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Determination of neonicotinoids and other residues in orange pollen by micro-QuEChERS and UHPLC–MS/MS

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ABSTRACT

Producers of citrus crops use different types of insecticides, such as neonicotinoids, to combat pests and diseases of these cultures. Neonicotinoids are systemic insecticides and are translocated to the plant tissue, which may leave residues in pollen and cause adverse effects on bees. Pesticide contamination of pollen is also a food safety issue in human nutrition since the product is consumed as a food supplement. Therefore, the determination of these pesticides in pollen is important to help understand their effects on bees because pollen is their main nutrient source. In this work, we developed and validated a method using UHPLC–MS/MS to determine pesticide residues in orange pollen using micro-QuEChERS extraction, which uses low consumption of solvent, sample, and reagents. The analytical method demonstrated linearity over the range of 50 to 1000 μg kg^{-1}, with a correlation coefficient of ≥0.990. Trueness and precision were assessed using spiked samples, analyzed in quintuplicate at concentrations of 50, 100, and 200 μg kg^{-1}. Trueness values ranged between 81 and 115 %, while precision, expressed as relative standard deviation (RSD), was consistently below 20 %. The limit of quantification (LOQ) was established at 50 μg kg^{-1}, with acceptable precision and accuracy observed at this level. Experimental treatment samples showed high concentrations of clothianidin, imidacloprid, and thiamethoxam, while in commercial samples, the analytes were below the LOQ or not detected.

Introduction

Brazil is a very important producer of citrus crops such as orange, lime, and tangerine. According to the Brazilian Institute of Geography and Statistics (IGBE), the Brazilian production of oranges in 2023 reached 17.615.667 tons, making it one of the most important producers in the world [1]. Despite the growth in orange production, the emergence of pests and diseases is a threat to the Brazilian and world production of this culture. The advancement of diseases such as greening (huanglongbing—HLB), citrus variegated chlorosis (CVC) and citrus bacterial canker is a world concern [2]. It has been lost to many producers because effective control is the immediate elimination of symptomatic plants, avoiding spreading in healthy trees.

To work around this problem, producers use different types of pesticides. The most commonly used pesticides for the orange crop are abamectin, azoxystrobin, carbendazim, and a group of insecticides known as neonicotinoids (imidacloprid, thiamethoxam, and clothianidin) [3]. Neonicotinoids are systemic insecticides that are applied in the soil or sprayed and translocated to the plant tissue, which has been widely discussed in the scientific community because of the possible correlation of the use of this insecticide class with the decline in the number of bees, known as colony collapse disorder (CCD) [4–9]. Therefore, it is important to emphasize that the CCD is also related to other factors, such as climate change and inappropriate use of pesticides, which can be associated with the lack of knowledge and training of the producer, and the misuse of application techniques [4,10].

Concerns over neonicotinoids are reflected in European Union Regulation [11], which restricts substances such as imidacloprid, clothianidin, and thiamethoxam due to their impact on pollinators. The European Community banned the use of this insecticide class in 2018 for all crops [5,12,13] while Brazil began to have restrictive use only in 2022 [14]. This highlights the importance of monitoring such pesticides, reinforcing the relevance of our study in Brazil.

Consumption or contact with contaminated pollen and nectar, which

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can occur through both direct and indirect exposure, may lead to neurological damage in bees, including direction loss, cognitive ability loss, and even death. Bees can be directly exposed to neonicotinoids through contaminated surfaces or pesticide spraying, and indirectly through consuming contaminated pollen or nectar. Direct exposure causes acute toxicity, while indirect exposure leads to chronic health issues [15]. Due to these effects on bees, the detection or quantification of these pesticides in nectar and pollen can help to understand the extent of the damage caused by pesticide residues in these matrices [16–18]. Besides bee health, pesticide contamination of pollen loads is also a food safety issue in human nutrition since the product is consumed as a food supplement [19].

Due to the complexity and poor availability of these nectar and polen for analysis, associated with their low concentration, it is an analytical challenge to develop a method capable of measuring these compounds with high sensitivity and accuracy [8,16,18,20,21]. A study by Moreno-González and collaborators used nanoflow liquid chromatography orbitrap tandem mass spectrometry to find neonicotinoids in pollen and nectar using a miniaturized QuEChERS [8]. In this work, we had the challenge of developing and validating a method using ultra high performance liquid chromatography coupled to mass spectrometry (LC–MS/MS) to quantify neonicotinoids such as clothianidin, imidacloprid, and thiamethoxam, as well as other commonly used pesticides in orange pollen as abamectin, azoxystrobin, and carbendazim, using micro-QuEChERS.

Materials and methods

Chemicals, reagents, and apparatus

Abamectin-certified standards were acquired from Sigma Aldrich (Darmstadt, Germany), azoxystrobin, carbendazim, clothianidin, imidacloprid from Chem Service (West Chester, Pennsylvania, USA) and thiamethoxam from TRC Canada (Toronto, Canada), all >98 % purity.

C18 silica gel spherical sorbent (particles of 40–75 μm) from Sulpeco (Bellefonte, Pennsylvania, USA), primary secondary amine (PSA) from Agilent Technologies (Wilmington, Delaware, USA), and magnesium sulfate anhydrous (≥99.5 %) from Sigma–Aldrich (St. Louis, Missouri, USA). The solvents acetonitrile and methanol were purchased from Mallinckrodt (Phillipsburg, New Jersey, USA), and ultrapure water was obtained from a Direct UV3® gradient system from Millipore (Molsheim, France).

An AY220 balance from Shimadzu (Kyoto, Japan), a Multi Reax from Heidolph (Schwabach, Germany), a Hettich® MIKRO 220/220R centrifuge, two mL Eppendorf® Safe-Lock microcentrifuge tubes (China) and pipettes of different volumes from Transferpette® (Wertheim, Germany) were used.

Sample

Dehydrated organic bee pollens (Breyer®) were used as a blank sample for the development and validation of the method. The samples were homogenized with the aid of a mortar and pestle.

Sampling was performed at the experimental station of Embrapa Environment (Jaguariúna-SP, Brazil; coordinates: −22.716732, −47.018613) (Fig. S1). The collection of orange flowers was performed during spring flowering (rootstock: Flying Dragon, variety: Valence) (October 2019). The flowers were collected in the preopening stage, ensuring no pollen contamination by drift.

Sample preparation optimization

The micro-QuEChERS procedure was based on the literature [8] with some modifications. The chromatographic method also showed differences in the mobile phase, equipment, and pesticides used.

In a two mL microcentrifuge tube, 100 mg of pollen and 500 μL of water were added, and after vortexing for two minutes, the suspension rested for 15 min to ensure water sorption in the pollen. For the extraction step, one milliliter of acetonitrile was added to the microcentrifuge tube and vortexed for two minutes.

The entire content was transferred to another microcentrifuge tube containing 50 mg of NaCl and 50 mg of $MgSO_4$. Due to the exothermic reaction of water and $MgSO_4$, the tubes with salt were placed in an ice bath before transferring the solution. The tubes were vortexed for two minutes and then centrifuged at 14,000 rpm for five minutes. In the clean-up step, 800 μL of the supernatant was transferred to a microcentrifuge tube containing 20 mg of PSA, 20 mg of C_{18}, and 50 mg of $MgSO_4$. The tube was vortexed for two minutes and then centrifuged at 14,000 rpm for five minutes.

The final step was to resuspension 500 μL of the final extract in 500 μL of methanol, dry it with a gentle nitrogen flux, and then filter it at 0.22 μm using a Millex-GV Filter (0.22 μm pore size hydrophilic PVDF membrane, Millipore Sigma, Burlington, MA, USA). Ten microliters of the final extract were diluted in water at a ratio of 1:4 (v/v) and injected into the LC–MS/MS instrument. All extraction steps are described in Fig. S1.

Liquid chromatography coupled to tandem mass spectrometry

An ultra high performance liquid chromatograph (UPLC, Waters Acquity UPLC™ System) coupled to a tandem mass spectrometer (MS/MS, Waters TQ Quattro Model XQ) with electrospray ionization source in positive ion mode (ESI—Electrospray Ionization) and analysis using multiple reaction monitoring (SRM) mode was used. Data acquisition was performed using MassLynx software version 4.1 from Waters (Milford, Massachusetts, USA). The separation of the compounds used a Phenomenex KINETEX core-shell technology column (2.1 × 100 mm; 1.7 μm). The mobile phase was 98:2 (v/v) water with 0.1 % formic acid: methanol (Phase A) and methanol with 0.1 % formic acid (Phase B). The flow rate was 0.225 mL min^{-1} with an injection volume of 20 μL, column temperature set at 35 °C and gradient mode. The mass spectrometer conditions were as follows: dwell time 0.03 s, desolvation gas flow (N_2): 500 L h^{-1}, gas flow of cone: 54 L h^{-1} and desolvation gas flow: 1050 L h^{-1} (both gases were nitrogen), desolvation temperature: 40 °C; capillary voltage: 3.0 kV; gas flow in the cone: 200 L h^{-1}, flow gas collision (argon): 0.15 mL min^{-1}, source temperature: 120 °C, capillary voltage: 3.0 kV, extractor voltage: 3 V; collision-induced dissociation (CID) was performed using argon at a pressure of 4×10^{-3} mbar. The optimized conditions for the selected pesticides were obtained from direct infusion into the mass (Table S1). Fig. S2 shows the chromatograms from the selected ions in the SRM mode of spiked samples after the QuEChERS procedure for LC–MS/MS.

Field trials

The trial was carried out at the Embrapa Meio Ambiente experimental station in 2018 and 2019 in a total area of 3000 m^2. The experimental plot consisted of 10 plants grown in a row. The planting spacing was 2 m between plants and 5 m between planting rows. Spraying insecticides from the pyrethroid class was a regular method of pest control. Applications of the neonicotinoid insecticides thiamethoxam and imidacloprid were performed annually; that is, the interval between the last application of neonicotinoids before the trial of this study was approximately one year. The insecticides thiamethoxam (Actara 250 WG—treatment T1) and imidacloprid (Provado 200 SC—treatment T2) were applied to the soil using an aqueous solution. The applied doses were calculated according to the manufacturers' recommendations and based on the plants' average height. In 2018, 0.32 g and 0.71 g per plant of thiamethoxam and imidacloprid were applied, respectively. In 2019, 0.45 g per plant of thiamethoxam and 1.02 g per plant of imidacloprid were applied. Applications were carried out in four replications in August 2018 and September 2019, immediately after the first flowering after the dry period characteristic of the study region.

Table 1
Values of limit of quantification (LOQ, μg kg^{-1}), trueness and precision (R% ± RSDr (%)), matrix effect (ME, %), linear regression, and coefficient of correlation (r^2) for each of the studied pesticides.

		Trueness and precision R (%) ± RSD_R (%)[b]			Precision R (%) ± RSD_{wR} (%)[c]	ME (%)	(r^2)[d]	Linear regression
Compounds	LOQ[a] (μg kg^{-1})	50 μg kg^{-1}	100 μg kg^{-1}	200 μg kg^{-1}	50 μg kg^{-1}			
Abamectin	50	85 ± 9	83 ± 16	–	99 ± 20	−9.5	0.992	$y = 0.9779x - 4.8338$
Azoxystrobin	50	102 ± 6	114 ± 10	94 ± 18	105 ± 4	+2.1	0.990	$y = 60.765x - 293.24$
Carbendazim	50	104 ± 6	108 ± 7	85 ± 4	111 ± 9	−4.0	0.996	$y = 136.91x - 580,79$
Clothianidin	50	93 ± 7	101 ± 10	93 ± 21	101 ± 11	+15.6	0.990	$y = 4.7823x + 8.8614$
Abamectin	50	81 ± 20	115 ± 7	88 ± 13	91 ± 15	−30.0	0.990	$y = 7.1679x + 70.523$
Azoxystrobin	50	86 ± 20	86 ± 20	100 ± 19	100 ± 19	+19.3	0.993	$y = 2.0771x + 66.165$

[a] LOQ—Limit of quantification.
[b] R (%)—recovery and RSD (%)—relative standard deviation.
[c] RSD_{wR} (%)—within - laboratory reproducibility.
[d] r^2—correlation coefficient estimated linearity.

Fig. S3 shows the sample collection process in the field and the collected samples.

Results and discussion

The micro-QuEChERS method was based on work proposed by Moreno-González and collaborators [8] with modifications: (1) an ice bath for the salting out and clean-up steps; (2) the chromatographic method used methanol instead of acetonitrile as the mobile phase; (3) the methods differ from the pesticides detected, having in common three neonicotinoids; and (4) the equipment used was a UHPLC, with a faster run time. The limits of detection in the literature [8] are much lower than those we got with our method. However, the authors used a nano-LC system coupled with a high-resolution mass spectrometer, which is more sensitive and efficient than the UHPLC system used in this work. On the other hand, UHPLC system coupled to triple quadrupole mass (TQD) is the equipment that pesticide residue laboratories use the most frequently, and the limits found in the developed method meet the maximum residue level (MRL) that the National Health Surveillance Agency (ANVISA) of Brazil uses for citrus [3].

If the original QuEChERS developed by Anastassiades et al. (2003) [22] is compared, ten times fewer reagents and 100 times less sample quantity were used in this work. Therefore, as in the original QuEChERS method, the sample:acetonitrile ratio is 1:1, and in the adopted procedure, it is 1:10. That is, a tenfold dilution factor in the final extract should be considered. Miniaturization has other advantages, such as lower consumption of reagents and greater flexibility in sample preparation, thus reducing the total analysis time, lower cost, and less waste generation.

Method validation

The method was validated following the parameters required by SANTE [23] guidelines (European Commission, 2022): specificity, linearity, precision, trueness, limit of quantification (LOQ), and matrix effect (ME).

The specificity of the method was ensured when blank pollen samples were analyzed, and any compounds detected. To determine if the analytical curves were linear, we performed a linear regression calculation in an Excel® table using the ordinary least squares method (OLS) (Fig. S4). The smallest point on the curve corresponds to a value less than or equal to the MRL and ranges from 50 to 1000 μg L^{-1} (or μg kg^{-1}) with a correlation ≥0.99. The ME was evaluated by comparing the angular coefficients of the analytical curves of pesticides in the solvent with the analytical curves in pollen extracts. To prevent enhancing or reducing the chromatographic response that the matrix-induced, the final extracts were 5 times diluted before analysis [24]. The results showed an increase in signal for azoxystrobin, clothianidin, and thiamethoxam and suppression for carbendazim and abamectin of less than ±20 %. However, only imidacloprid suppressed the medium-effect signal (−30 %). For this reason, the analysis becomes more reliable when the analytical curves are performed on the matrix and not on the solvent.

Trueness and precision were calculated from the spiked sample in quintuplicate for each level evaluated at concentrations of 50, 100, and 200 μg kg^{-1} on the same day by the same analyst and using the same method and equipment. The results showed trueness values between 81 and 115 %, and the precision was calculated as the relative standard deviation [RSD_R (%)] with values <20 %. The within-laboratory precision (RSD_{wR}) derived from ongoing method validation was <20 % for the pesticides studied and was evaluated on two different days. These results were within the range required by the EU guidelines. The LOQ evaluated was the lowest concentration that showed results with acceptable precision and accuracy and was 50 μg kg^{-1}. The results of the trueness, precision, ME, linear range, coefficient of determination, LOQ, and precision for each compound are shown in Table 1.

Some methods have already been described in the literature for the determination of pesticides in pollen using different analytical techniques and extraction methods and can be seen in Table 2. The methods, as detailed by various authors, offer advantages and limitations, which are addressed in the table.

Techniques such as solid-phase extraction (SPE), as used by Garcia-Chao et al. [4] and López-Fernández et al. [28], and modified QuEChERS, applied by Wiest et al. [18], Dively and Kamel [25], Chen et al. [26], and others, often used with chromatography coupled with mass spectrometry, provide low limits of quantification, making them highly sensitive for detecting pesticide residues. However, these methods typically require large amounts of pollen, which can be challenging to obtain, and involve additional steps, such as the use of SPE cartridges or dispersive solid-phase extraction (dSPE), that increase both the cost and complexity of the analysis, as noted by David et al. [27] and Hall et al. [32]. The use of high-performance liquid chromatography (HPLC), as mentioned by Chen et al. [26] and Codling et al. [10], contributes to longer run times. Moreover, the high-resolution mass spectrometry (HRMS) and nano-LC systems, while offering enhanced sensitivity, are less accessible due to their higher costs and specialized equipment requirements, as pointed out by Moreno-González et al. [8]. The newly developed methods that utilize reduced pollen quantities, such as the miniaturized QuEChERS by Moreno-González et al. [8], address some of these limitations by minimizing the sample size needed while maintaining low detection limits. However, they still face challenges related to the availability and cost of advanced instrumentation, such as nano-LC-HRMS and UHPLC systems. Overall, while each study, including those by Garcia-Valcarcel et al. [30] and Wood et al. [31], provides valuable insights for environmental monitoring and bee health, the trade-offs between sensitivity, sample size, cost, and practicality must be carefully considered.

Table 2
Main remarks of the methods developed for the extraction and analysis of pesticides in pollen.

Reference	Amount of pollen	Extraction technique	Instrumental technique	LOQ	Main remarks
Garcia-Chao et al. [4]	1 g	SPE[a]	UHPLC–MS/MS[b]	0.85–1.00 ng g^{-1}	**Pros:** Low limit of quantification. **Cons:** A large amount of pollen is needed, and it is difficult to obtain this amount of pollen; Use of SPE cartridges that raise the cost per analysis.
Wiest et al. [18]	2 g	Modified QuEChERS[c]	GC-TOF-MS[d] and HPLC–MS/MS[e]	3.0–70.4 ng g^{-1}	**Cons:** A large amount of pollen is needed, and it is difficult to obtain this amount of pollen; The use of HPLC leads to longer run times.
Dively and Kamel [25]	3 g	Modified QuEChERS	UHPLC–MS/MS	0.2 ng g^{-1} (LOD)	**Pros:** Low limit of quantification. **Cons:** A large amount of pollen is needed, and it is difficult to obtain this amount of pollen.
Chen et al. [26]	2 g	Modified QuEChERS	HPLC–MS/MS	0.1–0.5 ng g^{-1}	**Pros:** Low limit of quantification using dry pollen. **Cons:** A large amount of pollen is needed, and it is difficult to obtain this amount of pollen. The use of HPLC leads to longer run times.
David et al. [27]	0,1 g	Modified QuEChERS	UHPLC–MS/MS	0.02–2.5 ng g^{-1}	**Pros:** Low limit of quantification using dry pollen; Low amount of pollen. **Cons:** Additional extraction step after d-SPE to extract the pesticides that were adhered to the sorbents. This step can lead to increased matrix effect and extract unwanted components, and increase sample preparation time
López-Fernández et al. [28]	5 g	SPE	UHPLC–MS/MS	0.4–4.3 µg kg^{-1}	**Pros:** Low limit of quantification. **Cons:** A large amount of pollen is needed, and it is difficult to obtain this amount of pollen; Use of SPE Cartridges that raise the cost per analysis
Sánchez-Hernández et al. [9]	2 g	Solvent extraction	UHPLC–MS/MS	2.0–4.0 µg kg^{-1}	**Pros:** Low limit of quantification; **Cons:** A large amount of pollen is needed, and it is difficult to obtain this amount of pollen;
Codling et al. [7]	2 g	Modified QuEChERS	HPLC–MS/MS	0.1–4.0 ng ml^{-1}	**Cons:** A large amount of pollen is needed, and it is difficult to obtain this amount of pollen; The use of HPLC leads to longer run times.
Valverde et al. [29]	1 g	Modified QuEChERS	UHPLC-qTOF-MS[f]	6.0–12.0 µg kg^{-1}	**Pros:** Low limit of quantification **Cons:** A large amount of pollen is needed, and it is difficult to obtain this amount of pollen; High-resolution MS is less common and more expensive than the MS triple quadrupole, making it less accessible for acquisition and application in routine analysis.
Moreno-González et al. [8]	0,1 g	miniaturized QuEChERS	nanoLC-HR-MS[g]	500 ng kg^{-1}	**Pros:** Low limit of quantification using dry pollen; Low amount of pollen. **Cons:** Nano LC equipment is less common and more expensive than the UHPLC system, making it less accessible for acquisition and application in routine analysis. High-resolution MS is less common and more expensive than the MS triple quadrupole, making it less accessible for acquisition and application in routine analysis.
Garcia-Valcarcel et al. [30]	1 g	Modified QuEChERS	HPLC–MS/MS	1.0–2.0 ng g^{-1}	**Pros:** Low limit of quantification; **Cons:** A large amount of pollen is needed, and it is difficult to obtain this amount of pollen; The use of HPLC leads to longer run times.
Wood et al. [31]	3 g	Modified QuEChERS	UHPLC–MS/MS	0.33 ppb	**Pros:** Low limit of quantification using; **Cons:** A large amount of pollen is needed, and it is difficult to obtain this amount of pollen
Hall et al. [32]	0,2 g	dSPE[h]	UHPLC–MS/MS	0.4–1.0 ng g^{-1} (LOD)	**Pros:** Low limit of quantification; Low amount of pollen. **Cons:** The absent of the extraction stage assisted by salting out may reduce insecticides recovery. dSPE is efficient for the elimination of interfering, requiring a previous extraction step, such as extraction with acetonitrile
Tu et al. [33]	1 g	Salting-Out Assisted Liquid–Liquid Extraction Combined with DPX[i]	HPLC-DAD[j]	300.0 µg kg^{-1}	**Pros:** DAD detector is more common and cheaper than mass spectrometers **Cons:** A large amount of pollen is needed, and it is difficult to obtain this amount of pollen; Use of DPX pipette that raise the cost per analysis.
Wen et al. [34]	5 g	Modified QuEChERS	UHPLC–MS/MS	0.6–10.0 µg kg^{-1}	**Pros:** Low limit of quantification. **Cons:** A large amount of pollen is needed, and it is difficult to obtain this amount of pollen;

[a] SPE—Solid phase extraction.
[b] UHPLC–MS/MS—Ultra-high performance liquid chromatography coupled to tandem mass spectrometry.
[c] QuEChERS—Quick, Easy, Cheap, Effective, Rugged and Safe.
[d] GC-TOF-MS—Gas chromatography coupled to time-of-flight mass spectrometry.
[e] HPLC- MS/MS—High-performance liquid chromatography coupled to tandem mass spectrometry.
[f] UHPLC-qTOF-MS—Ultra-high performance liquid chromatography quadrupole time-of-flight mass spectrometry.
[g] nanoLC-HR-MS—Liquid chromatography high-resolution mass spectrometry.
[h] dSPE—Dispersive solid phase extraction.

[i] DPX—Disposable pipette extraction.
[j] HPLC-DAD—High-performance liquid chromatography diode array detector.

Table 3
Pesticide concentrations in pollen samples from the experimental field and commercial sample.

Concentration (μg kg^{-1})			
Pesticide	Treatment 1	Treatment 2	Commercial
Abamectin	ND[a]	ND	< LOQ[b]
Azoxystrobin	ND	ND	ND
Carbendazin	ND	ND	ND
Clothianidin	67 (±6)	64 (±6)	< LOQ
Imidacloprid	< LOQ	146 (±4)	< LOQ
Thiamethoxam	826 (±3)	673 (±3)	< LOQ

[a] ND—Not detected.
[b] LOQ—Limit of quantification.

Method application to commercial and experimental field samples

Table 3 shows the results for orange flower samples collected on different days and by different groups of people in the Embrapa during experimental field trials (Treatment 1 and Treatment 2), as well as a store-bought sample.

In the experimental field of Embrapa (Treatments 1 and 2), the pesticides abamectin, azoxystrobin, and carbendazim were applied only in the preparation and maintenance of the field, before carrying out the experiments. For treatment 1, the presence of the pesticide thiamethoxam was expected because it was applied in the field; the presence of clothianidin can be explained because it is a metabolite generated from thiamethoxam. For treatment 2, only the presence of imidacloprid was expected; however, the three neonicotinoids were found, and their presence can be attributed to a drift or improper application of pesticides, among other factors. In other works, residues of neonicotinoids were also found. Codling et al. [10] analyzed samples from the apiary, and some samples presented values of 912 μg kg^{-1} imidacloprid and 53 μg kg^{-1} thiamethoxam. According to the European Union Pesticides Database [35] maximum residue limit (MRL) values for honey range from 0.01 to 1 mg/kg, but no MRLs apply to other apicultural products. To this end, EU 62/2018 [36] specifies that MRLs for honey should not be extended to other apiculture products until each unique product within this category has been identified and included in the list, taking into account their distinct chemical features. Carrera et al. [37] compared the concentration of pesticides found in the analyzed bee pollen samples with the MRLs reported on the European Union Pesticide Database for honey and other beekeeping products since there was a lack of specific MRLs for bee pollen to perform a tentative risk assessment, as both products share the same source and some chemical characteristics.

In this study, it was possible to reduce the amount of sample needed for the analysis of pesticides, with a 100-fold reduction in sample volume compared to conventional techniques. Additionally, it was possible to develop a robust method capable of detecting low concentration limits. In Brazil, there is no specific legislation for pesticides on pollen, but their presence at the levels found in this work may indicate a risk to the health of bees. For this reason, with the help of the developed methodology, new studies can be carried out to understand the translocation of neonicotinoids and their impacts on the health of bees, assisting in the elaboration of legislation for pollen.

CRediT authorship contribution statement

Gabriela Brito Almeida: Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jordana Alves Ferreira:** Writing – original draft, Validation, Methodology, Investigation. **Robson R.M. Barizon:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Sonia C.N. Queiroz:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Carla Beatriz Grespan Bottoli:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.greeac.2025.100223.

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