


## Pesticide residues in honey and wax from *Apis mellifera* bees from municipalities of the Yucatán Peninsula



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### Abstract:

Bees as pollinators are crucial for global crop production and for maintaining ecosystem biodiversity. Nevertheless, like other pollinators, they are threatened by various factors, among which the indiscriminate use of pesticides stands out. These are substances that, depending on the dose acquired, could affect the foraging behavior, longevity, and learning

of bees, and can accumulate in the products of the hive. Studies on the presence of pesticides in honey and wax in the Yucatán Peninsula are limited. For this reason, this research aimed to identify and quantify the presence of pesticides in honey and wax from honeybee colonies in municipalities of the Yucatán Peninsula. Between June and October 2023, a total of 31 samples of honey and 31 of wax were collected in the three states of the Yucatán Peninsula, from apiaries near agricultural or fruit crops and apiaries located in areas of primary vegetation. The samples were analyzed by gas chromatography with triple quadrupole mass spectrometry (GC-MS/MS) detection. The honey had traces of two pesticides, whereas residues of 11 pesticides were identified in the wax. The pesticides with the highest frequency and quantity were cis-1,2,3,6-tetrahydrophthalimide and coumaphos. It is concluded that honeycomb wax presented a greater quantity and diversity of pesticide residues, mainly those samples collected in apiaries near agricultural crops.

**Keywords:** Identification and quantification, Colony collapse, Agricultural crops, Native vegetation.

Received: 16/08/2024

Accepted: 08/07/2025

## Introduction

Worldwide, Mexico stands out among the largest honey-exporting countries, ranking seventh as a producer, with 58,033 ton of honey annually<sup>(1)</sup>. The Yucatán Peninsula, which includes the states of Campeche, Quintana Roo, and Yucatán, is nationally the primary region engaged in beekeeping and honey production<sup>(2)</sup>. It stands out for its exuberant vegetation and diversity of vegetable, herbaceous, shrubby, and tree species, both native and introduced, which bloom at different times of the year, allowing bees to make honey of excellent quality, flavor, and aroma. Approximately 95 % of the region's honey production is destined for the international market, and due to its characteristics, it is one of the most consumed honeys in Europe and the United States<sup>(3)</sup>.

Honey is a natural product composed of monosaccharide sugars, predominantly fructose and glucose. It also contains, in smaller quantities, other substances such as organic acids, enzymes, and solid particles derived from the collection. The color of honey can range from colorless to dark brown. Its consistency is fluid, viscous, and totally or partially

crystallized<sup>(4)</sup>. The taste, aroma, and composition of honey will depend on the floral origin and the climatic conditions in which the plants develop<sup>(5)</sup>.

There are health regulations designed to control the presence of chemical residues in foods, such as honey, that put the consumer's health at risk; therefore, all foods must be free of any type of chemical residue or have permitted levels or Maximum Residue Limits (MRLs). Honey and other hive products may contain substances classified as harmful to human health, such as pesticides, which also influence the health of honeybee colonies<sup>(6)</sup>. In recent years, this topic has been of interest, as the presence of pesticide residues of 173 different compounds has been identified in samples of bee products (honey and wax)<sup>(7)</sup>. Pesticides can reach hive products, such as honey and wax, through several routes, including the exposure of honeybees through contact with the drift of sprays or contaminated dust, and by ingestion of residues in vegetation and water bodies<sup>(8)</sup>.

During flights in search of food resources, bees present in agricultural and industrial ecosystems tend to be in contact with the largest amount of pesticide residues on the flowers of such crops; likewise, pesticides can be found in the surrounding natural bloom and therefore be brought to the hive, so that they are present in honey, wax, and other products of the hive<sup>(9)</sup>.

In the last century, a transformation in the earth's surface has been caused worldwide due to human activity and intensive agriculture<sup>(10)</sup>; in this sense, in the last decade, due to agricultural activities and human development in the Yucatán Peninsula, natural and semi-natural habitats have been reduced, generating fragmentation and significantly affecting the diversity and floral abundance of the tropical rainforest<sup>(11,12)</sup>, which results in an impact on the abundance and health of bees<sup>(10)</sup>.

From 2016 to date, in the states of the Yucatán Peninsula, beekeepers and the collective "Mayan Alliance for the Bees in the Yucatán Peninsula" have reported a massive loss of bee colonies, due to the indiscriminate use of pesticides applied to soybean, sorghum, watermelon, melon, and chili crops and deforestation, affecting the beekeeping activity of the region<sup>(13)</sup>. Due to the lack of studies on the detection of pesticides in honey and wax samples, the purpose of this research was to identify and quantify the presence of pesticides in honey and wax in honeybee colonies in municipalities of the Yucatán Peninsula.

## Material and methods

### Sampling areas

The study was conducted in the Yucatán Peninsula, which encompasses the states of Campeche, Yucatán, and Quintana Roo, in Mexico. The sampling areas were selected through field reconnaissance tours to identify the following two types of vegetation. 1) Primary and acahual vegetation (PV): areas that have not experienced significant changes due to human activities or natural disturbances; these areas have a vegetation cover with a structure and floristic composition representative of the type of forest in the region. 2) Secondary vegetation or fruit plantations and agricultural crops (SV): areas where natural vegetation has been modified or altered by human or natural factors for the establishment of fruit plantations and agricultural crops. For PV, apiaries located within a 5 km radius were selected, ensuring the absence of agricultural or fruit crops in the vicinity. As for SV, apiaries were located within a radius of no more than 2 km, maintaining the characteristics mentioned above. This criterion was based on studies that proved that bees generally forage within a radius of up to 2 km from their hive<sup>(14)</sup>. After selecting the type of vegetation, beekeepers were contacted to obtain their consent to collect samples from their apiaries, and that they had a minimum of 10 hives.

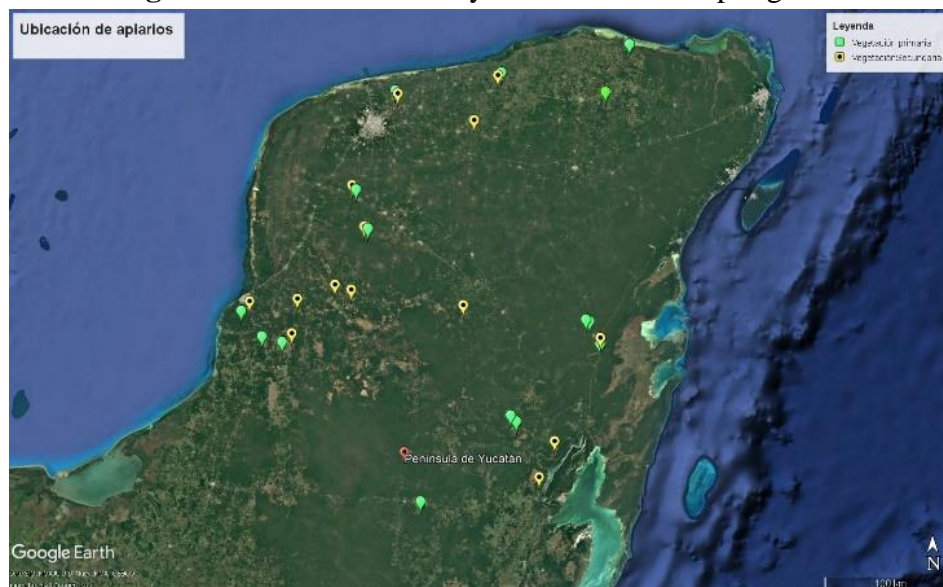
### Sampling

Between June and October 2023, 31 samples of honey and 31 of wax were collected in various apiaries of the Yucatán Peninsula. The distribution of the samples by state and type of vegetation was as follows: 1) Campeche: five samples of honey and four of wax from PV; five samples of honey and six of wax from SV. 2) Yucatán: five samples of honey and wax in PV; six samples of honey and wax from SV. 3) Quintana Roo: five samples of honey and four of wax from PV; five samples of honey and six of wax in SV.

To collect the samples, a disposable cutter was used to cut a piece of honeycomb with honey and another with wax, measuring approximately 12 cm<sup>2</sup>. The honey was deposited in Eppendorf conical centrifuge tubes with a capacity of 14 ml, and the wax in plastic bags. Each sample was identified by its origin and type of vegetation, and each apiary was georeferenced by GPS (Figure 1).

Once the honey and wax samples were collected from the apiary, they were stored under freezing conditions at  $-20\text{ }^{\circ}\text{C}$  until analysis by gas chromatography with triple quadrupole mass spectrometry (GC-MS/MS) detection. These samples were analyzed in the Analytical Services Laboratory of the Center for Research and Assistance in Technology and Design of the State of Jalisco, A. C. (CIATEJ, for its acronym in Spanish) at its Northeast headquarters.

**Figure 1:** Location of honey and beeswax sampling sites



Yellow, apiaries near secondary vegetation. Green, apiaries near primary vegetation.

## Analysis of pesticide residues in honey and wax

### Chemicals and solutions

A total of 75 pesticides were analyzed, which were available in the laboratory at the time. The following pesticide analytical standards were obtained from ChemService, Inc. (West Chester, PA, USA): 2,4'-DDT, 2,4'-D 2-ethylhexyl ester, 2,4'-D isopropyl ester, heptachlor epoxide isomer B, bifenthrin, gamma-cyhalothrin, lambda-cyhalothrin, dieldrin, difenoconazole, diflubenzuron, paclobutrazol, pentachloroaniline, pyridaben, pyriproxyfen, tebuconazole, alpha-HCH. From Sigma-Aldrich-Fluka (St. Louis, MO, USA): 2-phenylphenol, aldrin, carfentrazone-ethyl, chlorfenvinphos, chlorpyrifos-ethyl, chlorpyrifos-methyl, coumaphos, deltamethrin, diazinon, dichlofenthion, dicofol, dimethoate, spiromesifen, ethion, fenclorophos, fipronil-sulfone, fipronil-sulfide, fipronil-desulfinyl, fludioxonil, flusilazole, fonofos, phthalimide, hexachlorobenzene, malathion, oxyfluorfen,

pendimethalin, pentachloroanisole, pentachlorophenol, permethrin, pertan, phenthoate, phorate, phosmet, profenofos, propetamphos, quinoxifen, pyridaphenthion, pyrimethanil, quinalphos, spirodiclofen, tebuthiuron, tecnazene, terbufos, trichlorfon, tetradifon, trifloxystrobin, triazophos. Cis-1,2,3,6-tetrahydrophthalimide, endosulfan I, endosulfan II, fenitrothion, parathion-ethyl, parathion-methyl, and triphenyl phosphate (internal standard) were acquired from Sigma-Aldrich-Supelco (Bellefonte, PA, USA). Fenthion and pirimiphos-methyl were obtained from Accustandard (New Haven, CT, USA) and ULTRA Scientific (N. Kingstown, RI, USA), respectively. Formic acid (MS grade) and ammonium formate (trace metal base) were acquired from Sigma-Aldrich. HPLC-grade acetonitrile and HPLC-grade water were purchased from Tedia High Purity Solvents (Fairfield, OH). The “Quick, Easy, Cheap, Effective, Rugged, and Safe” (QuEChERS) extraction salts (AOAC method) and SPE dispersion kits (Bond Elut) were purchased from Agilent Technologies (Santa Clara, CA, USA).

### **Sample preparation and extraction**

From each sample, 7 g of honey and 3 g of wax were taken. The extraction of pesticide residues was carried out according to a modification of the “QuEChERS” analytical method, previously validated in the Analytical Services Laboratory of the Northeast Headquarters of CIATEJ<sup>(15,16)</sup>.

### **Extraction of pesticides from honey**

In a 50 ml plastic centrifuge tube, 7 g of honey was weighed, and 10 mL of deionized water was added, and this was stirred manually for one minute. A total of 15 mL of acetonitrile acidified with 1 % acetic acid (v/v) was added, and this was manually stirred again for 1 min. Subsequently, 6 g of magnesium sulfate ( $\text{MgSO}_4$ ) and 1.5 g of sodium acetate ( $\text{CH}_3\text{COONa}$ ) were used; all samples were stirred for 1 min and centrifuged at 4,000 rpm for 5 min. To clean the extract, 8 mL of the supernatant was used and transferred to a 15 mL tube containing 400 mg of primary-secondary amine (PSA), 1,200 mg of  $\text{MgSO}_4$ , and 400 mg of extra clean octadecylsilane (C18-EC); this was stirred for 1 min and centrifuged at 4,000 rpm for 5 min.

### **Pesticide extraction in wax**

For its extraction, Niell *et al*'s method<sup>(17)</sup> was used. In a 50 mL plastic centrifuge tube, 3 g of wax was weighed, and 15 ml of acetonitrile acidified with 1 % acetic acid (v/v) was added. The tubes were kept in a water bath at 80 °C until the wax melted; once the wax was melted, they were stirred manually for 20 sec and placed back in the water bath to melt; the processes of melting and manual stirring were repeated three more times; the samples were kept at rest until they reached room temperature and were placed in a freezer at -20 °C for 2 h so that the wax precipitated. To clean the extract, 8 mL of the supernatant was extracted and transferred to a 15 mL tube with 400 mg of primary-secondary amine (PSA), 1,200 mg of MgSO<sub>4</sub>, and 400 mg of EC-C18, and this was stirred for 1 min and centrifuged at 4,000 rpm for 5 min.

### **Chromatography and detection conditions**

For gas chromatography (GC-MS/MS), a 7890A gas chromatograph, coupled to a 7000B triple quadrupole mass spectrometer with electron impact ionization (EI), was used, which was equipped with a 7693A autosampler (Agilent Technologies). Chromatographic separation was performed using two DB-5 MS ultra inter capillary columns (15 m × 0.250 mm × 0.25 µm film thickness; Agilent Technologies). A purged ultimate union was used to connect the two columns, and a flush was performed after each run. Two microliters of the supernatant extract were injected in splitless mode (5 min at 21.1 psi), with a constant flow of 1.0 mL/min (column 1) and 1.2 mL/min (column 2). High-purity helium was used as a carrier gas. The injector setting was 65 °C (hold 0.2 min) to 310 °C at 600 °C/min. The oven temperature was programmed from 60 °C (1 min) to 170 °C at 40 °C/min to 310 °C (4 min). The mass spectrometer was operated in electron impact ionization mode (ionization energy 70 eV), whereas the transfer line and ion source temperatures were set at 300 °C. For the selection and quantification of analyses, the selected ion monitoring (SIM) mode was used with a minimum of three ions for each analysis. The scan speed for each segment was set to approximately two scans to obtain a minimum of 10 data points per peak.

## **Results**

All honey samples from the state of Campeche showed traces of fipronil, both in primary vegetation (PV) and in secondary vegetation (SV). In the wax samples from PV, two pesticides were detected, from traces up to 0.02 mg/kg, and the samples from SV showed the

greatest diversity of pesticides (7), with the organophosphate coumaphos presenting concentrations of up to 1.133 mg/kg (Table 1).

In Yucatán, all the honey samples analyzed presented concentrations of 0.026 and 0.0014 mg/kg of cis-1,2,3,6-tetrahydrophthalimide in PV and SV, respectively. The highest diversity of pesticides (four) was present in the wax from PV, followed by SV with three pesticides (Table 1).

For the state of Quintana Roo, traces of fipronil were detected in the honey from PV and SV. For wax, two pesticides were detected in PV, and four in SV (Table 1).

## Discussion

The results obtained indicate that the presence of pesticides found in honey and beeswax is related to the region and type of vegetation where the samples were collected; these findings could indicate a significant risk factor for bee health<sup>(6)</sup>, and that contamination of honey and wax by pesticides likely occurred through two main routes: indirect route, which refers to the transport of toxic substances by foraging bees when collecting nectar, pollen, water, and resins. Direct route, considered the most important, involves the contamination of bee products with acaricides, antibiotics, and volatile pesticides, mainly as a result of beekeeping practices<sup>(18)</sup>. In this study, the detection of agricultural pesticides, such as fipronil and cis-1,2,3,6-tetrahydrophthalimide, together with the acaricide coumaphos (applied directly to the hive<sup>(19)</sup>) supports these routes.

A greater diversity and quantity of pesticides were observed in the wax; first, eight insecticides were found, followed by two fungicides and one acaricide. This is in contrast to honey, where an insecticide and a fungicide were found. These results coincide with those reported by Vargas-Valero *et al*<sup>(20)</sup> in the north-central region of Mexico, reporting a greater quantity and diversity of pesticides in wax. This difference could be attributed to the hydrophobic nature of most pesticides, which gives them high stability and facilitates their transfer to wax<sup>(21)</sup>; additionally, inadequate management practices, such as wax recycling and the prolonged permanence of honeycombs in hives, may contribute to the accumulation of these compounds.

## Primary vegetation

Traces and low levels of pesticides were detected in the honey and wax samples collected in the PV areas in the three states of the Yucatán Peninsula. The most frequent compounds were: fipronil, present in 63 % of the honey samples, cis-1,2,3,6-tetrahydrophthalimide, which is a metabolite derived from the fungicide captan and was detected in 32 % of the honey samples and 93 % of the wax samples, and coumaphos, which was found in 53 % of the total wax samples from PV (Table 1). When comparing these results with the MRLs established by the European Union<sup>(22)</sup>, it was observed that none exceeded the maximum limits for honey: fipronil 0.005 mg/kg, cis-1,2,3,6-tetrahydrophthalimide 0.05 mg/kg, and coumaphos 0.1 mg/kg. These results are similar to those published by Fluton *et al*<sup>(23)</sup>, who found an interaction between different types of landscapes (agricultural, urban, forest, and grasslands) and pesticide exposure. In the context of the present research, where apiaries in PV were specifically selected for the absence of agricultural/fruit crops, the presence of traces of fipronil and the metabolite of captan could be attributed to contamination of commercially acquired embossed wax used for constructing new honeycombs.

In the case of coumaphos, its presence could be due to the use of this acaricide by beekeepers to control the Varroa mite or the hive beetle. Likewise, factors such as low honeycomb replacement and wax recycling could contribute to the accumulation of this compound. In addition, the possibility that they have collected these pesticides during foraging is not ruled out, considering their ability to fly up to 14 km in search of food<sup>(24)</sup>. Since a definitive explanation for the presence of pesticide residues in PV samples has not yet been established, it is recommended to continue with long-term sampling and analysis.

## Secondary vegetation

Traces of fipronil were detected in honey samples from SV in the states of Campeche and Quintana Roo, and the metabolite of the fungicide captan derivative (cis-1,2,3,6-tetrahydrophthalimide) was detected in honeys from Yucatán (Table 1). As for the wax from the three states, the most frequent pesticides in the total samples were the captan metabolite (67 %), followed by the organophosphorus compound coumaphos (50 %). Coumaphos is a highly persistent, lipophilic synthetic insecticide and acaricide; it is distributed through trophallaxis and contact between bees, acting both through contact and systemically<sup>(25)</sup>. Despite not being authorized in Mexico for the treatment of Varroa in bees, some beekeepers from the Yucatán Peninsula use an unregistered product called “magic powder”, which contains coumaphos in concentrations of 639.55 and 900 mg/kg<sup>(19)</sup>. This powder is applied

to control the small hive beetle and the Varroa mite. The MRLs for coumaphos vary by legislation; it is 0.15 mg/kg in honey and 45 mg/kg in wax for the United States<sup>(26)</sup>; 0.1 mg/kg in honey for the European Union<sup>(22)</sup>, and 0.02 mg/kg in honey and 0.1 mg/kg in wax for Canada<sup>(27)</sup>. The results of this study showed that 63 % of the wax samples from SV contained coumaphos concentrations below the U.S. MRLs, except for one sample that reached 1,133 mg/kg. This concentration differs from what was found by Valdovinos-Flores *et al* (2016) and Fulton *et al* (2019), who reported values of 0.155 to 2.220 and 15.5 mg/kg, respectively<sup>(19,23)</sup>. The use of “magic powder” is suspected to continue in the region, contributing to the presence of coumaphos.

The reuse of wax and medication of colonies with coumaphos can also contribute to the accumulation of this pesticide in the wax, even contaminating virgin wax<sup>(28)</sup>. The accumulation of coumaphos residues in honeycomb wax could have implications for colony strength and survival. Therefore, studies are required to determine the concentration of pesticides in commercial wax in the region.

The lower amount and diversity of pesticides in PV compared to SV is possibly due to the smaller agricultural area near the PV apiaries. The planted areas vary between the states: Campeche (334,905.29 ha), Yucatán (706,130.06 ha), and Quintana Roo (127,604.11 ha)<sup>(29)</sup>. Campeche is the largest producer of soybeans in Mexico<sup>(29)</sup> and uses large amounts of pesticides, which may have possibly influenced the results, especially the presence of cis-1,2,3,6-tetrahydrophthalimide, which was found at high frequencies in SV wax. Captan is used in soybean, tomato, chili, and fruit crops in the region.

Fipronil was detected in trace amounts in SV honey from Campeche and Quintana Roo; it is a systemic insecticide highly toxic to bees, even in sublethal doses<sup>(30)</sup>. Its bioaccumulation capacity and enhanced toxicity over time pose a significant risk<sup>(31)</sup>. Due to the above, it is not ruled out that quantifiable levels can be found; therefore, it is necessary to continue with monitoring. Finally, the diversity and quantity of pesticides detected in honey and wax from the Yucatán Peninsula, compared to northern and central Mexico<sup>(19,20)</sup>, is probably due to a smaller area of agricultural crop areas, in addition to the fact that areas of native vegetation still predominate; however, it is necessary to continue the monitoring over the years to observe the behavior.

## Conclusions and implications

The highest concentration and diversity of pesticides were detected in beeswax collected in apiaries near agricultural and fruit crops. Additionally, the possible use of unauthorized

chemicals in Mexico for controlling the mite *Varroa destructor* and the hive beetle was identified. Regarding honey, the detection of traces of pesticides suggests the need for additional studies to monitor their presence in quantifiable amounts. According to the results, these were lower compared to the north of the country; this is possibly due to the lower density of agricultural crops, and despite deforestation, there are still large areas with the presence of native vegetation in the region where the study was conducted. It is crucial to continue monitoring the presence of pesticides in hive products, expanding the study area and the number of samples in the region and nationally. It is recommended to carry out sampling at different times of the year to determine if the amount and diversity of pesticides found are related to agricultural activities in each region of the country.

### **Acknowledgements and conflict of interest**

The authors wish to express their deep gratitude to beekeepers in the states of Campeche, Quintana Roo, and Yucatán for their valuable collaboration and for facilitating access to their apiaries for the collection of samples. They also thank the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP, for its acronym in Spanish) for the funding it made possible through the project: 1311935685 Presence of pesticides in honey, pollen, and wax from bee colonies in the Yucatán Peninsula.

The authors declare that they have no conflict of interest.

**Table 1:** Concentration of pesticides (mg/kg) found in honey and wax from honeybee colonies collected in different types of vegetation in the states of the Yucatán Peninsula

	Pesticide	Toxicological group	Class	Samples analyzed	Positive samples (%)	Concentration (mg/kg)	
						Min	Max
<b>PV:</b>							
	Honey	Fipronil	Phenylpyrazole	Insecticide	5	5 (100)	< 0.005
	Wax	Cis-1,2,3,6-tetrahydrophthalimide	Captan metabolite	Fungicide	4	3 (75)	< 0.005 0.010
		Coumaphos	Organophosphorus	Insecticide/acaricide	4	2 (50)	< 0.005 0.026
<b>SV:</b>							
Campeche	Honey	Fipronil	Phenylpyrazole	Insecticide	5	5 (100)	< 0.005
		Coumaphos	Organophosphorus	Insecticide/acaricide	6	3 (50)	< 0.005 1.133
		Cis-1,2,3,6-tetrahydrophthalimide	Phthalimide	Fungicide	6	2 (33)	< 0.005 0.081
	Wax	Alpha-BHC	Lindane isomer	Insecticide	6	1 (17)	< 0.005
		Dieldrin	Organochlorine	Insecticide	6	1 (17)	< 0.005
		Boscalid	Chlorinated	Fungicide	6	1 (17)	< 0.005
		Pentachloroanisole	Pentachlorophenol	Insecticide/fungicide	6	2 (33)	< 0.005
		Acrinathrin	Pyrethroid	Insecticide/acaricide	6	2 (33)	< 0.005
<b>PV:</b>							
Yucatán	Honey	Cis-1,2,3,6-tetrahydrophthalimide	Captan metabolite	Fungicide	5	5 (100)	< 0.005 0.026
		Cis-1,2,3,6-tetrahydrophthalimide	Captan metabolite	Fungicide	5	5 (100)	< 0.005 0.022
	Wax	Coumaphos	Organophosphorus	Insecticide/acaricide	5	3 (60)	< 0.005 0.067
		Pentachloroanisole	Pentachlorophenol	Insecticide/fungicide	5	3 (60)	< 0.005
		Dicofol	Organochlorine	Insecticide/acaricide	5	1 (20)	< 0.005

		<b>SV:</b>						
<b>Quintana Roo</b>	Honey	Cis-1,2,3,6-tetrahydrophthalimide	Captan metabolite	Fungicide	6	6 (100)	< 0.005 0.014	
		Cis-1,2,3,6-tetrahydrophthalimide	Captan metabolite	Fungicide	6	4 (67)	< 0.005 0.022	
	Wax	Coumaphos	Organophosphorus	Insecticide/acaricide	6	3 (50)	< 0.005 0.051	
		Pirimiphos-methyl	Organophosphorus	Insecticide/acaricide	6	1 (17)	< 0.005	
			<b>PV:</b>					
	Honey	Fipronil sulfide	Fipronil metabolite	Insecticide		5 (100)	< 0.005	
Wax	Cis-1,2,3,6-tetrahydrophthalimide	Captan metabolite	Fungicide		3 (75)	< 0.005 0.00		
	Coumaphos	Organophosphorus	Insecticide/acaricide		4 (100)	< 0.005 0.023		
		<b>SV:</b>						
Honey	Fipronil	Phenylpyrazole	Insecticide		5 (100)	< 0.005		
	Fipronil sulfone	Fipronil metabolite	Insecticide		1 (20)	< 0.005		
Wax	Coumaphos	Organophosphorus	Insecticide/acaricide		3 (50)	< 0.005 0.010		
	Trans-permethrin	Pyrethroid	Insecticide		1 (17)	< 0.005 0.006		
	Cis-1,2,3,6-tetrahydrophthalimide	Captan metabolite	Fungicide		6 (100)	< 0.005 0.011		
	Fipronil-sulfone	Fipronil metabolite	Insecticide		1 (17)	< 0.005		

PV= primary vegetation; SV= secondary vegetation.

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