apidologie*, 44 (5): 563-574.

ERBAN T., HARANT K., CHALUPNIKOVA J., KOCOUREK F., STARA J., 2017.- Beyond the survival and death of the deltamethrin-threatened pollen beetle *Meligethes aeneus*: an in-depth proteomic study employing a transcriptome database.- *Journal of Proteomics*, 150: 281-289.

FENT K., SCHMID M., CHRISTEN V., 2020.- Global transcriptome analysis reveals relevant effects at environmental concentrations of cypermethrin in honey bees (*Apis mellifera*).- *Environmental Pollution*, 259: 113715.

FRIEDRICH A., THOMAS U., MÜLLER U., 2004.- Learning at different satiation levels reveals parallel functions for the cAMP–protein kinase A cascade in formation of long-term memory.- *Journal of Neuroscience*, 24 (18): 4460-4468.

FULLER Z. L., NIÑO E. L., PATCH H. M., BEDOYA-REINA O. C., BAUMGARTEN T., MULI E., MILLER W., 2015.- Genome-wide analysis of signatures of selection in populations of African honeybees (*Apis mellifera*) using new web-based tools.- *BMC Genomics*, 16: 518.

GALLAI N., SALLES J. M., SETTELE J., VAISSIÈRE B. E., 2009.- Economic valuation of the vulnerability of world agriculture confronted with pollinator decline.- *Ecological Economics*, 68 (3): 810-821.

GAO J., JIN S. S., HE Y., LUO J. H., XU C. Q., WU Y. Y., DIAO Q. Y., 2020.- Physiological analysis and transcriptome analysis of Asian honey bee (*Apis cerana cerana*) in response to sublethal neonicotinoid imidacloprid.- *Insects*, 11 (11): 753.

GAZZEA E., BATÁRY P., MARINI L., 2023.- Global meta-analysis shows reduced quality of food crops under inadequate animal pollination.- *Nature Communications*, 14 (1): 4463.

GOULSON D., LYE G. C., DARVILL B., 2008.- Decline and conservation of bumble bees.- *Annual Review of Entomology*, 53: 191-208.

GUEZ D., SUCHAIL S., GAUTHIER M., MALESZKA R., BELZUNCES L. P., 2001.- Contrasting effects of imidacloprid on habituation in 7- and 8-day-old honeybees (*Apis mellifera*).- *Neurobiology of Learning and Memory*, 76 (2): 183-191.

HENRY M., BEGUIN M., REQUIER F., ROLLIN O., ODOUX J. F., AUPINEL P., DECOURTYE A., 2012.- A common pesticide decreases foraging success and survival in honey bees.- *Science*, 336 (6079): 348-350.

KESSLER S. C., TIEDEKEN E. J., SIMCOCK K. L., DERVEAU S., MITCHELL J., SOFTLEY S., WRIGHT G. A., 2015.- Bees prefer foods containing neonicotinoid pesticides.- *Nature*, 521 (7550): 74-76.

KHALIFA S. A., ELSHAFIEY E. H., SHETAIA A. A., EL-WAHED A. A. A., ALGETHAMI A. F., MUSHARRAF S. G., EL-SEEDI H. R., 2021.- Overview of bee pollination and its economic value for crop production.- *Insects*, 12 (8): 688.

KLATT B. K., HOLZSCHUH A., WESTPHAL C., CLOUGH Y., SMIT I., PAWELZIK E., TSCHARNTKE T., 2014.- Bee pollination improves crop quality, shelf life and commercial value.- *Proceedings of the Royal Society B: Biological Sciences*, 281: 20132440.

KLEIN A. M., VAISSIÈRE B. E., CANE J. H., STEFFAN-DEWENTER I., CUNNINGHAM S. A., KREMEN C., TSCHARNTKE T., 2007.- Importance of pollinators in changing landscapes for world crops.- *Proceedings of the Royal Society B: Biological Sciences*, 274 (1608): 303-313.

KLEIN B. A., KLEIN A., WRAY M. K., MUELLER U. G., SEELEY T. D., 2010.- Sleep deprivation impairs precision of waggle dance signaling in honey bees.- *Proceedings of the National Academy of Sciences*, 107 (52): 22705-22709.

KUMAR D., BANERJEE D., CHAKRABARTI P., SARKAR S., BASU P., 2022.- Oxidative stress and apoptosis in Asian honey bees (*A. cerana*) exposed to multiple pesticides in intensive agricultural landscape.- *Apidologie*, 53 (2): 25.

KUNIEDA T., FUJIYUKI T., KUCHARSKI R., FORET S., AMENT S. A., TOTH A. L., MALESZKA R., 2006.- Carbohydrate metabolism genes and pathways in insects: insights from the honey bee genome.- *Insect Molecular Biology*, 15 (5): 563-576.

LEAL W. S., CHEN A. M., ERICKSON M. L., 2005.- Selective and pH-dependent binding of a moth pheromone to a pheromone-binding protein.- *Journal of Chemical Ecology*, 31: 2493-2499.

LI H., WU F., ZHAO L., TAN J., JIANG H., HU F., 2015.- Neonicotinoid insecticide interact with honeybee odorant-binding protein: implication for olfactory dysfunction.- *International Journal of Biological Macromolecules*, 81: 624-630.

LI Z., YU T., CHEN Y., HEERMAN M., HE J., HUANG J., SU S., 2019.- Brain transcriptome of honey bees (*Apis mellifera*) exhibiting impaired olfactory learning induced by a sublethal dose of imidacloprid.- *Pesticide Biochemistry and Physiology*, 156: 36-43.

LIMA Y. S., DE CASTRO LIPPI I. C., DA LUZ SCHEFFER J., LUNARDI J. S., ALVAREZ M. V. N., KADRI S. M., DE OLIVEIRA ORSI R., 2024.- Food contamination with fipronil alters gene expression associated with foraging in Africanized honey bees.- *Environmental Science and Pollution Research*, 31: 52267-52278.

LUNARDI J. S., ZALUSKI R., ORSI R. O., 2017.- Evaluation of motor changes and toxicity of insecticides fipronil and imidacloprid in Africanized honey bees (Hymenoptera: Apidae).- *Sociobiology*, 64 (1): 50-56.

MARTINS J. R., NUNES F. M., CRISTINO A. S., SIMÕES Z. L., BITONDI M. M., 2010.- The four hexamerin genes in the honey bee: structure, molecular evolution and function deduced from expression patterns in queens, workers and drones.- *BMC Molecular Biology*, 11 (1): 1-20.

MATSUDA K., BUCKINGHAM S. D., KLEIER D., RAUH J. J., GRAUSO M., SATTELLE D. B., 2001.- Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors.- *Trends in Pharmacological Sciences*, 22 (11): 573-580.

MOORE D., 2001.- Honey bee circadian clocks: behavioral control from individual workers to whole-colony rhythms.- *Journal of Insect Physiology*, 47 (8): 843-857.

PALMER M. J., MOFFAT C., SARANZEWA N., HARVEY J., WRIGHT G. A., CONNOLLY C. N., 2013.- Cholinergic pesticides cause mushroom body neuronal inactivation in honeybees.- *Nature Communications*, 4: 1634.

PISA L. W., AMARAL-ROGERS V., BELZUNCES L. P., BONMATIN J. M., DOWNS C. A., GOULSON D., WIEMERS M., 2015.- Effects of neonicotinoids and fipronil on non-target invertebrates.- *Environmental Science and Pollution Research*, 22 (1): 68-102.

POTTS S. G., BIESMEIJER J. C., KREMEN C., NEUMANN P., SCHWEIGER O., KUNIN W. E., 2010.- Global pollinator declines: trends, impacts and drivers.- *Trends in Ecology & Evolution*, 25 (6): 345-353.

RAYMANN K., MOTTA E. V., GIRARD C., RIDDINGTON I. M., DINSER J. A., MORAN N. A., 2018.- Imidacloprid decreases honey bee survival rates but does not affect the gut microbiome.- *Applied and Environmental Microbiology*, 84 (13): e00545-18.

R DEVELOPMENT CORE TEAM, 2013.- *R: a language and environment for statistical computing*.- R Foundation for Statistical Computing, Vienna, Austria.

RINCON-LIMAS D., JENSEN K., FERNANDEZ-FUNEZ P., 2012.- *Drosophila* models of proteinopathies: the little fly that could.- *Current Pharmaceutical Design*, 18 (8): 1108-1122.

RSTUDIO, 2015.- *RStudio: integrated development environment for R*.- RStudio, PBC, Boston, MA, USA.

SÁNCHEZ-BAYO F., GOULSON D., PENNACCHIO F., NAZZI F., GOKA K., DESNEUX N., 2016.- Are bee diseases linked to pesticides? - A brief review.- *Environment International*, 89-90: 7-11.

SÁNCHEZ-GRACIA A., VIEIRA F. G., ROZAS J., 2009.- Molecular evolution of the major chemosensory gene families in insects.- *Heredity*, 103 (3): 208-216.

SCHNEIDER C. W., TAUTZ J., GRÜNEWALD B., FUCHS S., 2012.- RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*.- *PLoS ONE*, 7 (1): e30023.

SIMON-DELSO N., AMARAL-ROGERS V., BELZUNCES L. P., BONMATIN J. M., CHAGNON M., DOWNS C., WIEMERS M., 2014.- Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites.- *Environmental Science and Pollution Resear*ch, 22 (1): 5-34.

TACKENBERG M. C., GIANNONI-GUZMÁN M. A., SANCHEZ-PEREZ E., DOLL C. A., AGOSTO-RIVERA J. L., BROADIE K., MCMAHON D. G., 2020.- Neonicotinoids disrupt circadian rhythms and sleep in honey bees.- *Scientific Reports*, 10: 17929.

TISON L., HAHN M.-L., HOLTZ S., RÖßNER A., GREGGERS U., BISCHOFF G., MENZEL R., 2016.- Honey bees' behavior is impaired by chronic exposure to the neonicotinoid thiacloprid in the field.- *Environmental Science & Technology*, 50 (13): 7218-7227.

UDDIN S., BROOKS P. R., TRAN T. D., 2022.- Chemical characterization, α-glucosidase, α-amylase and lipase inhibitory properties of the Australian honey bee propolis.- *Foods*, 11 (13): 1964.

VIDAL M. D. F., 2021.- Mel natural: cenário mundial e situação da produção na área de atuação do BNB.- *Escritório Técnico de Estudos Econômicos do Nordeste - ETENE*, 6 (157): 1-9.

WILLIAMS G. R., TROXLER A., RETSCHNIG G., ROTH K., YAÑEZ O., SHUTLER D., GAUTHIER L., 2015.- Neonicotinoid pesticides severely affect honey bee queens.- *Scientific Reports*, 5: 14621.

WILLIAMSON S. M., WRIGTH G. A., 2013.- Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honeybees.- *Journal of Experimental Biology*, 216: 1799-1807.

WILLIAMSON S. M., MOFFAT C., GOMERSALL M. A., SARANZEWA N., CONNOLLY C. N., WRIGHT G. A., 2013.- Exposure to acetylcholinesterase inhibitors alters the physiology and motor function of honeybees.- *Frontiers in Physiology*, 4: 13.

WINFREE R., GROSS B. J., KREMEN C., 2011.- Valuing pollination services to agriculture.- *Ecological Economics*, 71: 80-88.

WOODCOCK B. A., BULLOCK J. M., SHORE R. F., HEARD M. S., PEREIRA M. G., REDHEAD J., PYWELL R. F., 2017.- Country-specific effects of neonicotinoid pesticides on honey bees and wild bees.- *Science*, 356 (6345), 1393-1395.

ZHANG E., NIEH J. C., 2015.- The neonicotinoid imidacloprid impairs honey bee aversive learning of simulated predation.- *Journal of Experimental Biology*, 218 (20), 3199-3205.

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