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Chapter

Prevention and Control of American Foulbrood in South America with Essential Oils: Review

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Abstract

American foulbrood (AFB) is the most severe bacterial disease that affects honey bees, having a nearly cosmopolitan distribution. AFB's causative agent is *Paenibacillus larvae*. AFB kills infected honey bee larvae; however, it eventually leads to the collapse of the entire colony when left untreated. The infection takes place by the ingestion of the spores with the food provided by adult worker bees to the larvae. In South America (SA) the disease was first described in 1989 in Argentina, constituting the first sanitary challenge for beekeepers to overcome. Prevention and control measures of AFB in SA countries generally include vigilance for early diagnosis, isolation of apiaries with cases of AFB, and multiplication of healthy colonies with hygienic queens, among others. The extensive use of tetracycline hydrochloride in Argentina has led to the development of resistant *P. larvae* isolates. In this context, the development of alternative and effective methods for the control and prevention of AFB disease is crucial. Currently, alternative strategies for the prevention and treatment of AFB are being studied, mainly based on essential oils.

Keywords: *Paenibacillus larvae*, essential oils, quorum sensing, American foulbrood, *Apis mellifera*

1. Introduction

Along with wild bees, honeybees are the most important crop pollinators [1, 2]. *Apis mellifera* pollinates 77% of the plants responsible for producing food resources which sustain the global human population [1]. Since 1998, individual beekeepers have reported the unusual weakening and mortality of colonies, particularly in France, Belgium, Switzerland, Germany, the United Kingdom, the Netherlands, Italy, Spain, and North America [3, 4]. Most scientists agree that there is no single explanation for the extensive colony losses, but that interactions between different stressors are likely involved [5].

American foulbrood (AFB) is the most severe bacterial disease that affects honey bees, having a nearly cosmopolitan distribution (**Figure 1**) [6]. AFB only kills

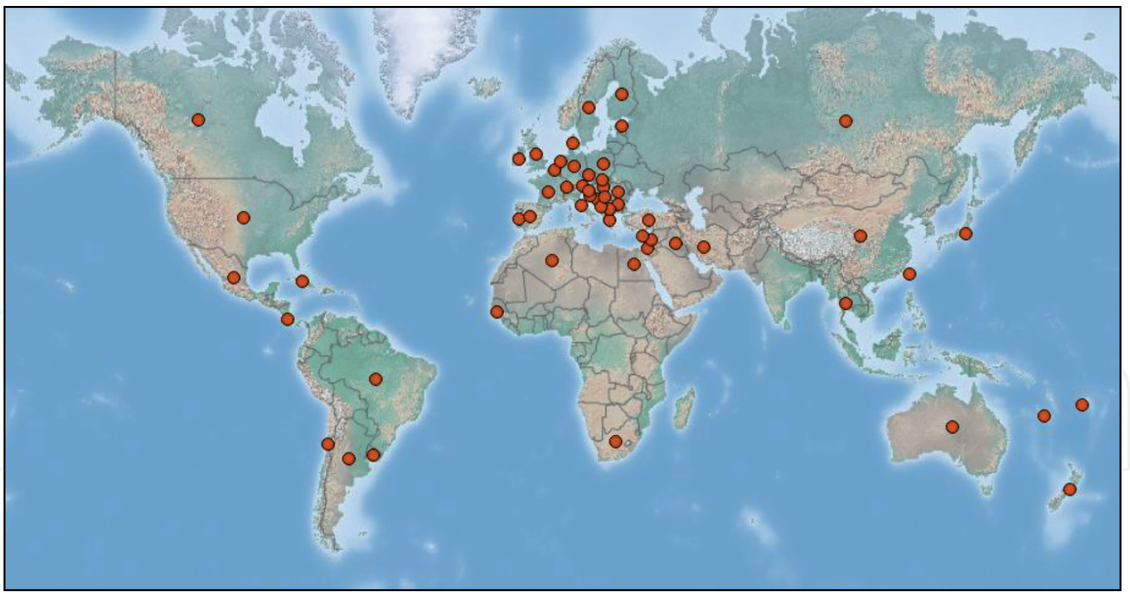

Figure 1.
Distribution of American foulbrood. (https://www.cabi.org/isc/datasheet/78183).

infected honey bee larvae; however, it eventually leads to the collapse of the entire colony when left untreated. AFB is considered to be very contagious; therefore, it is a notifiable disease in most countries [7]. AFB's causative agent is *Paenibacillus larvae*, which is a flagellated gram-positive bacterium, whose main characteristic is the formation of highly resistant endospores. This pathogen affects the breeding during the larval or pupal stages [8]; its spores being the infectious form. Honey bee larvae are more susceptible to infection during the first 36 h after egg hatching [9], indeed only 10 spores are required to make a larva of less than 24 h old ill [10]. However, at later larval developmental stages, spore doses needed to successfully infect a larva are too high to occur under natural conditions [11]. The infection takes place by the ingestion of the spores with the food provided by adult worker bees (nurses) to the larvae [12]. The spores after germinating in the midgut of the larvae proliferate for several days. After this, *P. larvae* reaches the peritrophic matrix, penetrates the epidermal cells, produces septicemia causing death of the larva. Finally, dead larvae are digested by vegetative bacterial cells and converted to dry flakes containing millions of spores of *P. larvae* [12, 13]. The most evident symptoms of AFB are the irregular coating of the offspring, which show cells with cap and uncovered irregularly dispersed through the frames of the offspring; dark, sunken, and often perforated caps emitting a characteristic AFB odor; remnants of brown glue from the dead larvae forming a characteristic cord thread when removed with a wooden stick or an inlay; and a hard scale of larval residues at the bottom of the cell. The traditional diagnosis is made based on the observation of these clinical symptoms in the hive and in the microbial culture of material from infected colonies [14].

AFB was first described in South America (SA) in Argentina, in 1989, constituting the first sanitary challenge for beekeepers to overcome. It was hypothesized that the entrance of *P. larvae*, into the country was through bees imported from the USA [15]. AFB quickly spread to most important beekeeping centers of the country [16], with incidences as high as 30% in some geographic areas [17]. At least 30–45% of the colonies were lost due to AFB during those years (Eguaras, unpublished data). AFB was extended to Chile in 2002 and was controlled. New outbreaks were detected in 2005 in different regions [18] (**Table 1**).

In some countries the use of antibiotics, particularly tetracycline hydrochloride (OTC) [6, 12], is the most common method for prevention and treatment of

Argentina	Restricted distribution	[15, 84]
Bolivia	No information available	[84]
Brazil	Present	[84]
Chile	Present	[84]
Colombia	Disease never reported	[84]
Ecuador	Disease never reported	[84]
French Guiana	Disease never reported	[84]
Peru	Disease not reported	[84]
Uruguay	Present	[84]
Venezuela	Disease never reported	[84]

Table 1.
Distribution of P. larvae in South America.

infected colonies. However, in most European countries the use of antibiotics is banned, since their use is known to generate several problems including the presence of chemical residues in the beehive products (honey, pollen and wax), which eventually may even affect consumer health. Moreover, antibiotic application can affect life of bees and can increase the risk of occurrence of resistant strains [19]. To date, the presence of OTC resistant strains has been reported in Argentina, United States, Italy, New Zealand and United Kingdom [16, 20].

Prevention and control measures of AFB in SA countries generally include vigilance for early diagnosis, isolation of apiaries with cases of AFB, and multiplication of healthy colonies with hygienic queens, among others [21]. Brazilian, Chilean, and Uruguayan authorities specifically recommend the burning of colonies containing clinical signs of the disease in order to control the outbreaks [21]. The use of antibiotics in SA is not allowed, except in Argentina [18]. The extensive use of OTC in this country has led to the development of resistant *P. larvae* isolates [16], which is a major concern for Argentine beekeepers. In contrast, in Uruguay and Chile, where their use is not authorized, no resistant strains have been detected [22]. The endospore resistance of *P. larvae* is an important problem in the control and prevention of AFB because these individuals can survive for more than 35 years in honey and/or beekeeping material and is resistant to high temperatures as well as to the most used disinfectants [10]. Most treatments are based on the use of broad spectrum antibiotics, which, in most cases, have been used continuously and excessively. In fact, different antibiotics, such as sulfathiazole and OTC, are able to inhibit the growth of *P. larvae*, but its use and abuse during the last years has led to the appearance of resistant strains and residues that contaminate the products of the hive. For these reasons, the use of antibiotics for the treatment and prevention of AFB is prohibited in several countries, and the affected colonies must be destroyed [23].

In this context, the development of alternative and effective methods for the control and prevention of AFB disease is crucial. These methods may consider the evidence of the bacteria-resistant phenomenon and meet the strict EU standards, as well as current trends in green consumption [24, 25]. Currently, alternative strategies for the prevention and treatment of AFB are being studied, mainly based on essential oils [25–27], probiotics and propolis [28].

1.1 Essential oils

In light of developments in the scientific field, the medicinal properties of plants have received great interest due to their low toxicity, pharmacological activities

and economic viability [29]. These studies have focused on the benefits of phytochemicals extracted from plants and their effect on human health. The additives naturally obtained from plants can be individual compounds, groups of compounds or essential oils (EOs). In recent times, there has been an increase in the interest of the food industry in natural compounds, either by direct addition or by its use in synergy with other compounds. It has been reported that the direct addition of essential oils and extracts of aromatic plants to food products exerts its antioxidant or antimicrobial effect [30].

Plants and other natural sources can provide a wide variety of complex and structurally diverse compounds. Plant extracts and essential oils have antifungal, antibacterial and antiviral properties and have been evaluated worldwide as potential sources of new antimicrobial compounds, agents that promote food preservation and alternatives to treat infectious diseases [31, 32]. It has been reported that essential oils possess significant antiseptic, antibacterial, antiviral, antioxidant, antiparasitic, antifungal, and insecticidal activities [33, 34]. Therefore, essential oils can serve as powerful tools to reduce bacterial resistance [33]. Oily aromatic liquids called essential oils (also called volatile oils) are obtained from plant materials (leaves, buds, fruits, flowers, herbs, branches, bark, wood, roots and seeds).

Being natural mixtures of very complex nature, the essential oils can consist of approximately 20–60 components at quite different concentrations. Essential oils are characterized by two or three main components that are present in fairly high concentrations (20–70%) compared to other components that are present in trace amounts. The amount of different components of essential oils varies between different parts of plants and different plant species since they are derived chemically from terpenes and their oxygenated derivatives, i.e., terpenoids which are esters of aromatic and aliphatic acid, and phenolic compounds. An important characteristic of essential oils and their components is their hydrophobicity, which allows them to interact with the lipids present in the cell membrane of bacteria and mitochondria, making them more permeable by altering their cellular structures. This eventually results in the death of bacterial cells due to the leakage of critical molecules and ions from the bacterial cell. Some compounds modulate drug resistance by targeting efflux mechanisms in several species of gram-negative bacteria [35]. An important function of essential oils in nature is the protection of plants by acting as antifungal, antibacterial, antiviral and insecticidal agents and also protection against herbivores by reducing the appetite of herbivores for plants with such properties. Health Services and Human Services Public Health Services have recognized essential oils as safe substances, and some of them contain compounds that can be used as antibacterial additives [33]. The efficacy of EOs has been reported in several studies against pathogens and food contaminants [36], suggesting their applications in the food industry [34, 37]. Several EOs have been evaluated for the *in vitro* and *in vivo* control of *P. larvae* (**Table 2**), as well as their acute oral toxicity to *Apis mellifera* (**Table 3**).

1.1.1 In vitro assays to control P. larvae

EOs from *Achyrocline satureioides*, *Carum carvi*, *Cinnamomum* spp., *Cinnamomum zeylanicum*, *Citrus paradise*, *Cuminum cyminum*, *Cymbopogon citratus*, *Eucalyptus cinerea*, *Melaleuca alternifolia*, *Mentha piperita*, *Minthostachys verticillata*, *Origanum majorana*, *Origanum vulgare*, *Polygonum bistorta*, *Salvia officinalis*, *Salvia sclarea*, *Syzygium aromaticum*, *Tagetes minuta*, *Thymus vulgaris*, *Verbena*, *Pimenta dioica* (L.) Merr., *Litsea cubeba* Pers., *Trachyspermum ammi* L., *Mentha arvensis* L., *Mentha spicata* L., *Illicium verum* Hook.f, *Myristica fragrans* Gronov., *Cinnamomum camphora* (L.) J. Presl., *Ocimum tenuiflorum* L., *Daucus carota* L., *Zingiber officinale* Rosc., and

Essential oil	Technique	Activity	Amount tested	MIC[a]	MBC[b]	References
Acantholippia seriphioides A. Gray	Broth macrodilution	Inhibitory		236 mg/L		[26]
	Broth macrodilution	Inhibitory		300 mg/L		[41]
Achyrocline satureioides Lam.	Agar diffusion	Inhibitory	10 μl			[33]
Artemisia absinthium L.	Broth microdilution	Inhibitory		416 mg/L	647 mg/L	[42]
Artemisia annua L.	Broth microdilution	Inhibitory		402 mg/L	624 mg/L	[42]
Aloysia polystachia Griseb.	Broth microdilution	Inhibitory		700–800 mg/L	900 mg/L	[42]
Carapa guianensis Aubl.	Broth microdilution	Inhibitory		25% (v/v)		[27]
Carum carvi L.	Agar diffusion	Inhibitory	5, 10 μl			[35]
Chamomilla recutita L.	Agar diffusion	Non-inhibitory	5, 10 μl			[35]
Cinnamomum aromaticum L.	Agar diffusion	Inhibitory	10 μl			[34]
	Agar diffusion	Inhibitory		0.015% (v/v) (strong activity)		[34]
Cinnamomum camphora (L) J. Presl.	Agar diffusion	Inhibitory	10 μl			[36]
	Broth microdilution	Inhibitory	3200–0.78	286.2 ± 27.9 μg/ml	375.0 ± 34.8	[36]
Cinnamomum glandulifera Nees.	Agar dilution	Inhibitory		700 μg/ml		[40]

Essential oil	Technique	Activity	Amount tested	MIC[a]	MBC[b]	References
Cinnamomum zeylanicum L.	Agar diffusion	Inhibitory	2 mg/ml			[36, 59]
	Broth macrodilution	Inhibitory		58–83 μg/ml	108–112 μg/ml	[42]
		Inhibitory		25–100 mg/L	25–100 mg/L	[45]
		Inhibitory		38–50 μg/ml		[46]
		Inhibitory		25–67 μg/ml		[6]
Cinnamomum zeylanicum + *Thymus vulgaris* L.	Broth macrodilution	Inhibitory		66.6 μg/ml	95.83 μg/ml	[42]
Citrus limon L.	Broth microdilution	Inhibitory		764 mg/L	2293 mg/L	[26]
Cinnamomum zeylanicum + *Thymus vulgaris* L.	Broth macrodilution	Inhibitory		66.6 μg/ml	95.83 μg/ml	[42]
Citrus limon L.	Broth microdilution	Inhibitory		764 mg/L	2293 mg/L	[42]
Citrus nobilis Lour	Broth microdilution	Inhibitory		815 mg/L	2447 mg/L	[42]
Citrus reticulata var. *madurensis* Blanco	Agar diffusion	Inhibitory	10 μl			[34]
	Agar dilution	Inhibitory		0.12–1.0% (v/v)		[34]
Copaifera officinalis L.	Broth microdilution	Inhibitory		1.56% (v/v)		[27]
Copaifera officinalis L. nanoemulsion		Inhibitory		0.39% (v/v)		[50]
Cymbopogon citratus + *Thymus vulgaris* L.	Agar dilution	Inhibitory		25–100 μg/ml		[40]
C. citratus + *T. vulgaris* + *Satureja hortensis* L. + *Origanum vulgare* L. + *Ocimum basilicum* L.	Agar dilution	Inhibitory		25–175 μg/ml		[40]
C. citratus + *T. vulgaris* + *O. basilicum*	Agar dilution	Inhibitory		50–350 μg/ml		[40]

Essential oil	Technique	Activity	Amount tested	MIC[a]	MBC[b]	References
Cymbopogon martini Stapf.	Broth microdilution	Inhibitory		1195 mg/L	1208 mg/L	[42]
Cymbopogon nardus L.	Broth microdilution	Inhibitory		319 mg/L	595 mg/L	[42]
Daucus carota L.	Agar diffusion	Inhibitory	10 μl			[36]
	Broth microdilution	Inhibitory	3200–0.78 μg/ml	412.8 ± 26.0 μg/ml	589.6 ± 48.2 μg/ml	[36]
Eucalyptus cinerea F. Muell	Agar diffusion	Inhibitory	10 μl			[33]
Eugenia spp.	Agar diffusion	Inhibitory				[32]
Illicium verum Hook.f.	Agar diffusion	Inhibitory	10 μl			[36]
Illicium verum Hook.f.	Broth microdilution	Inhibitory	3200–0.78	278.6 ± 21.2 μg/ml	365.0 ± 32.1	[36]
Lavandula officinalis L.	Broth macrodilution	Inhibitory		350–400 μg/ml		[45]
Laurus nobilis L.	Broth microdilution	Inhibitory		1000 μg/ml		[39]
	Broth microdilution	Inhibitory		12,879 μg/ml		[36]
Lepechinia floribunda Benth.	Broth microdilution	Inhibitory		394 mg/L	518 mg/L	[26]
Lippia turbinata Griseb	Broth macrodilution	Inhibitory		866 mg/L		[26]
Litsea cubeba Pers.	Agar diffusion	Inhibitory	10 μl			[36]
Litsea cubeba Pers.	Broth microdilution	Inhibitory	3200–0.78 μg/ml	85.0 ± 7.9 μg/ml	186.0 ± 21.2 μg/ml	[36]

Essential oil	Technique	Activity	Amount tested	MIC[a]	MBC[b]	References
Melaleuca alternifolia Maiden & Betche	Agar diffusion	Inhibitory	10 µl			[34]
	Agar dilution	Inhibitory		0.015–0.12% (v/v) (strong activity)		[34]
	Broth microdilution	Inhibitory		1095 mg/L	1187 mg/L	[42]
		Inhibitory		0.18–1.5% (v/v)		[27]
		Inhibitory		331 mg/L	585 mg/L	[42]
	Broth microdilution	Inhibitory		1000–1800 µg/ml	1600–2000	[81]
Mentha arvensis L.	Broth microdilution	Inhibitory		144.7 ± 17.2 µg/ml	248.0 ± 23.4 µg/ml	[36]
Mentha (hybrid)	Broth microdilution	Inhibitory		600–700 µg/ml	1000–1200 µg/ml	[81]
Mentha rotundifolia L.	Broth microdilution	Inhibitory		600–1000 µg/ml	1600 ≥ 2000 µg/ml	[81]
Mentha spicata L.	Agar diffusion	Inhibitory	10 µl			[36]
	Broth microdilution	Inhibitory	3200 to 0.78 µg/ml	145.6 ± 15.4 µg/ml	256.0 ± 26.5 µg/ml	[36]
Minthostachys mollis Kunth.	Broth macrodilution	Inhibitory		775 mg/L		[42]
Minthostachys verticillata Griseb	Agar diffusion	Inhibitory	10 µl			[33]
Myristica fragrans Gronov.	Agar diffusion	Inhibitory	10 µl			[36]
Myristica fragrans Gronov.	Broth microdilution	Inhibitory	3200–0.78	285.8 ± 29.2 µg/ml	371.3 ± 29.0	[36]
Ocimum basilicum L.	Agar dilution	Inhibitory		350–450 µg/ml		[40]
		Inhibitory		0.06–0.12% (v/v)		[34]

Essential oil	Technique	Activity	Amount tested	MIC[a]	MBC[b]	References
Ocimum tenuiflorum L.	Agar diffusion	Inhibitory	10 μl			[36]
	Broth microdilution	Inhibitory	3200–0.78	412.8 ± 26.0 μg/ml	589.6 ± 48.2	[36]
Pimenta dioica (L.) Merr.	Agar diffusion	Inhibitory	10 μl			[36]
	Broth microdilution	Inhibitory	3200–0.78 μg/ml	78.0 ± 8.2 μg/ml	162.0 ± 18.2 μg/ml	[36]
Pimpinella anisum L.	Agar diffusion	Inhibitory	5 μl			[35]
		Inhibitory	10 μl			[35]
	Broth macrodilution	Inhibitory		300 μg/ml		[46]
Salvia officinalis L.	Agar diffusion	Inhibitory	5 μl			[35]
		Inhibitory	10 μl			[35]
Salvia sclarea L.	Agar diffusion	Inhibitory	10 μl			[34]
	Agar dilution	Inhibitory		0.06% (v/v) (strong activity)		[34]
Satureja odora Griseb.	Broth microdilution	Inhibitory		700–800 mg/L	900 mg/L	[42]
Schinus molle L.	Broth macrodilution	Inhibitory		666 mg/L		[42]
Syzygium aromaticum L.	Agar diffusion	Inhibitory	10 μl			[34]
Syzygium aromaticum L.	Agar diffusion	Inhibitory	5 μl			[35]
Syzygium aromaticum L.	Agar diffusion	Inhibitory	10 μl			[35]
	Agar dilution	Inhibitory		0.015% (v/v) (strong activity)		[34]

Essential oil	Technique	Activity	Amount tested	MIC[a]	MBC[b]	References
Tagetes minuta	Agar diffusion	Inhibitory	10 µl			[34]
	Agar dilution	Inhibitory		500–650 µg/ml		[40]
	Agar dilution	Inhibitory		700–800 µl/L		[48]
	Broth macrodilution	Inhibitory		900–1000 mg/L		[41]
		Inhibitory		833 mg/L		[42]
Thymol (component of *Thymus vulgaris*)	Broth macrodilution	Inhibitory		100–133 µg/ml	133 µg/ml	[26]
Trachyspermum ammi L.	Agar diffusion	Inhibitory	10 µl			[36]
	Broth macrodilution	Inhibitory	3200–0.78 µg/ml	137.0 ± 12.2 µg/ml	224.8 ± 25.6 µg/ml	[36]
Verbena officinalis L.	Broth microdilution	Inhibitory		700–800 mg/L	850 mg/L	[42]
Wedelia glauca Ortega	Broth microdilution	Inhibitory		700–800 mg/L	950 mg/L	[42]
Zingiber officinale Rosc.	Agar diffusion	Inhibitory	10 ml			[36]

[a]*MIC, Minimal Inhibitory Concentration.*
[b]*MBC, Minimal Bactericidal Concentration.*

Table 2.
Essential oils for the in vitro Paenibacillus larvae control.

Essential oil	Technique	Toxicity	Amount tested	References
Carapa guaianensis	Spraying procedure	Non-toxic	25% (v/v)	[27]
Carapa guaianensis nanoemulsion	Complete exposure	Non-toxic	10% (v/v)	[50]
	In-vivo against larva	Slightly toxic		[50]
Copaifera officinalis	Spraying procedure	Non-toxic	1.56% (v/v)	[27]
Cymbopogon citratus	Systemic administration	Moderately toxic (>2 μg EO/bee)	1, 2, 4, 8, 16 and 32 μg EO/bee	[47]
Cymbopogon citratus + *Thymus vulgaris* (20:80, v/v)	Systemic administration	Slightly toxic (24 h-LD_{50} = 15.94 μg b.e./bee)	0.19, 0.37, 0.75, 1.50, 3.0 and 6.0 μg b.e./bee	[47]
Cymbopogon citratus + *Thymus vulgaris* + *Satureja hortensis* + *Origanum vulgare* + *Ocimum basilicum* (5:11:21:26:37, v/v/v/v/v)	Systemic administration	Not determined	1.19, 2.37, 4.75, 9.50, 19.0 and 28.0 μg b.e./bee	[47]
Cymbopogon citratus + *Thymus vulgaris* + *Ocimum basilicum* (10:20:70, v/v/v)	Systemic administration	Virtually non-toxic (24 h-LD_{50} = 122 μg b.e./bee)	0.625, 1.25, 2.5, 5.0, 10.0 and 20.0 μg b.e./bee	[47]
Cinnamomum zeylanicum	Systemic administration	Virtually non-toxic	2000, 4000, 8000 and 16,000 μg/ml	[46]
Eucalyptus globulus	Complete exposure	Non-toxic	2.5, 5, 10 and 20 ml per cage of EO	[44]
Eugenia spp.	Systemic administration	Non-toxic	400 μg/ml	[32]
Melaleuca alternifolia	Spraying procedure	Toxic/non-toxic the nanoparticles of *M. alternifolia*	6.25% (w/v)	[49]
Origanum vulgare	Systemic administration	Moderately toxic (≥ 3 μg EO/bee)	3, 6, 12, 24, 48 and 96 μg EO/bee	[47]
Rosmarinus officinalis	Complete exposure	Non-toxic	2.5, 5, 10 and 20 μl per cage of EO	[51]
Tagetes minuta	Spraying procedure	Non-toxic	5% (w/v)	[48]
Thymus vulgaris	Systemic administration	Moderately toxic (>8 μg EO/bee)	2, 4, 8, 16, 32 and 64 μg EO/bee	[47]

Table 3.
Essential oils toxicity assays on Apis mellifera.

Pelargonium graveolens L., were able to inhibit the growth of *P. larvae* by the agar diffusion technique [38–45].

EOs from *Cymbopogon citratus*, *Cinnamomum aromaticum*, *Citrus reticulata* var. madurensis, *Citrus paradisi*, *Heterothalamus alienus*, *Melaleuca alternifolia*, *Mentha piperita*, *Origanum majorana*, *Origanum vulgare*, *Salvia sclarea*, *Syzygium aromaticum*, *Tagetes minuta*, *Thymus vulgaris*, as well as the mixtures of *Cymbopogon citratus* and *Thymus vulgaris* EOs (20:80, v/v), and *Cymbopogon citratus*, *Thymus vulgaris*, *Satureja hortensis*, *Origanum vulgare*, and *Ocimum basilicum* EOs (5:11:21:26:37, v/v/v/v/v) showed antibacterial activity against *P. larvae* [44, 46, 47].

EOs from *Citrus sinensis*, *Cinnamomum* spp., *Eugenia* spp., *Thymus vulgaris*, *Verberna* spp., *Acantholippia seriphioides*, *Cinnamomum zeylanicum*, *Heterothalamus alienus* Spreng., *Pimpinella anisum*, *Foeniculum vulgare*, and *Eucalyptus globulosus*, and the mixture of *Thymus vulgaris* EO, thymol and *Cinnamomum zeylanicum* EO (62.5:25:12.5, v/v/v) exhibited antibacterial activity against *P. larvae* by the broth macrodilution technique [40, 48–52].

1.1.2 Toxicity assays on Apis mellifera

Citrus sinensis, *Cinnamomum* spp., *Cinnamomum zeylanicum*, *Cuminum cyminum*, *Eugenia* spp., *Thymus vulgaris*, and *Verbena* spp. EOs were non-toxic for adult honey bees when they were fed with candy and the EO at different concentrations by systemic administration [40, 53]. *Cymbopogon citratus*, *Thymus vulgaris* and *Ocimum basilicum* EOs, as well as *Cymbopogon citratus* and *Thymus vulgaris* EO mixture (50:50, v/v) were moderately toxic to adult honey bees. However, the *Cymbopogon citratus*, *Thymus vulgaris* and *Coriandrum sativum* EO mixture (33.3:33.3:33.3, v/v/v) presented negative mortality curves, meaning that there was less mortality at high doses. This fact disclosed that bees did not consume candy with high quantities of *Coriandrum sativum* EO [54]. When a solution containing a certain amount of EO was sprayed over a group of honey bees, *Tagetes minuta*, *Carapa guianensis* and *Carapa officinalis* EOs resulted to be non-toxic for adult bees [27, 55]; whereas *Melaleuca alternifolia* EO caused the death of the bees after 7 days of treatment. Nevertheless, the use of nanoparticles of *Melaleuca alternifolia* EO did not produce any toxic effect on honey bees [56]. *Eucalyptus globosus* and *Rosmarinus officinalis* EOs and the nanoemulsion of *Carapa officinalis* EO were not toxic for adult worker honey bees when they were completely exposed to the EO, that is, bees were in contact with the EO and ingested the EO [50, 57, 58]. The nanoemulsion of *Carapa guianensis* EO exhibited a toxic effect for larvae and adult honey bees, whereas the nanoemulsion of *Carapa officinalis* EO, a low toxic effect on larvae [57].

1.1.3 Mechanism of action of essential oils on P. larvae

Different mechanisms of action of EOs on bacteria have been reported, among others: degradation of the cell wall, affecting the cell morphology and damaging the cytoplasmic membrane; damage of membrane protein, disruption of cell wall, leading to leakage of the cell contents, reduction of proton motive force, reduction of intracellular ATP pool, via decreasing ATP synthesis; inhibition of quorum sensing and alteration of cell division [59]. The alteration of the membrane permeability can be detected by the crystal violet assay [35] and the determination of the released UV-absorbing material assays [60]. The crystal violet assay is based on the fact that the compound enters easily when the cell membrane is defective. The released of UV-absorbing material assays is based on the fact that EOs can disrupt the cell membrane leading to a leakage of the cell content which is measured in the UV spectrum. The relationship between the chemical composition of EOs

and their antimicrobial mode of action against *P. larvae* has not been systematically researched so far. EOs are complex mixtures of low molecular weight volatile constituents biosynthesized by plants, which mainly include two biosynthetically related groups, i.e., terpenes and terpenoids, and aromatic and aliphatic constituents [61]. Most antimicrobial compounds are constitutively expressed by the plants, but others are synthesized as mechanism of defense in response to pathogens [59, 62]. Pellegrini et al. [62] demonstrated that the essential oils of *Acantholippia seriphioides*, *Aloysia polystachia*, *Buddleja globosa*, *Lippia turbinata*, *Minthostachys mollis*, *Schinus molle* and *Solidago chilensis* permeabilized and altered the cell membrane and the cytoplasmic membrane of *P. larvae* causing the leakage of cytoplasmic constituents.

1.1.4 Anti-quorum sensing and antimicrobial activity of essential oils

Antúnez et al. (2010) [70] determined that during the division *P. larvae* produces and secretes different proteins with proteolytic activity, such as metalloproteases and enolase, these proteins are secreted and remain on the surface of the spores, producing a response in the immune system of *A. mellifera* and are probably involved in the degradation of larval tissue.

In recent years, the detection of quorum sensing (QS) detection signals in bacteria has added a new dimension to study the infection process. Through QS, bacteria depending on population density can activate specific genes [63–66]. The QS can regulate the expression of virulence factors, bioluminescence, sporulation, biofilm formation and conjugation [67–69]. Many bacteria coordinate the expression of multiple virulence factors, such as toxins, active redox compounds, siderophores, exoproteases, lipases and biofilm formation, thus maximizing the chances of infection and allowing better propagation [70, 71].

The QS signals occur while the bacterial population grows until it reaches a threshold concentration perceived by the bacteria and results in the activation or repression of specific genes. The accumulation of a stimulant amount of such molecules can occur only when a specific number of cells, known as a quorum, is present. These self-inducing molecules have been identified as acylated homoserine lactones in gram-negative and oligopeptide bacteria, thiolactone/lactone peptide, lanthionines, isoprenyl groups [65] and even acylated homoserine lactones in gram-positive bacteria [72, 73]. Similar signaling mechanisms have not yet been demonstrated in *P. larvae*. It is possible that larval infection by *P. larvae* is influenced by phenotypes regulated by QS, such as proteases exported by bacteria to their environment. The concept of QS has encouraged the development of a new non-antibiotic antibacterial therapy through the use of QS inhibitor compounds [74, 75].

The increase in resistance to multiple drugs of the bacteria against traditional medicines drastically reduces the efficacy of conventional antibiotics. This multiple resistance is now recognized as a global problem [76]. Therefore, it is necessary to develop a new therapeutic strategy to prevent this type of multidrugging. A promising mechanism is to block cell-to-cell communication, establishing a strategy called quorum extinction [77]. Although traditional antimicrobial agents cause cell death of the pathogen, the use of systems that alter the QS sensors adopts a less aggressive strategy [78]. There are several sources of QS inhibitors (quorum quenchers), but so far the most diverse and abundant are derived from natural sources such as algae and plants. There are cases of QS inhibitors in bacteria, fungi, algae, bryozoans, corals, sponges [79], plant extracts [80], essential oils [42], compounds isolated from bacteria [81] and furanones, among others.

Essential oils extracted from plants, such as *Cymbopogon citratus*, *Cymbopogon martini*, *Rosmarinus officinalis*, *Mentha piperita*, *Pelargonium odoratissimum* and

Negundo vitex, and different products, such as citral, geraniol, thymol and the linalool, have been used to evaluate its protease inhibitory activity, constituting one of the virulence factors of bacteria that can be regulated by QS [82].

Pellegrini et al. [62] propose that the EO will act by inhibiting the production of proteases, inhibiting its transportation and secretion, inhibiting the detection of quorum or avoiding the loading of proteases. All extracellular bacterial proteases are synthesized as an inactive pre-proenzyme consisting of a signal peptide, a prosequence and a maturity sequence. The peptide functions as a signal for the translocation of the pre-proenzyme to the membrane. The pre-proenzyme is processed in the proenzyme by the peptidase signal. The accusation acts as a molecular chaperone that leads to a self-cleavage of the peptide bond that links the pro and mature sequences [83]. The EOs acted at some point in this regulatory mechanism. The inhibition of larval proteases by EO could be a form of therapeutic intervention; the blocking of bacterial virulence factors does not destroy or inhibit the growth of pathogenic bacteria. It is expected that this strategy will generate little pressure on the selection of bacteria and, therefore, could diminish the appearance of bacterial resistance and avoid the interruption of the microbiota of benefits in urticaria. In future investigations, it will be interesting to isolate and characterize automatically the potential autoinductors of *P. larvae* and study their relationship with protease regulation. EOs studies are promising to use EOs in hives with symptoms of Foulbrood for the control of damage caused by *P. larvae*.

2. Conclusion

The research carried out to study the *in vitro* and *in vivo* antimicrobial activity of essential oils against *P. larvae*, their toxicity in adult honey bees, as well as their mode of action (degradation of the cell wall, affecting cell morphology and damaging the cytoplasm membrane, coagulation of the cytoplasm, etc.) and anti-QS activity (inhibiting the production of proteases, inhibiting transportation and secretion of proteases, inhibiting the detection of quorum, etc.), has been thoroughly reviewed throughout this chapter. As far as honeybee larvae are the target of AFB disease, future research should focus on studying the effect of essential oils that are effective *in vitro* and non-toxic for adult honey bees on honeybee larvae. In addition, more studies are still needed on the distribution and effects of these natural products in hives, adult honey bees, larvae, honey, royal jelly and other bee products to understand the pharmacokinetics and pharmacodynamics within the hive. As well, research on the effectiveness of these natural antimicrobials in field conditions is imperative. Moreover, further studies should be conducted on the sporicidal properties of these natural substances to destroy spores of *P. larvae* for the prevention of AFB disease. And last but not least, the development of adequate delivery modes of the essential oils within the hives for *in vivo* treatment and prevention of the disease is another important issue that requires further research, to put these natural strategies into practice under true hive conditions.

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