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Pesticides residues in honey and wax from bee colonies in La Comarca Lagunera

Residuos de plaguicidas en miel y cera de colonias de abejas de La Comarca Lagunera

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ABSTRACT

Honeybees are important for food security and biodiversity preservation. There has been a collapse of the colonies caused by exposure to pesticides. The aim was to determine and quantify the presence of pesticides in honey and wax from bee colonies, under collapse (BC) and with (CA) and without antecedent of collapse (SA). Five honey samples and five colony wax samples were analyzed from colonies CA y SA, as well as two of honey and two wax from colonies CB; samples were analyzed by LC-QTOF and GC-MS/MS. 24 pesticides were detected in honey and wax analyzed. Acetamiprid was found in all samples. In colonies, CB the wax and honey had high averages levels of acetamiprid (0.402 and 0.633 mg kg-1 respectively). For wax and honey from colonies CA, the averages of acetamiprid were 0.686 and 0.266 mg kg-1 respectively. In wax and honey from colonies SA the averages of acetamiprid were 0.234 and 0.404 mg kg-1 respectively. In conclusion, the colonies CA had the greatest diversity of pesticides, followed by the group SA and finally BC. Our results suggest the participation of pesticides as a cause of colony collapse.

Keywords: *Apis mellifera*, Colony Collapse Disorder, México and QuEChERS.

RESUMEN

Las abejas melíferas son importantes para la seguridad alimentaria y el mantenimiento de la biodiversidad. Se ha presentado un colapso de las colonias ocasionado por la exposición a plaguicidas. El objetivo fue determinar y cuantificar la presencia de plaguicidas en miel y cera de colonias de abejas bajo colapso (BC), con (CA) y sin antecedentes de colapso (SA). Se analizaron cinco muestras de miel y cinco de cera de colonias CA y SA, así como dos de miel y de cera BC; las muestras fueron analizadas por LC-QTOF y GC-MS/MS. Se detectaron en total 24 plaguicidas en miel y cera. El acetamiprid se encontró en el 100% de las muestras. En las colonias BC, presentaron en promedio altos niveles de acetamiprid en cera y miel (0.402 y 0.633 mg kg^{-1} respectivamente). Para las colonias CA, los promedios de acetamiprid fueron 0.686 y 0.266 mg kg^{-1} para cera y miel respectivamente, en las colonias SA, los promedios del acetamiprid en cera y miel fueron 0.234 y 0.404 mg kg^{-1} respectivamente. En conclusión, las colonias CA presentaron la mayor diversidad de plaguicidas seguidas por SA y BC. Estos resultados podrían sugerir la participación de los plaguicidas como causa del colapso de las colonias.

Palabras clave: *Apis mellifera*, Desorden del Colapso de las Colonias, México y QuEChERS.

INTRODUCTION

Honeybees are important pollinators of a wide diversity of native crops and plants, the pollination carried out by these insects is necessary for 35% of crops intended for human consumption (Ollerton, 2017). However, worldwide they have suffered a decrease in their populations, a phenomenon called Colony Collapse Disorder (CCD) (Brutscher *et al*., 2016). The characteristics of the collapsed colonies are: the partial or total death of the colony with the presence of dead bees in or near the hive, the partial or total disappearance of the colony, with the abandonment of food reserves and the young and weakening of the colony through slow development during spring, under optimal conditions (Simon-Delso *et al*., 2014).

Although various factors have been reported such as the probable causes of colony collapse, one of the most important is exposure to pesticides (Calatayud-Vernich *et al*., 2018; Sánchez-Bayo *et al*., 2016; Traynor *et al*., 2016). Bees are exposed to pesticides when they search for nectar-polliniferous resources, especially if the colonies are located near agricultural areas (O'Neal *et al*., 2018). Exposure to sub-lethal doses of pesticides has been shown to affect bee behavior (Balbuena *et al*., 2015), foraging (Cresswell y Thompson, 2012), its longevity (Wu *et al*., 2011), thermoregulation (Tosi *et al*., 2016); as well as their olfactory learning and memory (Lu *et al*., 2014).

Pesticide residues can accumulate on bee bread, honey, and wax (Johnson *et al*., 2010; Lozano *et al*., 2019); presenting in the latter the residual storage capacity of pesticides (Benuszak *et al*., 2017). Therefore, from a contaminated honeycomb, the residues can be transferred to the stored honey, presenting a risk to consumers. Also, the consumption of "honey in honeycomb", as a food additive in fruit treatment, food supplement or as a flavoring, represents a health risk (Wilmart *et al*., 2016).

In the semi-desert of northern Mexico, the presence of pesticides in low concentrations has been found in honey and wax samples (Alcántar-Rosales *et al*., 2016), and a decrease of up to 2010 has been reported for the period from 2010 to 2017. 35% of bee colonies (SIAP, 2018).

Therefore, the objective of this research was to determine and quantify the presence of pesticides in honey and wax samples from bee colonies, with and without a history of collapse.

MATERIAL AND METHODS

Sampling of the hives´ matrices.

The sampling was carried out in the semi-desert of northern Mexico (25º 05 'and 26º 54' LN and 101º 40 'and 104º 45' LO), between the months of June to September 2017, based on the pattern of beekeepers registered in the Beekeeping Product System Committee of the Lagunera Region A.C, under the criterion of having at least 10 colonies; 43

beekeepers were selected, of which, by means of a random sampling of 20% of the colonies of each apiary and under natural conditions. A total of 132 honeycomb samples with honey and wax of approximately 12 cm^2 were obtained; Of these, 12 samples were randomly selected for analysis of pesticides, of which five were classified as coming from colonies with a history of collapse (CA); five as asymptomatic or from colonies with no history of collapse (SA), and two samples of apiaries that at the time of sampling suffered collapse (BC). The classification was made according to the data obtained from the beekeeper and based on the criteria defined by Simon-Delso *et al*. (2014).

With a disposable cutter, a piece of honeycomb with honey and wax of approximately 12 cm^2 was cut, placed in a plastic bag with their respective identification and later they were transported to the Biology laboratory of the Autonomous Agrarian University Antonio Narro, Laguna Unit in Torreón, Coahuila, to store them at -20 °C, until their analysis in the laboratory of the Center for Research and Assistance in Technology and Design of the Jalisco State, A.C (CIATEJ), Apodaca, Nuevo León. 165 pesticides were determined for each of the samples (honey and wax).

Chemical products and solutions. Pesticide analytical standards were obtained from ChemService, Inc. (West Chester, PA, USA): Sigma-Aldrich-Fluka (St. Louis, MO, USA), Sigma-Aldrich-Supelco (Bellefonte, PA, USA), Accustandard (New Haven, CT, USA), and ULTRA Scientific (N. Kingstown, RI, USA). Formic acid (MS grade) and ammonium formate (trace metal base) were purchased from Sigma-Aldrich. HPLC grade Acetonitrile and HPLC grade water were purchased from Tedia High Purity Solvents (Fairfield, OH). The extraction salts "Quick, Easy, Cheap, Effective, Rugged, and Safe" (QuEChERS) (AOAC Method) and the dispersion SPE kits (Bond Elut), were purchased from Agilent Technologies (Santa Clara, CA, USA).

Preparation and extraction of samples. 7 g of honey and 3 g of wax were taken from each sample, which were previously thawed at room temperature. The extraction of pesticide residues was carried out according to a modification of the analytical method “QuEChERS” (Valdovinos-Flores *et al*., 2017), previously validated in the Analytical Services Laboratory of the Northeast Headquarters of CIATEJ. This method consists of two steps: (1) the separation of pesticides from the matrix with acetonitrile, and (2) the cleaning extract. Matrix-matched calibration curves were used, the analytes and the internal standard were added after weighing the samples, before adding solvents. 300 µL of extract was transferred to 2 mL vials. A sample was injected into a Liquid Chromatography system, coupled to a time-of-flight mass spectrometer (LC-QTOF); and another on a Gas Chromatograph coupled to a Triple Quadrupole Mass Spectrometer (GC-MS/MS).

Extraction in honey. According to the "QuEChERS" analytical method modified by Valdovinos-Flores *et al*. (2017), in a 50 mL plastic centrifuge tube, 7 g of honey were weighed, to which 10 mL of deionized water were added; Samples were shaken manually for one minute, 15 mL of 1% acetonitrile acidified with acetic acid (v/v) was added and stirred again for 1 min. Subsequently, 6 g of $MgSO_4$ and 1.5 g of sodium acetate were used. All samples were shaken for 1 min and centrifuged at 4000 rpm for 5 min.

To clean the extract, 8 mL of the supernatant were used, and transferred to a 15 mL tube, with 400 mL of primary-secondary amine (PSA), 1200 mg of $MgSO_4$ and 400 mg of EC-C18; they were stirred for 1 min and centrifuged at 4000 rpm for 5 min.

Wax extraction. For the extraction of this matrix, the method of de Niell *et al*. (2014). 3 g of wax were weighed into a 50 mL plastic centrifuge tube, 15 mL of acetonitrile acidified 1% with acetic acid (v/v) were added. The tubes were placed in a water bath at 80 °C until the wax melted. Once the wax was melted, they were stirred for 20 sec. and they were placed again in the bathroom so that it melts. The casting and stirring process was repeated three more times. The samples were placed at room temperature and then kept in a freezer at -20 °C for two hours.

To clean the extract, 8 mL of the supernatant was extracted and transferred to a 15 mL tube, with 400 mL of primary-secondary amine (PSA), 1200 mg of $MgSO_4$ and 400 mg of EC-C18. They were stirred for 1 min. and centrifuged at 4000 rpm for 5 min.

Liquid chromatography coupled to a time-of-flight mass spectrometer (LC-QTOF). For LC analysis, an Agilent 1200 series HPLC system (Agilent Technologies) was used, with a binary pump coupled to a G6530A Q-TOF mass spectrometer (Agilent Technologies). Chromatographic separation was achieved using an Eclipse Plus C18 column (100 mm x 2.1 mm x 1.8 µm, Agilent Technologies). The mobile phases consisted of water with 0.01% formic acid+10 mM ammonium format (Solvent A) and methanol with 0.01% formic acid+10 mM ammonium format (Solvent B).

Injection was performed using an autosampler solution, in which 3 µL of extract were mixed with 15 µL of solvent A. The elution gradient was as follows: 20-50% B at 0-3.5 min, 50-90% B at 3.25-8.81 min, 90-100% B at 8.81-10 min, 100% B at 10-12.8 min and rebalancing at initial conditions from 12.9 min to 18 min. For the mass spectrometry analysis, an Agilent Jet-Stream electrospray ionization source was used, operating in positive ionic mode, with the following operating parameters: TOF MS acquisition mode, acquisition range of 50–950 m/z, N_2 at 180 ˚C and 13 L/min as drying gas, nebulizer pressure at 40 psi, nozzle voltage at 0 V, sheath gas at 300 ˚C and 10 L/min, capillary voltage at 4000 V , skimmer voltage at 65 V, fragmentation voltage at 150 V, octapole RF at 750 V. Agilent Mass Hunter, Workstation was used for data acquisition and analysis.

Gas chromatography coupled to a triple quadrupole mass spectrometer (GC-MS/MS). For gas chromatography, a 7890A gas chromatograph was used, coupled to a 7000B triple quadruped mass spectrometer, with electron impact ionization (EI), equipped with a 7693A auto-sampler (Agilent Technologies). Chromatographic separation was performed using two ultra-interesting DB-5 MS capillary columns (15m × 0.250mm × 0.25 µm film thickness; Agilent Technologies). A purged final joint was used to connect the two columns, and a wash was performed after each run. 2 µL of the extract was injected in an undivided 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 configuration was 65 ˚C (contain 0.2 min) at 310 ˚C at 600 ˚C/min. The oven temperature was programmed from 60 ˚C (1 min) to 170 ˚C at 40 ˚C/min at 310 ˚C (4 min). The mass spectrometer was operated in the electron impact ionization mode (70 eV ionization energy); while the transfer line and the ion source temperatures were set at 300 ˚C.

Ion monitoring mode (SIM) was used for the selection and quantification of analyzes, selected with a minimum of three ions for each analysis. The scanning speed for each segment was established in approximately two scans, in order to obtain a minimum of 10 data points per peak.

RESULTS

Table 1. Pesticides found in wax and honey samples in colonies of honey bees under collapse (BC), with a history of collapse (CA) and without a history of collapse (SA), by LC-QTOF and GC-MS/MS

Group	Type of simple	Insecticides	Fungicides	Acaricides	Herbicides	Total
BC	Wax	4	1	0	0	5
	Honey	2	1	0	0	3
CA	Wax	13	4	1	1	19
	Honey	5	2	0	0	7
SA	Wax	11	4	1	1	17
	Honey	5	0	0	0	5
	BC	6	2	0	0	8
	CA	18	6	1	1	26
	SA	16	4	1	1	22

Pesticides detected in wax and honey from colonies BC, CA and SA. In wax, a greater diversity of pesticides (insecticides, fungicides, acaricides and herbicides) was found, compared to the honey samples (insecticides and fungicides). On the other hand, the greatest number of pesticides by groups was found in colonies with a history of collapse (20), followed by colonies without a history (19) and finally with those under collapse (7). This same behavior was observed when separating pesticides by category, finding in all cases a greater diversity in CA, followed by SA and finally by BC (Table 1).

Regarding the amount of pesticides, the insecticide acetamiprid was the only one detected in all the wax samples analyzed and was present in a greater amount in the colonies CA (0.686 mg kg^{-1}), followed by BC (0.402 mg kg^{-1}), and finally by SA (0.234 mg kg^{-1}) (Table 2). This same insecticide was present in all the honey samples, but in greater quantity in the BC colonies (0.633 mg kg^{-1}), followed by the SA (0.404 mg kg^{-1}), and finally the CA (0.266 mg kg^{-1}) (table 3).

On the other hand, the insecticide malathion was found in 11 wax samples from the colonies CB, CA and SA; however, the amounts were low. It should be added that cis permethrin was found only in six wax samples CA (0.087 mg kg^{-1}) and SA (0.002 mg kg^{-1}) (Table 2).

Table 2. Average of pesticides (mg kg^{-1}) in wax from colonies of honey bees under (BC), with (CA) and without (SA) history of collapse, detected by LC-QTOF and GC-MS/MS

Plaguicide / Sample	BC		CA					SA					Positives	BC (x̄)	CA (x̄)	SA (x̄)
	1	2	1	2	3	4	5	1	2	3	4	5				
A	0.569	0.235	1.716	0.062	0.64	0.735	0.28	0.198	0.072	0.311	0.35	0.237	12	0.402	0.686	0.234
	CL	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**	**CL**				
B			0.009	0.032	T	0.008							4			
			CL	**CL**	**CL**	**CL**										
C								0.006					1			
								CL-CG								
D			0.025	0.024	0.023	0.025	0.025		0.024	0.021	0.023	0.023	9		0.025	0.023
			CL-CG	CL-CG	CL-CG	CL-CG	CL-CG		CL-CG	CL-CG	CL-CG	CL-CG				
E							T						1			
							CL									
F			0.018		0.009		0.008	0.006				0.008	5		0.012	0.007
			CL		**CL**		**CL**	**CL**				**CL**				
G	0.004		0.004		0.007		0.006	0.01	0.022	0.025			7		0.006	0.019
	CL-CG		CL-CG		CL-CG		CL-CG	CL-CG	CL-CG	CL-CG						
H						T				T			2			

						CL				**CL**						
I			0.011	0.013	0.059	T	0.02		0.004	0.01	0.008	0.003	9		0.026	0.006
			CL-CG	CL-CG	CL-CG	CL-CG	CL-CG		CL-CG	CL-CG	CL-CG	CL-CG				
J					0.116			0.052					2			
					CL			**CL**								
K			T				T						2			
			CL				**CL**									
L	T	0.005	0.034	0.019	0.008	0.041	0.006	0.008	0.008		0.009	T	11	0.005	0.022	0.008
	CL-CG	CL-CG	CL-CG	CL-CG	CL-CG	CL-CG	CL-CG	CL-CG	CL-CG		CL-CG	CL-CG				
M			0.015	0.018	0.007	0.013	0.014			0.028	0.015	0.01	8		0.013	0.018
			CL	**CL**	**CL**	**CL**	**CL**			**CL**	**CL**	**CL**				
N						0.007				0.007			2			
						CL				**CL**						
Ñ						T		T					2			
						CL		**CL**								
O	0.006		T	0.004	T		T		T	T	T	T	9			
	CL-CG		CL-CG	CL-CG	CL-CG		CL-CG		CL-CG	CL-CG	CL-CG	CL-CG				
P			0.323	0.012	0.002		0.01		0.001	0.003			6		0.087	0.002
			CL-CG	CL-CG	CL-CG		CL-CG		CL-CG	CL-CG						
Q			0.206	0.007	0.001	T	0.003		0.001	0.001			7		0.054	0.001
			CL-CG	CL-CG	CL-CG	CL-CG	CL-CG		CL-CG	CL-CG						
R			T				T						2			
			CL				**CL**									
S						0.005							1			
						CL										
T		T						T					2			
		CL						**CL**								
U								0.009					1			
								CL-CG								

A: Acetamiprid**, B:** Carbendazim**, C:** Carfentrazone ethyl**, D:** Chlorpyrifos**, E:** Chlorantraniliprole**, F:** Coumaphos**, G:** Deltamethrin**, H:** Dimethoate**, I:** Diphenylamine**, J:** Fluoxastrobin**, K:** Imidacloprid**, L:** Malathion**, M:** Malaoxon**, N:** Methoxyfenozide**, Ñ:** Propargite**, O:** Pentachlorophenol**, P:** Permethrin**,** cis-**, Q:** Permethrin**,** trans-**, R:** Pyraclostrobin**, S:** Tebutiuron**, U:** Trifloxystrobin**. T:** Traces**,** CL**:** Liquid Chromatography *CG*: Gas Chromatography

DISCUSSION

Diversity of pesticides in wax and honey. The wax samples presented a greater diversity of pesticides (insecticides, fungicides, acaricides and herbicides), compared to those of honey. In the same way Johnson *et al*. (2010), report the occasional presence of pesticide residues in honey, since most pesticides are hydrophobic and can be transferred

more easily through interactions of honey bees towards their wax (Calatayud-Vernich *et al*., 2018). Therefore, pesticides can be found more frequently in wax than in honey; or, although the levels of pesticides in honey are low, they tend to contaminate the wax due to their lipophilic nature (Valdovinos-Flores *et al*., 2017); as well as a low wax replacement in the hive and recycle to reintroduce. Therefore, highly hydrophobic and stable pesticides are the main factors for the storage of pesticides in wax (Calatayud-Vernich *et al*., 2018).

Table 3. Average of pesticides (mg kg^{-1}) in honey from honey bee colonies under (BC), with (CA) and without (SA) history of collapse, detected by LC-QTOF

		Pesticides in honey							
	Sample	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
BC	1	0.579	0.004				0.006		
	2	0.687	traces						
CA	1	0.255				traces	0.006		
	2	0.141	0.008		traces	traces			
	3	0.105						0.002	
	4	0.600						0.004	
	5	0.231		0.008				0.003	
SA	1	0.787				traces	0.007	0.005	
	2	0.101						0.014	traces
	3	0.290				traces			
	4	0.487					0.009		
	5	0.353							
Positives		12	3	1	1	4	4	5	1
Average BC		0.633							
Average CA		0.266						0.003	
Average SA		0.404						0.010	

A: Acetamiprid, **B**: Carbendazim, **C**: Imidacloprid, **D**: Fluoxastrobin, **E**: Dimethoate, **F**: Malaoxon, **G**: Methamidophos, **H**: Ometoate.

From the total pesticides, insecticides were the main ones found in the wax samples, with 59.1%, which correspond mainly to organophosphates (chlorpyrifos, malathion, coumaphos, dimethoate and malaoxon), pyrethroids (deltamethrin, cis and trans permethrin) , neonicotinoids (acetamiprid and imidacloprid), and organochlorines (pentachlorophenol). Likewise, organophosphates (malaoxon dimethoate, methamidophos and omethoate), and neonicotinoids (acetamiprid and imidacloprid) were

found in honey; representing 75.0% of the samples. The presence of insecticides in honey and beeswax represents the greatest risk for pollinating insects (Botías y Sánchez-Bayo, 2018; Ostiguy *et al*., 2019); therefore, the detection and quantification of these in the samples analyzed reflect their high exposure to bees, causing irreversible damage in some colonies.

Fungicides in wax (27.3%) and honey (25.0%), were the second class of pesticides found with the highest presence. Botías y Sánchez-Bayo (2018) point out that some fungicides can increase the toxicity of insecticides by reducing the detoxification capacity of bees. Colony fungicide residues have also been found to be related to the prevalence of disease in bees (Simon-Delso *et al*., 2014). Furthermore, it is suggested that the effect of fungicides on pollinators is not due to direct toxicity, but due to the alteration of the microbiome present in the pollen and nectar of the treated and / or contaminated plants that the bees feed on and their own bacterial flora (VanEngelsdorp *et al*., 2009); which has important consequences on the nutrition and health status of bees.

Finally, herbicides (9.1%) and acaricides (4.5%) are the pesticides with the lowest presence in the wax samples analyzed. Herbicides do not represent acute toxicity to pollinating insects (Botías y Sánchez-Bayo, 2018); however, its use indirectly affects bees, because they eliminate large numbers of wild plants and reduce floral diversity, which is the main source of food (Bohnenblust *et al*., 2016); the low presence of herbicides in the samples analyzed can be attributed to this.

In the case of acaricides, which are used to control Varroa destructor, they can act additively or synergistically with insecticide residues in bee colonies (Johnson *et al*., 2013); however, in our case, the acaricides found in the samples was 4.5% of the total pesticides, which is why these possibly cause minor adverse effects in the bee colonies.

Diversity of pesticides in wax and honey from colonies BC, CA and SA. Colonies with a history of collapse had a greater diversity of pesticides (20), compared to colonies without a history (19), and colonies with a collapse (7); This is also reflected by disaggregating pesticides into insecticides, fungicides, acaricides and herbicides; except for the latter two cases, where there was no presence in BC, and the diversity was similar in the CA and SA colonies.

Alcántar-Rosales *et al*. (2016), report similar data in honey and wax regarding the reduced diversity of pesticides for the colonies that collapsed, finding two pesticides (neonicotinoids and organophosphates) in the honey samples, and four (organophosphates, benzimidazole, pyrethroids and derivatives of pyridine) in the wax; however, these are in low concentrations. In relation to this, the wax contaminated with pesticides, being in contact with the developing egg until the bee emerges, can cause sub-lethal effects in worker bees, mainly affecting larval development and longevity of bees. (Wu *et al*., 2011); being able to cause indirect effects in the colony, such as

premature changes in the role of bees and in foraging activity. For the present case, the colonies that collapsed presented the least diversity of pesticides, but in high quantities; possibly showing that the presence of insecticides and fungicides affected bees, causing their collapse.

It is also important to highlight that the presence of pesticides is due to the management of the hives (stationary and migratory), as well as the location of the apiary, among other factors (Ostiguy *et al*., 2019). For our case, in the three groups (CA, SA and BC), the majority of hives are mobilized, and it has been reported that the mobilization of hives intended for pollination causes greater exposure to pesticides (Traynor *et al*., 2016). In our case, beekeepers mobilize them, for two reasons: the search for flowering, and when they are leased for crop pollination; this management can cause stress to bees, making them more susceptible to pesticide poisoning (Sánchez-Bayo *et al*., 2016).

The location of the apiaries is also an important factor, due to the effect that the surrounding vegetation will have on the hives. Intensified agriculture has been shown to cause loss of natural habitats; therefore, intensive cultivation and, in general, the lack of plant biodiversity limit the quantity of food, which causes a decrease in abundance and richness of pollinators; as well as an impact on the health of honey bees (Kovács-Hostyánszki *et al*., 2017).

In our case, the surrounding vegetation at the time of sampling and the crops that are mostly planted are as follows: for the BC group, the predominant vegetation was pinabete (*Tamarix* spp.); and the crops close to the apiaries were corn (*Zea mays*), alfalfa (*Medicago sativa*) and cotton (*Gossypium* spp.). For the CA group, the predominant vegetation was pinabete (*Tamarix* spp.) and mesquite (*Prosopis* laevigata), with crops of maize (*Z. mays*), sorghum (*Sorghum vulgare*), cotton (*Gossypium* spp.), Alfalfa (*M. sativa*), watermelon (*Citrullus lannatus*), melon (*Cucumis melo*), chili (*Capsicum annuum*), and squash (*Cucurbita pepo*). Finally, for the SA group, the main predominant vegetation was mesquite (*P. laevigata*) and pinabete (*Tamarix* spp.), with crops of alfalfa (*M. sativa*), corn (*Z. mays*), sorghum (*S. vulgare*) and cotton (*Gossypium* spp.). This gives evidence that the hives in the region are widely exposed to pesticides, which means that agricultural areas contribute to the high presence of pesticides in the hive products (Traynor *et al*., 2016).

The greatest diversity of pesticide residues was found in honey and wax in the CA colonies, it could be related to the amount of crops near the colonies where the samples were taken; however, the amount of pesticides was less compared to collapsed colonies with the presence of high amounts of pesticides in honey and wax. This was possibly due to the fact that these colonies presented greater exposure to the treated crops, or due to a recent application of pesticides during the colonies' stay with respect to CA and SA; since they presented lower levels of pesticides, even being in the same region.

Chronic exposure of bees to pesticides at sublethal doses can affect neurological functions, such as memory and behavior; symptoms that may occur before the collapse of the hive (Lu *et al*., 2014); In addition to this, exposure to pests, diseases, poor nutrition, or the interaction between pesticides and pathogens, contribute to the mortality of bee colonies (Broadrup *et al*., 2019), which possibly occurred in BC colonies.

Amount of pesticides in honey and wax samples. The insecticide found in all the honey and wax samples was acetamiprid, which was also the one that had the highest amount on average, both in honey (0.385 mg kg^{-1}) and in wax (0.316 mg kg^{-1}) ; exceeding the Maximum Residue Limits (MRLs) of the European Union (EU) (0.05 mg kg^{-1}). Data reported by Gaweł *et al.* (2019) in honey differ from ours, whose concentrations are low and range from 0.001 to 0.13 mg kg^{-1}. Also, Da Silva *et al*. (2015), report an average of 0.0025 mg kg^{-1}.

Studies carried out by El Hassani *et al*., (2008) indicate that the consumption of this pesticide at sublethal doses of 0.1 µg/bee affects its behavior and olfactory learning, due to its immunosuppressive effect (Di Prisco *et al*., 2013), causing a greater susceptibility to infection by the microsporidium *Nosema* (Broadrup *et al*., 2019). Furthermore, the immune weakening favors the spread of the Varroa mite in the honey bee colonies, which is a source of virus transmission (Di Prisco *et al*., 2016); such as the Deformed Wings Virus (DWV), the Israeli Acute Paralysis Virus (IAPV), the Acute Paralysis Virus (ABPV) and the Kashmir Virus (KBV) (Belsky y Joshi, 2019; Brutscher *et al*., 2016). The combination of these diseases with neonicotinoid insecticides, contribute to the collapse of the hives (Sánchez-Bayo *et al*., 2016). Furthermore, acetamiprid can present synergistic effects when combined with other pesticides (Wang *et al*., 2019), which possibly explains the collapse of the colonies in this region; in addition, they probably had a longer time of exposure to pesticides.

The second pesticide in frequency was Malathion, which was found in 11 wax samples with levels from 0.005 to 0.041, and an average of 0.015 mg kg^{-1}; figure not exceeding the EU MRLs of 0.05 mg kg^{-1}. For the north of Mexico Valdovinos-Flores *et al*. (2017), report the presence of Malathion in 100% of the wax samples, with levels ranging from 0.006 to 1,532 mg kg^{-1}, with an average of 0.018 mg kg^{-1}. Malathion, used in agriculture as an insecticide and acaricide and for the control of urban pests, has low persistence and high toxicity in insects (Toxnet, 2019).

The results of this study demonstrate a high incidence of organophosphate Malathion in northern Mexico, and although it has low persistence, it is of high incidence in the samples analyzed. This is probably explained by the application of organophosphate near the hives before the samples were collected, since, as previously mentioned, most of the hives move around in search of flowering and are located mainly near agricultural crops. .

Finally, cis- permethrin was found less frequently (six wax samples), but in greater quantity (0.087 mg kg^{-1}); however, the EU does not specify its MRL. Similar data are reported by Johnson *et al*. (2010), with values of 0.133 mg kg^{-1}. However, this insecticide is highly toxic to bees, with a topical LD50 of 0.024 µg/bee (Piccolomini *et al*., 2018). Prolonged exposure of pyrethroids can affect cellular and humoral immunity; as well as the decrease in immunity in bees (Qi *et al*., 2019). Permethrin is mainly used as an insecticide and acaricide for the treatment of forest use seeds, and for vector control (Toxnet, 2019); and its wide use may be the reason for its greater quantity and presence in the samples analyzed, since some samples came from the proximity of agricultural areas.

It is important to note that La Comarca Lagunera has been a benchmark in terms of cotton planting, forage cultivation for cattle, and also horticultural production is on the rise (SIAP, 2019); making it a region where a great diversity of pesticides have been used, many of which have a residual effect (Vargas-González *et al*., 2016). Therefore, inadequate agricultural practices and the inefficient use and management of pesticides (Esquivel-Valenzuela *et al*., 2019) have created a serious public health problem, due to poisoning by agrochemicals, as well as for the environment; highlighting the damage caused to beekeeping by its effect on the collapse of hives**.**

CONCLUSIONS

The greatest amount of pesticides were found in the wax of the colonies with a history of collapse; thus they also present the greatest diversity (insecticides, fungicides, acaricides and herbicides). The presence of pesticides in honey and wax from the colonies under, with, and without a history of collapse may be the consequence of the phytosanitary treatments used in agriculture, so that their presence may be influenced by the origin of the sample, since that the radius of bees' action is up to ten kilometers. However, our data does not allow us to affirm that the presence of pesticides is the main or only cause of colony collapse; and therefore, it is required to continue with this type of research to determine the factors that affect the health of honey bees, such as the presence of pesticides, parasites and diseases in the region.

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