
El Colegio de la Frontera Sur

# Degradación de plaguicidas por la microbiota intestinal de abejas silvestres del Soconusco

Tesis
presentada como requisito parcial para optar al grado de
Maestría en Ciencias en Recursos Naturales y Desarrollo Rural
Con orientación en Entomología Tropical

Por

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*Anexo 9*

**PORTADILLA DE TESIS DE MAESTRÍA**

# El Colegio de la Frontera Sur

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Las personas abajo firmantes, miembros del jurado examinador de:

Augusto Campollo Ovalle

hacemos constar que hemos revisado y aprobado la tesis titulada

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para obtener el grado de **Maestro (a) en Ciencias en Recursos Naturales y Desarrollo Rural**

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**Tabla de contenido**

**Resumen**

Diversas especies de insectos han desarrollado resistencia o tolerancia a insecticidas. Aunque la presencia de mutaciones en genes relacionados con la degradación o insensibilidad a insecticidas se encuentra bien demostrada se han descubierto microorganismos endosimbiontes capaces de neutralizar la toxicidad de dichos compuestos, proporcionando resistencia a sus hospederos. En *Apis mellifera*, se han reportado microorganismos benéficos que aparentemente están involucrados en la degradación de pesticidas. El objetivo del trabajo fue determinar si la microbiota intestinal de *A. mellifera* y *Scaptotrigona mexicana* presentes en el Soconusco, Chiapas, es capaz de degradar al insecticida malatión debido al uso constante en campo; además se consideró al plaguicida spinetoram como un testigo de reciente utlización en campo al que no han desarrollado tolerancia. Se llevó a cabo el siguiente procedimiento experimental: 1) se realizaron colectas de abejas en apiarios y meliponarios en zonas agrícolas del Soconusco, expuestos continuamente a insecticidas; 2) se determinó la presencia de malatión en cuerpos de abejas mediante extracciones por el método de QuEChERS y se analizaron mediante cromatografía de gases; 3) se calculó la DL50 y CL50 de ambas especies a ambos insecticidas; 4) se disectaron los tractos digestivos de abejas, para evaluar los consorcios bacterianos en medio M9, enriquecido con ambos plaguicidas a diversas concentraciones. No se encontraron residuos de malatión en los especímenes analizados. La DL50 y la CL50 obtenida fue parecida a los obtenidos en estudios previos, indicando que las abejas no habían desarrollado tolerancia al malatión ni al spinetoram. No se obtuvieron consorcios bacterianos del tracto digestivo de abejas capaces de degradar malatión ni spinetoram.

Palabras clave: interacción insecto – microorganismos, endosimbiontes, tolerancia, pesticidas, exposición prolongada.

## Introducción

Las abejas son los principales polinizadores de plantas con flores en el mundo; polinizan alrededor de 73% de los vegetales que consumimos y el 75% de la vegetación silvestre (Diodato et al., 2008). Dentro de los cultivos de interés económico para la región del Soconusco que son polinizados por abejas se encuentran el rambután (*Nephelium lappaceum*), el café (*Coffea* spp.), el marañón (*Anacardium occidentale*) y en cierta medida el mango *(Mangifera indica*). Sin embargo, se ha registrado un decremento de las poblaciones en abejas asociado al aumento irracional de plaguicidas (Arizmendi, 2009; Schierow et al., 2012). El insecticida dicloro difenil tricloroetano (DDT), molécula organoclorada que tiene alta estabilidad y persistencia en el ambiente, fue usada ampliamente para el control de insectos desde inicios del siglo XX, pero debido a los daños que ocasionó a la salud de los mamíferos, fue prohibido su uso en la década de los setenta, lo que llevó a la búsqueda de plaguicidas menos agresivos. En la actualidad, los insecticidas más utilizados son aquellos provenientes de las familias de neonicotinoides, piretroides, macrólidos, y en mayor proporción los organofosforados, como el malatión, de amplio uso en la agricultura y que presenta una alta afinidad por tejido lipídico, acumulándose en órganos y tejidos de importancia vital para la salud de los mamíferos (Hazarika et al., 2003; Ruiz-Toledo, 2019). Se ha demostrado que los plaguicidas compuestos por moléculas organofosforadas actúan en la fosforilación del grupo hidroxilo de la serina del sitio activo de la acetilcolinesterasa (AChE) que se encuentra en las terminaciones nerviosas, lo que conlleva a la inactivación de esta enzima esencial, que tiene un papel importante en la neurotransmisión del sistema nervioso en insectos (Sudakin & Stone, 2011; Du et al., 2012). Estudios con organofosforados como malatión y clorpirifos en *Apis mellifera* han demostrado los daños que ocasionan en insectos, destacando problemas como la pérdida de visión, habilidad de vuelo, y desorientación al momento de forrajear. Estos efectos han llevado al colapso de colmenas y a la pérdida de aproximadamente un tercio de la riqueza de especies de abejas en Norteamérica (Kluser & Peduzzi, 2007; Oliveira et al., 2014). Actualmente, han surgido nuevas alternativas que ostentan ser biodegradables y

por tanto “amigables con el ambiente”. Sin embargo, a la fecha se desconoce el daño que pueda causar en la salud de los insectos benéficos. Uno de estos insecticidas compuesto por macrólidos es el spinetoram, insecticida semisintético que está compuesto por dos moléculas, espinosinas J y L, las cuales actúan sobre el sistema neurotransmisor de los insectos de manera más rápida que su antecesor el insecticida natural Spinosad. Las espinosinas, al momento de entrar en contacto o ser ingeridas por los insectos, interrumpen los receptores nicotínicos de acetilcolina y ácido γ-aminobutírico (GABA), provocando que su sistema neurotransmisor falle, teniendo como resultado daños letales o subletales en la vida del insecto (Hussain et al., 2018). Por consiguiente, ha crecido el interés por el estudio sobre las afectaciones de los pesticidas en polinizadores, así como la búsqueda de soluciones hacia estos efectos. No obstante, se ha demostrado el potencial de algunos insectos por tener resistencia o tolerancia hacia ciertos insecticidas. Algunos estudios han demostrado que ciertos insectos tienen abundante producción de enzimas como glutatión-S-transferasas (GST), monooxigenasas del citocromo P450 (P450s) y las carboxilo / colinesterasas (CCE) que ayudan en la degradación de los plaguicidas, provocando que estos insectos presenten resistencia y tolerancia a algunos plaguicidas (Yu et al., 1984; Claudianos et al., 2006). En moscas de *Ceratitis capitata*, se ha demostrado la producción de acetilcolinesterasa (AChE) mutante, que provoca la resistencia en malatión (Magaña et al., 2008). Otra de las formas en la que los insectos han generado tolerancia hacia los insecticidas se basa en interacciones con sus microorganismos simbiontes, que permiten la asimilación y transformación de compuestos dañinos a metabolitos digeribles y de menor impacto para las abejas (Engel & Moran, 2013). Algunos estudios han demostrado el potencial de las bacterias para degradar plaguicidas como el malatión; se han descrito especies como *Pseudomonas sp*., *Pseudomonas putida*, *Micrococcus lylae*, *Pseudomona saureofaciens* y *Acetobacter liquefaciens* que pueden degradar plaguicidas (Goda et al., 2010). En cucarachas se ha descubierto una simbiosis benéfica entre bacterias, donde los microorganismos *Pseudomonas aeruginosa, Streptomona maltophilia, Bacillus atrophaeus* y *Citrobacter amolonaticus* que forman parte de su

microbiota, degradaron endosulfán hasta en 85% (Ozdal et al., 2016). Por lo anterior, las abejas podrían beneficiarse de la microbiota capaz de degradar agentes agroquímicos, lo que implicaría un mecanismo indirecto de tolerancia no estudiado hasta la fecha en abejas. Ante el desconocimiento de la capacidad de degradación de insecticidas como malatión y spinetoram por la microbiota intestinal de abejas, se evaluó la capacidad de la microbiota intestinal de *A. mellifera y S. mexicana* para degradar estos plaguicidas.

# Microbial gut consortia from two highly social bee species is not involved on the degradation of the pesticides malathion and spinetoram

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**Abstract**

In many bee colonies around the world honey and pollen reserves are contaminated with residues of pesticides. Such chronic exposure might turn gut microorganisms capable to degrade pesticides. In this way, bees would become tolerant to insecticides, as shown in other insects. We tested this possibility in two bee species: the honey bee *Apis mellifera* and the native stingless bee *Scaptotrigona mexicana*. We evaluated the insecticides malathion, intensively and extensively used in the study region and spinetoram, a semisynthetic insecticide derived from spinosyns, which was recently introduced but it is scarcely used. We found that *A. mellifera* had larger LD50 to malathion and slightly smaller LD50 to spinetoram than *S. mexicana*. In previous works *A. mellifera* showed larger LD50 to both insecticides than in our study; unfortunately, no previous data for *S. mexicana* was available. Cultured guts did not grow bacteria in medium supplemented with malathion or spinetoram. We found no evidence that the studied species had developed any tolerance trait to any of the tested pesticides, nor the presence of bacteria capable of degrading them.

Keywords: LD50, LC50, *Apis mellifera*, *Scaptotrigona mexicana*, tolerance

**Resumen**

Se han reportado residuos de plaguicidas de polen y miel de colonias de abejas sociales en todo el mundo. Esta exposición crónica podría funcionar como un agente de selección de microorganismos simbiontes de las abejas, que desarrollarían la capacidad de degradar estas sustancias; de esta forma se incrementaría la tolerancia de las abejas hacia los plaguicidas, como se ha mostrado en otros insectos. El objetivo de este trabajo fue investigar esta posibilidad en dos especies de abejas: la abeja melífera *Apis mellifera* y la abeja sin aguijón *Scaptotrigona mexicana*. Se evaluaron los insecticidas malatión, el cual se usa intensiva y extensivamente en la región de estudio, y spinetoram, insecticida semisintético derivado de spinosyn, el cual es de reciente introducción y se ha utilizado muy poco. Se encontró que *A. mellifera* tuvo una LD50 a malatión mayor que *S. mexicana*, y una LD50 a spinetoram ligeramente menor. Trabajos previos en *A. mellifera* reportan LD50 mayores que los encontrados en el presente estudio para ambos insecticidas; desafortunadamente no se tienen datos de *S. mexicana* para comparar. El cultivo de los intestinos de las abejas en medios suplementados con malatión o spinetoram no presentó crecimiento bacteriano. Así, no encontramos evidencia de que las especies que estudiamos hayan desarrollado tolerancia alguna a malatión o a spinetoram, ni que tengan bacterias que degraden dichos insecticidas.

Keywords: DL50, CL50, *Apis mellifera*, *Scaptotrigona mexicana*, tolerancia

## Introduction

Bees provide us the fundamental ecological service of pollination, which enhances crop production (Michener, 2000; Klein et al., 2003). However, control of arthropod pests, which often involves the use of synthetic pesticides, threatens the populations of bees (McLaughlin & Mineau, 1995). Since the discovery of the insecticidal properties of the DDT, the variety and amount of synthetic chemicals used for crop protection has increased, causing a steady parallel decrease in the population of bees (Oberemok et al., 2015). For that reason it is not uncommon to find pesticides in pollen and honey in apiaries around the world (Ruiz-Toledo et al., 2018).

Acute exposure to lethal dose of pesticides causes the dead of bees (Johnson, 2015). Sublethal exposure contributes to the deterioration of organs such as the brain and midgut, and impairs vision, flying ability, and causes disorientation and reduction in longevity (Desneux et al., 2007), which eventually would cause the failure of the colony. However, bees somehow tolerate sublethal exposure to insecticides, otherwise it would be impossible to explain the presence of pesticides in pollen and honey in living colonies (Sanchez-Bayo & Goka, 2014). Such tolerance can be partially explained by the presence of detoxifying enzymes (Berenbaum & Johnson, 2015; Rand et al., 2015), though some studies seem to indicate that their role is less relevant in honey bees than in other insects (Yu et al., 1984; Claudianos et al., 2006). Other potential explanation can be found in the degrading role microbiota in the intestine of bees, with which have symbiotic relationships (Martinson et al., 2011). It is thought that damaging the gut bacteria community might have detrimental effects on bee health, since some of them are associated with the assimilation of nutritive substances (Vásquez et al., 2012). In fact, it has been shown in some insect species that gut symbionts can also degrade pesticides. For instance, *Pseudomonas aeruginosa*,

*Streptomona maltophilia*, *Bacillus atrophaeus* and *Citrobacter amolonaticus*, from the gut of cockroaches chronically exposed to insecticides, were able to degrade endosulfan (Ozdal et al., 2016). Other examples of symbiont-mediated insecticide resistance include *Burkholderia* sp which turns the stinkbug *Riptortus pedestris* resistant to fenitrothion (Kikuchi et al., 2012), the trichlorphon-degrading *Citrobacter* bacteria in the gut of the oriental fruit fly *Bactrocera dorsalis* (Cheng et al., 2017) and *Spodoptera frugiperda* with bacteria able to degrade lambda-cyhalothrin, deltamethrin, chlorpyrifos ethyl, spinosad and lufenuron (Almeida et al., 2017).

The presence of pesticides in stored pollen and honey (Ruiz-Toledo et al., 2018) invariably means that gut microbiota is exposed to such substances. Thus, it might be possible that some bacteria had developed the potential to degrade insecticides. Bees could benefit from such microbiota, because they would represent an indirect mechanism of tolerance not studied to date in bees. Therefore the aim of this study was to investigate the presence of intestinal bacteria, from the honey bee *Apis mellifera* and the stingless bee *Scaptotrigona mexicana*, able to degrade the spynosin spinetoram and the organophosphorous malathion. We chose these species because they are widely managed in the study region and we have been keeping colonies in conventional agricultural landscapes for over 20 years. Malathion is widely used for the control of several insect pests in several crops, while spinetoram is recently and scarcely used, thus representing a no-exposure pesticide for our bees. We expect that bees had developed some resistance to malathion but no to spinetoram, due to the presence of malathion-degrading bacteria.

## Materials and methods

Our samplings were carried out in February - April 2018. *Apis mellifera* workers were collected from three managed colonies located in our apiary in Tapachula, Chiapas, Mexico. *Scaptotrigona mexicana* workers were collected from three colonies from our meliponary located in Tuxtla Chico, Chiapas. Workers were subjected to any of the following procedures.

We investigated the degree of exposure to malathion by extracting the lipophilic substances of a macerate consisting of 25 foragers from each colony in one 13 x 100 glass tube, following the QUEChERS method by Mullin et al. (2010). After centrifugation at 10,000 rpm during 10 min, the liquid phase was separated into another tube and then analyzed by gas chromatography according to the method described by Ruiz-Toledo et al. (2018), using the same equipment, run conditions and materials, but using an analytical standard of malathion to identify the target pesticide (Sigma-Aldrich, Mexico). We did not carry out the identification of spinetoram since it was not reported by local growers to be used for pest control in our agricultural landscape at least in the last year.

In order to estimate the oral LC50 of commercial formulations of malathion and spinetoram we exposed 3-5 day-old workers of both species to several concentrations of malathion and spinetoram as follows: brood combs (with approximately 400 bees in the larval stage) from each colony were taken. Next they were placed in a plastic container of approximately 3 L with a 5 mL vial with 2M sucrose solution to feed the bees. They were maintained at a temperature of 29 ºC and 70% relative humidity and 12:12 light-darkness cycle. Every day we transferred the emerged bees to 100 ml plastic recipients to know exactly their age. Once they reached the age of exposure, were left starving for 24 h. Thirty bees from each colony were orally exposed to 10, 5, 2.5, 1, 0.1 and 0 ppm of spinetoram,

and 30, 20, 10, 5, 2.5, 2, 1 and 0 ppm malathion in 2M sucrose solution. We registered mortality 24 hours after exposure. We considered that a bee was dead when it did not respond to a gentle touch with a paintbrush. In order to be able to make a comparison between these species and data from literature we estimated the consumption of 2 M sucrose to calculate LD50: we placed 20 workers in separated 100 ml plastic recipients and one 10 μl glass capillary filled with sucrose solution was introduced. To measure the effect of evaporation we set 5 more recipients as mentioned before but without any worker inside.

Reagents used in the experiment to determine the presence of pesticide-degrading bacteria in the gut of bees (M9 minimal salts growth medium containing 33.9g/L $Na_2HPO_4$, 15g/L $KH_2PO_4$, 5g/L $NH_4Cl$, 2.5g/L NaCl, SIGMA-ALDRICH, United States; 5% glucose solution, Laboratorios PISA, Mexico; Palgus (Spinetoram 6%), Dow Agrosciences, Mexico; Malathion 83.6%, Agroquímica Tridente S.A de C.V., Mexico) were obtained in local shops. Three groups of ten workers were orally exposed to the LD0, LD5, LD25, LD50 or LD100 of both insecticides, as in the previous experiment. Next, all surviving workers were sacrificed by cold (5 min at -20 ° C), and then immediately disinfected from external microorganisms using sodium hypochlorite following Disayathanoowat et al. (2012). The intestines of the sacrificed workers, of the same species, LD and insecticide, were dissected, pooled and homogenized in 10 ml of sterile M9 medium (11.28g/L). One milliliter of the homogenate was mixed with 9 ml of any of the following treatments at 30°C, pH 7: 1) M9 medium alone as negative control. 2) M9 medium enriched with malathion or spinetoram at 22.5 μg as the sole carbon source, 3) M9 medium at 5% glucose as a positive control of bacterial growth and 4) M9 medium at 5% glucose + malathion or

spinetoram at 22.5 μg. We followed bacterial growth for 30 days by observing changes in turbidity in the media in all the rests.

Malathion was identified by comparing the peaks obtained in the extracts obtained by the QUEChERS method and that of previous runs with the analytical standard. LC50 was estimated by a log-logistic approach using the package drc v 3.0-1 (Ritz et al., 2015) in the R environment v 3.5.0 (R Development Core Team, 2012). LD50 was calculated by multiplying the average consumption of each bee by the LC50. Changes in turbidity in the growth medium were considered as positive if a change occurred or negative in the opposite case.

**Results**

We could not detect malathion in any of the pooled extracts, i.e. probably foragers did not carry in their body this pesticide back to the colony. Table 1 shows the LC50 and 95% confidence intervals for malathion and spinetoram in both species. *Scaptotrigona mexicana* seems to be far more sensitive to malathion than *A. mellifera*. *Scaptotrigona mexicana* consumed 2.6 μl / 24 h on average and *A. mellifera* 50 μl / 24 h. From these data it could be possible to estimate LD50 (Table 1).

Results from the experiment about pesticide-growing bacteria are shown in Table 2a, b. Control treatments T1 and T3 presented turbidity as expected: no turbidity and turbidity, respectively. No turbidity was observed in any of the tubes from treatment 2, so microbiota was unable to use directly any of the chemicals as the only source of carbon in the conditions of our experiments. Interestingly enough, in the case of *S. mexicana* some

tubes of T4 did not show turbidity, indicating some degree of inhibition by the pesticides. But, some form *S. mexicana* and all tubes of T4 in *A. mellifera* did show bacterial growth.

**Discussion**

We did not detect malathion in any worker, nor observed malathion/spinetoram-degrading bacteria, despite the beehives were located in an agricultural landscape where producers still use malathion to control insect pests. The lack of malathion/spinetoram degrading bacteria does not rule out that they are in contact with these compounds or that they cannot actually degrade these substances. It is possible we could not detect malathion due to the short half-life of malathion in honey and beeswax it is in the order of minutes, as compared to other insecticides (Shimshoni et al., 2019). Actually, in a recent work Ruiz-Toledo (2019) reported the presence of malathion in some pollen samples of the study region, indicating recent exposure. The minute amounts of malathion brought back by foragers perhaps did not represent a threat to our target species, and could be easily degraded by gut enzymes, as shown by our data of LD50 in spinetoram and malathion which are within the parameters described by other studies conducted in *A. mellifera* (Naggar et al., 2015; Shimokawatoko et al., 2012). Also it is possible that foragers receiving lethal doses die before returning to the colony, thus making it undetectable in our specimens and minimizing the contact with the colonies. Finally, the presence of organochlorines (OCs) in the samples analyzed by Ruiz-Toledo et al. (2018), indicates that exposure to organophosporous compound might be lower than expected, and that search should go in the direction of OCs-degrading bacteria.

To be able to develop some pesticide-degrading capacity, microorganisms should be exposed to the insecticide and survive; the insect harboring the microorganisms must also

survive to the exposure. And then, some means to reproduce the microorganisms beyond the dead of the host must be present. In the case of highly social bees, exposure to sublethal amounts of insecticides occurs worldwide (Mitchell et al., 2017; Ruiz-Toledo et al., 2018), so microorganisms are also exposed, but maybe at amounts that do not represent a selective factor in microbial communities; on the other hand, Tian et al. (2012) found tetracyclin-resistant gut microbiota in long-term exposed honey bee colonies that were directly under selective pressure. Once established, the transmission of microbiota within the hive seems to be through trophallaxis by nurse bees to younger stages (Powell et al., 2014). In the case of insecticides they potentially had little selective pressure over microbiota, so it was not possible to detect degrading bacteria. However, soil microorganisms are more likely to develop pesticide-degrading capabilities. For instance, the bean bug, *Riptortus pedestris*, acquire fenitrothion-degrading microorganisms from soil during larval stage (Kikuchi et al., 2012). Perhaps symbiont-conferred resistant is present in social and non-social bee species that use soil as the preferred nesting substrate, so it is less likely to find them otherwise. This has yet to be investigated.

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Table 1. LC50 in ppm (LD in ng/bee) of spinetoram and malathion in the tested species with showing 95% confidence interval.

Table 2a and 2b. Results of the experiments to detect insecticide-degrading bacteria in *Scaptotrigona mexicana* and *Apis mellifera*. LD column refers to the percentil of the lethal dose estimate in the corresponding experiment . T1: M9 medium alone as negative control. T2: M9 medium enriched with the insecticide at 22.5 μg, as the sole carbon source, T3: M9 medium at 5% glucose as a positive control of bacterial growth and T4: M9 medium at 5% glucose + insecticide at 22.5 μg. Turbidity is expressed as either positive (bacterial growth) or negative (no growth).

345 Table 1.

| Spinetoram (ppm) | | | | | |
|---|---|---|---|---|---|
| | LC50 (LD50) | Lower Limit | Upper Limit | Best Fit Model | LD50 in other studies |
| *S. mexicana* | 4.5 ppm (11.7 ng/bee) | 2.3 | 6.6 | Weibull | No study available |
| *A. mellifera* | 0.21 ppm (10.5 ng/bee) | 0.03 | 0.38 | Log-logistic | 24.8 ng/bee (Shimokawatoko et al., 2012) |
| Malathion (ppm) | | | | | |
| | LC50 (LD50) | Lower Limit | Upper Limit | Best Fit Model | LD50 in other studies |
| *S. mexicana* | 10.0 ppm (26 ng/bee) | 7.8 | 12.3 | Log-logistic | No study available |
| *A. mellifera* | 3.6 ppm (180 ng/bee) | 2.0 | 5.15 | Weibull | 335 ng/bee (Al Naggar et al., 2015) |

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Table 2a.

| *Scaptotrigona mexicana* | | TREATMENT | | | |
|---|---|---|---|---|---|
| Insecticide | LD | T1 | T2 | T3 | T4 |
| Malathion | 0 | Negative | Negative | Positive | Negative |
| Malathion | 5 | Negative | Negative | Positive | Negative |
| Malathion | 25 | Negative | Negative | Positive | Negative |
| Malathion | 50 | Negative | Negative | Positive | Positive |
| Malathion | 100 | Negative | Negative | Positive | Positive |
| Spinetoram | 0 | Negative | Negative | Positive | Positive |
| Spinetoram | 5 | Negative | Negative | Positive | Negative |
| Spinetoram | 25 | Negative | Negative | Positive | Positive |
| Spinetoram | 50 | Negative | Negative | Positive | Negative |
| Spinetoram | 100 | Negative | Negative | Positive | Negative |

Table 2b.

| *Apis mellifera* | | TREATMENT | | | |
|---|---|---|---|---|---|
| Insecticide | LD | T1 | T2 | T3 | T4 |
| Malathion | 0 | Negative | Negative | Positive | Positive |
| Malathion | 5 | Negative | Negative | Positive | Positive |
| Malathion | 25 | Negative | Negative | Positive | Positive |
| Malathion | 50 | Negative | Negative | Positive | Positive |
| Malathion | 100 | Negative | Negative | Positive | Positive |
| Spinetoram | 0 | Negative | Negative | Negative | Positive |
| Spinetoram | 5 | Negative | Negative | Positive | Positive |
| Spinetoram | 25 | Negative | Negative | Positive | Positive |
| Spinetoram | 50 | Negative | Negative | Positive | Positive |
| Spinetoram | 100 | Negative | Negative | Positive | Positive |

## Conclusiones

Con las condiciones empleadas en este trabajo, no se detectaron microorganismos capaces de degradar malatión, concordando con los datos obtenidos en cromatografía de gases en donde tampoco se logró detectar la presencia de malatión en las abejas *Apis mellifera* colectadas en campo. Es poco probable que se encuentren abejas expuestas a dosis sub-letales, dado que realizamos las colectas durante los periodos de mayor aspersión de malatión y spinetoram en los sitios de muestreo.

Se encontró que *A. mellifera* tuvo una LD50 a malatión mayor que *S. mexicana*, y una LD50 a spinetoram ligeramente menor.

Las colonias utilizadas durante los experimentos realizados no presentaron daños aparentes por las prácticas agrícolas realizadas en la zona.

Faltan pruebas a realizar entre especies solitarias y abejas sociales para poder comprender sí la susceptibilidad de las abejas está ligada a la procedencia de su microbiota intestinal.

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