Biological Control Methods for Arthropod and Disease Pests in Managed Hives of *Apis mellifera* in Campeche, Mexico[1]

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J. Entomol. Sci. 60(4): 000–000 (Month 2024)
DOI: 10.18474/JES24-71

Abstract Beekeeping activities involving *Apis mellifera* L. (Hymenoptera: Apidae) impact social, environmental, and economic aspects of daily life in Mexico. For this activity, the participation of women is crucial because they attend to, care for, and collect products from the hives. Beekeeping, however, can be significantly limited by arthropod and disease pests, thereby reducing production. This review was undertaken to identify agents or products of natural origin (i.e., biological control) that have been developed or have the potential to combat these pests. We found that managed hives in Campeche, Mexico are plagued by the varroa mite (*Varroa destructor* Anderson and Trueman) (Acari: Mesostigmata), the small hive beetle (*Aethina tumida* Murray) (Coleoptera: Nitidulidae), the greater wax moth (*Galleria mellonella* L.) (Lepidoptera: Pyralidae), and the microsporidia *Vairimorpha apis* (Zander) Tokarev et al. and *Vairimorpha ceranae* (Fries et al.) Tokarev et al. (Microsporidia: Nosematidae). Our review revealed at least 47 microorganisms or products of plant or microbial origin that have been assessed and/or developed for management of these pests in *A. mellifera* hives. All of these products can be easily obtained in Mexico and, thus, hold promise in the management of pests of *A. mellifera* hives in Mexico.

Key Words *Apis mellifera*, biological control, plant extracts, Campeche

Keeping honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae) in managed hives impacts global economic and environmental health (Baena-Díaz et al. 2022, Cordero 2021, Dzul Uuh 2022, Ramos and Pacheco 2016). Beekeeping usually refers to the production of honey, pollen, royal jelly, and propolis natural products; however, the ecological importance of bees in environmental balance and food production cannot be overestimated (INES 2024).

Mexico is considered among the main producers and exporters of honey worldwide (Ávila Ramos et al. 2022; Campos García et al. 2018; Chan Chi et al. 2018; INEGI 2024; SADER 2024a, 2024b). Beekeeping is an ancient activity in Mexico with important contributions to economic, social, and ecological aspects in different parts of the country (Báez Melendez et al. 2018, Contreras-Escareño et al. 2013, Elorza et al. 2024). Although beekeeping is an important component of the Mexican economy and society, this activity is threatened by arthropod and disease

[1]Received 11 July 2024; accepted for publication 20 October 2024.
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pests that affect the production and development of hives (Punina Gallegos 2022). Studies over the last decade have documented alarming losses of *A. mellifera* colonies throughout the world, losses reaching 20–30% annually mainly caused by arthropod and disease pests (Baena-Díaz et al. 2022, Fernández Chaguay 2022, Velasquez and Bautista 2016). In Mexico, the loss of hives in recent years has been linked to issues within the hives (Reyna Fuentes et al. 2022), such as pests that drastically affect honey production (Collantes and Del Cid 2022, Martínez-Puc et al. 2018, Reyna Fuentes et al. 2022). In addition to pest occurrence, other abiotic environmental factors have drastically affected hives, as occurred in the state of Campeche where 3,365 hives were lost. This loss was estimated as having an economic impact of 12,990,418 pesos and was likely due to the use and misuse of pesticides in agriculture (El Financiero 2023, SEMARNAT 2023, Wired 2023).

There are five beekeeping regions in Mexico, and the southeast region or Yucatán Peninsula is considered the most important due to its volume of honey production (32.4% of national production) and because most of the country's hives are located there. The region comprises Campeche, Yucatán, Quintana Roo, and part of the states of Chiapas (northeast) and Tabasco (east) (SADER 2022).

Honey production in Campeche, in southeastern Mexican, is one of the state's most important agricultural activities. This state is the second highest producer of honey nationwide, with a production that exceeds 8,000 tons annually (SADER 2019), of which 90% is destined for international markets. Campeche is considered one of the main honey producers in Mexico with Chiapas, Jalisco, Veracruz, and Yucatán (Chan Chi et al. 2018, Martínez Puc et al. 2022). INIFAP (2013) reported that hives in the state are affected by varroasis and nosemosis, which also occur in other states (Martínez-Cesáreo et al. 2016; Martínez-Puc et al. 2011, 2015; Medina-Flores et al. 2014).

Based these factors, we undertook a review of the literature to (a) identify the pests that currently affect *A. mellifera* hives and beekeeping in the state of Campeche and (b) catalog the naturally occurring biological control agents that might have potential for management of these pests. We defined biological control agents as those microorganisms and microbial or plant products that could be toxic or repellent to the identified pests.

Review Methodology

A review was conducted of available literature on the primary arthropod and disease pests that affect bee hives in the state of Campeche. This review included searches for books, documents, and scientific publications in Journal Citation Reports, Latindex, Scielo, and other databases and directories of scientific journals. Other sources, such as reports from local studies and available databases in Mexico, were also searched. Any omission of relevant available literature was unintentional.

Pests of Beekeeping in Campeche

We identified five species of pests of beekeeping in the state of Campeche. Those are the varroa mite (*Varroa destructor* Anderson and Trueman), the small hive beetle (*Aethina tumida* Murray), the greater wax moth (*Galleria mellonella* L.), and the microsporidia *Vairimorpha apis* (Zander) Tokarev et al. and *Vairimorpha ceranae* (Fries et al.) Tokarev et al. Various wasps and ants (Hymenoptera) are reported as occasional pests (Collantes and Del Cid 2022, Hernández Melchor and Williams 2024, Martínez-Puc et al. 2018, Reyna Fuentes et al. 2022). The diseases American foulbrood (caused by *Paenibacillus larvae* [Nakamura] Heyndricks et al.), European foulbrood (caused by *Melissococcus plutonius* [White] Bailey and Collins), and chalkbrood (caused by *Ascosphaera apis* [Maassen et Claussen] Olive et Spiltoir) also have been reported from the state (Calderón et al. 2009), and beekeepers should remain vigilante for occurrence of these diseases.

Varroa mite. The varroa mite is considered the most serious pest of honey bee colonies worldwide. This external parasite attacks adult bees and developing larvae, sucking hemolymph from infested individuals and causing a decline in the overall health and possibly death of the colony. The mites can also vector several viruses that can kill bees and eliminate colonies, including those that cause parasite mite syndrome. Varroa infestation in adult bees has been diagnosed in Mexico and other countries by using the method of De Jong et al. (1982a), which consists of washing the bees in a detergent solution, rinsing them, and counting the number of mites, and obtaining the ratio of the total number of mites observed and the total number of bees examined.

Varroa mite was first detected in Mexico in the state of Veracruz in 1992, at an infestation rate of 5% per hive (Amparán et al. 1992). In other states, the infestation rate varies. For example, in Yucatán, about 2% infestation was identified in managed and wild bee colonies (Martínez Puc et al. 2011). In the state of Mexico, infestation rates of 0.5–22% have been reported in individual colonies, but all colonies sampled were infested (Martínez-Cesáreo et al. 2016, Velasco and Novoa 2000). Ruíz-Flores et al. (2012) reported average infestation rate of 5% in Xochimilco, Mexico City; Martínez Puc et al. (2022) reported 4% in Tabasco; and Ceniceros-García et al. (2019) reported 3.4% in Durango, a figure lower than the recommended upper limit of 5% per hive. In Campeche, however, the prevalence of the varroa mite is 98–100% per hive, with 3.73–7.32 mites per 100 bees (Domínguez-Rebolledo et al. 2023).

Varroa infestation rates reported in Cuba are similar to those reported in Mexico, with 5% of adult bees infested (Sanabria et al. 2015). In Colombia, infestations of 1–4% have been reported by Salamanca Grosso et al. (2015). In Ecuador, infestations of 10.84% were detected, which were deemed high and affected the productivity of the colonies (Díaz-Monroy et al. 2019). In Brazil, infestations were 5.77%, 5.03%, 2.43%, 1.62%, 1.54%, and 1.04% for the months of September, August, July, June, April, and May, respectively, indicating higher levels when the nectar supply and propolis production increase (Manrique and Egea Soares 2004).

Díaz-Monroy et al. (2019) reported that when varroa mite infestation within the colonies reaches 10% of the adult bees, productivity of the colony will be reduced, and when the infestation reaches 30–40% of the bees, the colony will be eliminated. When the mite infestation exceeds 5% in a colony, the colony must receive immediate attention; otherwise, it will disappear in 2 years (Reyna-Fuentes et al. 2021, SAGARPA 2005). Numerous studies have indicated a significantly negative correlation between varroa infestation level and honey production by the colony (Arechavaleta-Velasco and Guzmán-Novoa 2000; Medina-Flores et al. 2011, 2014; Murilhas 2002). Callizaya Barrionuevo (2023) determined that an infestation level of 12.58% reduced honey yield to 5 kg/hive/yr from an average of 18 kg/hive/yr in hives with few or no mites.

The modification to the Mexican Official Standard NOM-001-ZOO-1994, National Campaign Against *Varroa* in Bees prescribes that when the infestation levels of a queen bee hatchery (an apiary for the production of nuclei and packages of bees) or any other beekeeping activity exceeds 5% and is under the supervision of the Secretariat, treatment must be applied to the entire hatchery or apiary in question (SAGARPA 2005). Once the treatment is completed, sampling must be repeated according to the regulations set in the Standard. A certificate of infestation levels will be granted only when infestation levels are $\leq$5%.

Microsporidian diseases. In the states of Mexico and Campeche, microsporidian diseases occur in adult bees and are caused by *V. apis* and *V. ceranae*. The microsporidian spores must be ingested by the adult bee to cause infection, which then weakens the bee host and shortens its life span (Tapia-González et al. 2017). Calderón-Fallas and Moreno-Morales (2022) used the Jaycox table, which provides an estimate of the number of spores per bee, as an acceptable method to evaluate the degree of infection by microsporidia (no infection, $<$10,000 spores/bee; very mild infection, 10,000–1,000,000; mild infection, 1,000,000–5,000,000; moderate infection, 5,000,000–10,000,000; semi-strong infection, 10,000,000–20,000,000; strong infection, $>$20,000,000). With *Vairimorpha* and other microsporidia, the higher the number of spores per host, the higher the mortality rate and shorter the time to lethality for the disease.

In the state of Jalisco, Tapia-González et al. (2017) reported microsporidian disease prevalence as 100%, with 1 to 5 million spores/bee, whereas Ramos-Cuellar et al. (2024) reported $>$310,000 spores/bee from the same state, indicating low infestation. In Yucatán, the level of disease in managed colonies had a mean ($\pm$SD) of 1,480,000 $\pm$ 232,000 spores/bee, similar to that recorded in wild swarms with 1,416,000 $\pm$ 264,000 spores/bee (Martínez Puc et al. 2011), whereas in Tabasco, infections had 133,738 $\pm$156,221 spores/bee (Martínez Puc et al. 2022).

Microsporidian disease has not been reported from the state of Mexico (Martínez-Cesáreo et al. 2016), and in some states, microsporidiosis does not represent a serious problem for beekeeping because of its low frequency (Medina-Flores et al. 2014). For Campeche, the prevalences of the *Vairimorpha* species were 85.9% and 88.0%, with reported spore counts of 7.8 million and 23. million per bee (Domínguez-Rebolledo et al. 2023). Similar results have been reported from other parts of the world. In Uruguay, spore counts were 50,000–2,750,000 spores/bee (Mendoza et al. 2013), whereas in Costa Rica, infections were rated as mild to severe, with spore counts of $<$5 million (mild) to $>$20 million (heavy) (Calderón-Fallas and Moreno-Morales 2022, Medina-Flores et al. 2014). Spore counts in bees from Argentina were 43,500 $\pm$ 40,941 to 104,633 $\pm$ 187,410 spores/bee (Marcos Raúl et al. 2022).

Hive beetle. Infestations of the small hive beetle, *A. tumida,* also cause economic losses for beekeepers (Martínez Puc and Cetzal-Ix 2018). *Aethina tumida* was first reported in Mexico in the state of Coahuila in 2007 (Canto et al. 2020, Saldaña-Loza et al. 2014). This beetle lays eggs in honey bee colonies, and its larvae feed on honey, pollen, and eggs, producing galleries in the honeycombs and destroying them. Because this beetle is an exotic pest of bees, its occurrence must be reported (SAGARPA 2013).

The small hive beetle is native to Africa and has been reported in the United States, Canada, Australia, Egypt, Italy, Korea, the Philippines, El Salvador, Nicaragua, Cuba, Jamaica, Guatemala, Brazil, Nicaragua, and several countries in South and Central America (FAO 2016, Lóriga Peña et al. 2014, Mutinelli et al. 2014, Neumann and Ellis 2008, WOAH 2018). In Mexico, this beetle is most successful in mainly warm and humid areas in the southeast (Yucatán Peninsula and the Lacandon area of Chiapas) where there are, coincidentally, a large number of managed bee hives (Bayona Célis et al. 2018). In Yucatán, the pest has been evaluated in several studies involving testing and development of alternative controls using plant extracts (Canto et al. 2020, Duarte-Chávez et al. 2017, Haas et al. 2020). The beetle has been present in Tamaulipas since its first report there in 2013 (Reyna-Fuentes et al. 2021). In Nayarit, this beetle was reported in San Blas, where it had not been reported prior to the study by Robles-Navarrete et al. (2024). In Campeche, the pest was reported for the first time in stingless bee colonies in 2012 (Canto et al. 2020, Hernández Torres et al. 2023). This beetle can now be found in the entire Yucatán Peninsula (Campeche, Quintana Roo, and Yucatán), Nuevo Leon, Guanajuato, Michoacan, Nayarit, Jalisco, Tamaulipas, and San Luis Potosi (Canto et al. 2020, Reyna-Fuentes et al. 2021, Robles-Navarrete et al. 2024). It has not been currently reported from other parts of the country, including Comarca Lagunera (Gurrola Aleman and Reyes Carrillo 2010, Lara Cruz 2009).

Greater wax moth. *Galleria mellonella* has been recorded in Campeche and is a worldwide pest of bee hives. Its economic impact is due to the feeding habits of the larvae and the tunnels they form in the middle of the honeycombs as they feed on hive wax, pollen, propolis, dead bees, and bee pupae. However, the development and metamorphosis of this moth are influenced by relative humidity and diet (Restrepo-García et al. 2019). Although the pest occurs in the state of Campeche, no data were found to indicate that this moth is a problem for beekeeping activity; the varroa mite is the main beekeeping problem encountered in Campeche.

Management with Biocontrol Agents and Plant Extracts

The arthropod and disease pests that were identified as affecting honey bee hives in Campeche are usually treated with chemical pesticides and/or biological control agents (Seshadri and Walker 2019). In Campeche, 47 types of biological control agents have been identified, developed, or used to mitigate or control pest species and their impacts on honey bees (Table 1).

These agents include some plant extracts and entomopathogenic fungi that are used against several of the pests. Extracts from plants tested against multiple pests include extracts from thyme (*Thymus vulgaris* L.), *Citrullus* spp. (including watermelon), laurel (*Laurus nobilis* L.), chamomile (*Matricaria chamomilla* L.), and marjoram (*Origanum majorana* L.). All of these extracts can be easily obtained and/or developed in Campeche because they are marketable and/or grown in the region. Thyme is an aromatic medicinal and culinary herb that concentrates various chemicals in its tissues, a product of secondary metabolism, giving it antimicrobial properties (Corrales et al. 2012) and inhibiting the growth of various phytopathogenic microorganisms (Rodríguez et al. 2021). The essential oil of thyme contains mainly

Table 1. Biological control agents for potential mitigation of pests of *A. mellifera* hives in Campeche, Mexico.

Pest	Biological Control Measures	References
Varroa destructor	Hop acids, formic acid, neem (*Azadirachta indica*) concentrate, *Citrullus colocynthis* extract, *Citrullus* chloroform extract (powder), *Citrullus* alcoholic extract (powder), *Beauveria bassiana* (balsam) (entomopathogenic fungus), thyme (*Thymus vulgaris*) extract, thymol, eucalyptol, menthol, camphor, garlic paste, *Eucalyptus* essential oil, thymol essential oil, laurel (*Laurus nobilis*) extract	Aziz et al. 2015, Dimetry et al. 2005, Emsen and Cengiz 2009, Emsen and Dodologlu 2009, Gregorc et al. 2018, Ibrahim 2015, Martínez-Puc et al. 2017, Reyna Fuentes et al. 2022, Sapna et al. 2010, Underwood 2005
Vairimorpha spp.	Formic acid, oxalic acid, resveratrol, *Aristotelia chilensis* (Chilean maque) extract, *Cryptocarya alba* extract (Chilean peumo), *Gevuina avellana* extract (Chilean hazelnut), laurel extract, chamomile (*Matricaria chamomilla*) extract, *Ugni molinae* (Chilean murta) extract, thymol, marjoram (*Origanum majorana*) extract, polysaccharide extracts, propolis extracts, resveratrol syrup, thymol syrup, honey and lemon juice, Api-Bioxalfi® probiotics, ApiHerbfi® probiotics, microbial symbionts of the bee digestive system (bifidobacteria and lactobacilli), *Bombella apis* sp. nov. without *Parasacharibacter apium*	Burnham 2019, Caamal and Ku 2022, Corby-Harris et al. 2016, Cuesta-Maté et al. 2021, Huang et al. 2013, Parish et al. 2022, Puetate et al. 2023, Reyna Fuentes et al. 2022, Xiaowen et al. 2024
Aethina tumida	Epazote (*Dysphania ambrosioides*) powder, basil (*Ocimum basilicum*) powder, oregano (*Origanum vulgare*) powder, slaked lime, powdered	Amparán and Amparán 2011, Buchholz et al. 2009, Ellis et al. 2010, Haas et al. 2020, Hill et al. 2016,

Table 1. Continued.

Pest	Biological Control Measures	References
	limestone, diatomaceous earth, pollen inoculated with *Kodamaea ohmeri* yeast, apple cider vinegar, *Metarhizium anisopliae, Beauveria bassiana, Heterorhabditis bacteriophora* (HP88strain), *Heterorhabditis indica*	Ma et al. 2013, Muerrle et al. 2006, Neumann and Ellis 2008, Nolan and Hood 2008, Sanchez et al. 2021
Galleria mellonella	*Billaea claripalpis, Bracon hebetor, B. bassiana, Steinernema* sp., neem, *Heterorhabditis indica, Metarhizum anisopliae, Trichoderma viride, Pseudomonas*	Bacca and Lagos 2014, Bogantes et al. 2018, Mosqueira Tucto 2023, Núñez 2011, Speranza et al. 2002

thymol, which in some varieties can have a concentration of up to 80%; thus, thyme is recognized for its antibacterial and antifungal activity (Alzate et al. 2009) and insecticidal properties (Yánez et al. 2014). Researchers have reported the antifungal activity of thyme essential oil at various concentrations and its effects on fungal cells, with lethal damage preventing mycelial development of the fungus and inhibiting sporulation and germination of spores and visible effects in less time than needed with commercial fungicides (Álvarez Hernández et al. 2020, Alzate et al. 2009, Lascano Vela 2020, Necha and Barrera 2008). In the case of *Citrullus,* some species have antifungal and antimicrobial activity. Extracts from the seeds of *Citrullus* spp. have been tested against *Staphylococcus* sp., *Escherichia coli, Proteus* sp., *Klebsiella* sp., and *Pseudomonas aeruginosa* (Adunola et al. 2015) and some species of fungi, such as *Aspergillus niger* and *Candida albicans* (Hassan et al. 2011). Extracts of *L. nobilis* have antifungal and antimicrobial activity (Centurion Paredes 2017, Santiago Pinazo 2015, Valverde Caballero 2019). In beekeeping, laurel and other species affect the exoskeleton, inhibit molting, and decrease the growth rate of *V. destructor* (Puetate et al. 2023). Chamomile possesses various chemicals, such as monoterpenes, sesquiterpenes, hydrocarbons, flavonoids, phenolic acids, oxygenated derivatives, aromatic compounds, and tannins, that give its essential oils antibacterial and antifungal properties (Torres Cunalata 2022), which is why chamomile has been used against diseases encountered by beekeepers. Marjoram, like the other plants species mentioned above, has acaricidal, antibacterial, and antifungal properties (Cueto Wong 2010, Falcón Bautista 2016, Tellez Monzon 2017, Vidal 2021).

The entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizum anisopliae* (Metchnikoff) Sorokin have broad host ranges and infect numerous species of insects from various taxonomic orders and thus are used against several of the arthropod pests of honey bees in managed hives.

Concluding Remarks

This literature review revealed that a large number of studies have been done worldwide and in Mexico on the arthropod and disease pests that attack *A. mellifera* hives. Most of those studies dealing with management through use of biological control agents were mainly assessments of plant extracts and entomopathogenic fungi. In Campeche, these products are not well known, so it is essential to disseminate information about them to beekeepers in the state and to develop products and extracts of aromatic species from this region for the same purpose. More studies are required to overcome the limitations and difficulties related to the production and development of bioinsecticides in Campeche.

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