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Beekeeping
New Challenges

Edited by Ramón Eduardo Rebolledo Ranz

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Contributors
Zahra Ayoub, Luciano Pilati, Paolo Fontana, Sandra Rosa Fuselli, Pablo Gimenez Martinez, Rosa Maria Alonso-Salces, Giselle Fuentes, Matias Maggi, Karuane Saturnino Da Silva Araújo, Bruno Araújo, Diego Carvalho Viana, Leonardo Oliveira, Eline Pacheco, Ila Raquel Cardoso, Jailson Honorato, Dark Luzia Neto, Virlane Kelly Hunaldo, Claudia Lima Dias, Ivaneide De Oliveira Nascimento, Djany Souza Silva, Ramón Eduardo Rebolledo Ranz

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Meet the editor

Dr. Ramón Eduardo Rebolledo Ranz has been an agronomist since 1986 at the Universidad Austral de Chile. He received his PhD in Agricultural Engineering from the Polytechnic University of Madrid. He has been working at the University of La Frontera since 1986, responsible for agricultural entomology, apiculture and zoology. He has been a guide professor of more than eighty undergraduate theses in the careers of agronomy, forestry engineering, and natural resources engineering. He has also directed theses of Master and Doctorate students. He participates in postgraduate examining commissions in different Chilean universities. He has participated in more than fifteen research projects on pests and bees with more than 80 scientific articles in national and international journals on the specialty. He has authored a book and three book chapters, in addition to being editor of two books and has created original class books for entomology subjects. He is currently working on writing a book on beekeeping and another on insects associated with sport fishing. He has presented more than 100 papers in national and international scientific congresses. He is recognized on the Who is Who platform. He has organized and chaired the organization of Scientific Congresses of Entomology, Management of Renewable Natural Resources and Apiculture of national and international character. He is a Scientific Advisor of the National Apicola Network of Chile. He is currently working in the organization of XIV Latin American Congress of Beekeeping to be held in Temuco (Chile) in 2020 and participates in the Commission that will apply to organize the World Beekeeping Congress (Apimondia) in 2023. He has held different management positions within the University of La Frontera where he works.

Contents

Preface

This book will be very useful for beekeepers, undergraduate and postgraduate students, public institutions, private companies, and independent people who look for a way of life and sustenance in bees.

Researchers of the highest academic level and with vast professional trajectory participated in the creation of this book, which contains articles to directly serve as a support to the productive and academic activity and, also in the elaboration of public policies in the field of beekeeping, nationally and globally.

The authors who participated in the preparation of this book are bee specialists from different countries, which ensures a systemic approach to the problems and the way to cope with such problems globally.

Finally, the editor of this book wishes to emphasize that special care has been taken to ensure the high quality of the written articles. Furthermore, he wants to thank and recognize the effort made by all the authors to produce this work. All the chapters published here correspond to results of cutting-edge research in the area of beekeeping worldwide.

Ramón Eduardo Rebolledo Ranz
University of La Frontera,
Chile

Chapter 1

Introductory Chapter: Actuality and Trends of Beekeeping Worldwide

Ramón Rebolledo Ranz

1. Introduction

Both biodiversity and food production rely heavily on the role that play pollinating insects, especially honeybees (*Apis mellifera*), so much so that of the 100 cultivated species that give 90% of the world's food, 70% are pollinated by bees and other native insects. In Europe it is estimated that 84% of commercial crops and 80% of natural plants depend on bee pollination. It is estimated that the contribution of bees to pollination corresponds to 22,000 million euros for Europe and 265,000 million dollars for the rest of the world.

Nonetheless, bee populations are suffering a sharp decline in their populations, due among other things to loss of environment, inadequate agricultural practices such as monocultures and pesticides, new diseases and parasites, and climate change. On the other hand, the significant increase in the world's population has brought with it a greater demand for food, which is increasingly innocuous and of better quality. To respond to this great demand, it has been necessary to resort to an increasing use of inputs such as fertilizers, pesticides, and growth regulators, among others, which affect various organisms of the ecosystem including natural pollinators and honeybees.

There is a growing concern in regards the colony collapse (CCD) issue over the world. There is a wide range of literature on how to solve this problem and which are the economic and ecological implications this represents. So much so that the European Economic Community has already banned the use of Neonicotinoid pesticides, considered as one of those responsible for the loss of hives in the world. Despite the aforementioned problems faced by beekeepers, the commercial exchange of honey has had an important increase of 12% annually in its value and 8% in quantity, being China, New Zealand, Argentina, Mexico, and Germany the main honey exporting countries (**Figure 1**), while the United States, Germany, France, the United Kingdom, and Japan are the main importing countries of honey in the world (**Figure 2**)

The honey production of bees has shown an important growth in the last decades with 1,860,712 tons (**Figure 3**), growth mainly given by the Asian continent. On the other hand, the number of hives has had a significant increase (**Figure 4**), this being higher than the 80 million hives.

In most of the continents, there have been few significant changes in honey production in the last 10 years, with the exception of Asia which has shown strong growth due to the important influence of China. However, even though there is growth in terms of the number of hives and the production of honey, new problems have appeared in the world concert, such is the case of adulterated and counterfeit

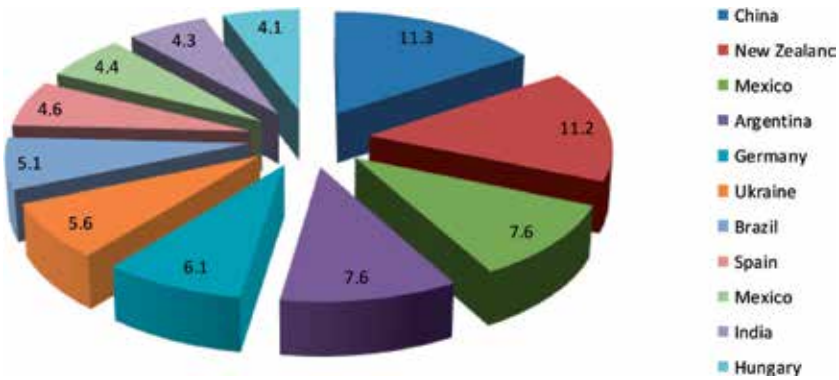

Figure 1.
Leading countries in the export of honey and percentage of participation in the world market year 2017 (FAO source, FAOSTAT) [1].

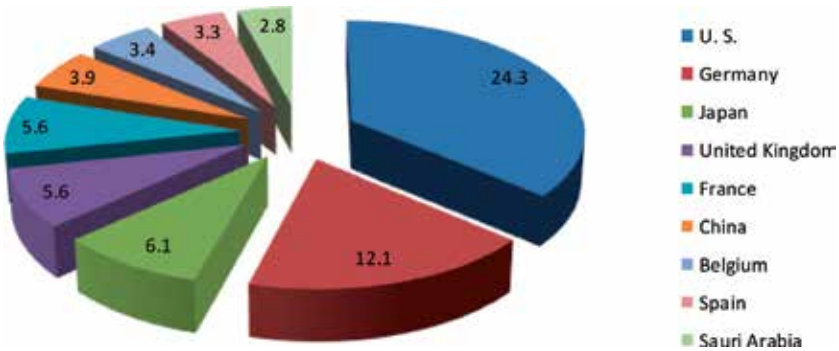

Figure 2.
Main countries importing honey and percentage of participation year 2017 (FAO source, FAOSTAT) [1].

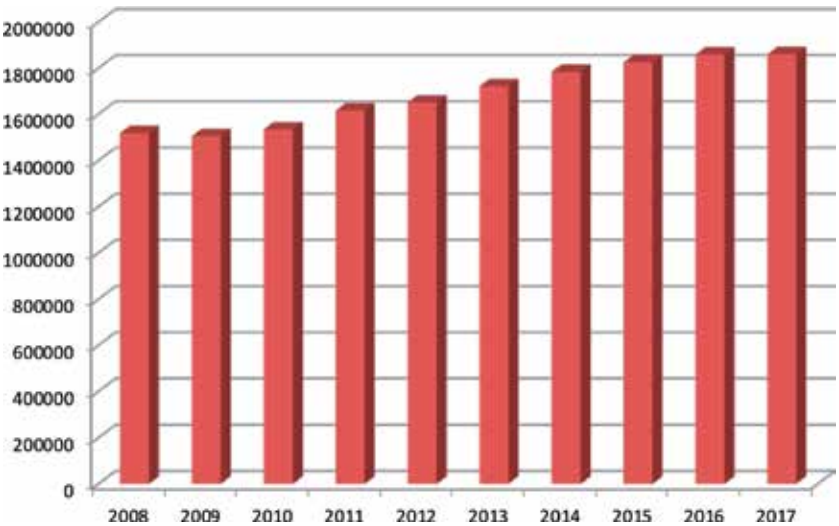

Figure 3.
World honey production year 2008–2017 in tons of honey.

honeys, which, in the particular case of China, has been discovered by commercializing counterfeit and adulterated honeys that seriously affect countries that cannot compete in these conditions. In recent years, the production of honey has

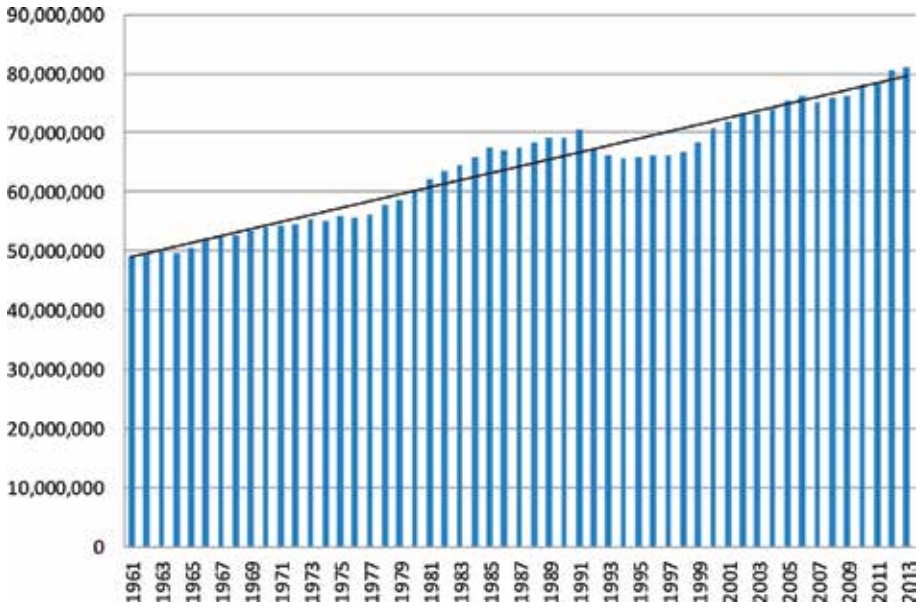

Figure 4.
Number of beehives worldwide.

suffered severe losses due to climate change conditions (with extreme droughts), and in particular, the application of glyphosate herbicide that appears in honey in crops and complicates and slows its commercialization is the main challenge facing beekeeping today.

Author details

Ramón Rebolledo Ranz
Faculty of Agricultural and Forestry Sciences, University of La Frontera, Temuco, Chile

*Address all correspondence to: ramon.rebolledo@ufrontera.cl

Reference

[1] FAO STAT 2018. Available at: www.fao.satat.org/statistic/e/ visited may 2019

Chapter 2

Sequencing the Movements of Honey Bee Colonies between the Forage Sites with the Microeconomic Model of the Migratory Beekeeper

Luciano Pilati and Paolo Fontana

Abstract

A beekeeper who moves his honeybee colonies from one forage site to another during the productive season does not passively follow a prefixed sequence, but must create one by comparing a wide range of forage sites. How can migratory beekeeper sequence the movements of his honeybee colonies from one forage site to another? The microeconomic model formalized in Section 3 offers a solution to this question. The model assumes that the migratory beekeeper is following, in conditions of certainty, a profitability target under the constraint that the time taken up by each sequence of sites is less than or equal to the duration of the honeybee colonies' annual biological cycle. Each forage site that the honeybee colonies visit contributes not just to the profitability but also to the sustainability of the sequence to which it belongs. Replacing one or more forage sites within a sequence therefore simultaneously affects the levels of profitability and sustainability. In Section 4, the sustainability of the sequence will be explained in terms of the characteristics of the sites, their agro-environmental context, the honey bee well-being and the timing and duration of the placement period of the honeybee colonies on the site.

Keywords: migratory beekeeper, forage sites, sequential movements, microeconomic model, ecosystem service, sustainability

1. Introduction

The honey bee colony is a moveable organism, which is easy to transport and manage; it is suitable for pollinating a very wide range of wild and cultivated vegetation [1, 2]. Thanks to its polylectic nature, the honey bee colony is widely used throughout the world for cross-pollination of crops.

The widespread use of managed honey bee colonies to pollinate crops is a response to the need to compensate for the pollination deficit resulting from the decline in wild pollinating insect populations [3–6]. Although growers use managed honey bee colonies, they are able to ensure consistent pollination of their crops, even when adverse climatic conditions limit the pollination range of wild pollinators.

Crop pollination is optimized by placing the honey bee colonies on the forage site once flowering is underway (when 20–40% of the flowers are open) in small batches according to a precise density per acre—the stocking density [7]—which varies according to the crop requiring pollination. The honey bee colonies must be removed before the resumption of antiparasite treatments on the forage site to avoid harming their health. The commercial pollination service therefore enters the cultivation process at a precise point in time determined by the flowering periods of the crops.

The spread of the practice of commercial crop pollination has given rise to a specific market [8–11] with prices, that is, with colony rental fees, varying from crop to crop and from year to year [12–14]. This market provides economic benefits both to the beekeeper and, perhaps even more so [15], to the grower. The former collects the colony rental fees paid by the grower for pollination and in addition, if the pollinated crop produces valuable nectar, obtains an income from honey production; the latter benefits from increased crop yield and/or improvement in the quality of the fruit [16, 17].

Plants flower in succession throughout the year and beekeepers can move their honey bee colonies from one forage site to another to meet the sequential demand for pollination services and/or to produce honey. Empirical observation reveals that crop pollination services take place mainly in the spring, while spontaneous/wild vegetation pollination services take place mainly in the summer. By moving the honey bee colonies between forage sites covered with spontaneous vegetation in full bloom, beekeepers can increase their honey yield and produce monofloral honey, which is highly sought-after by consumers.

The migratory beekeeper cannot passively follow a preset sequence because of changes over the years in pollination calendars, as a result of climate changes shifting the onset of the flowering period [18]; the price of honey and commercial pollination services; production factor costs; and forage sites available for the movement of honey bee colonies. In the USA, where commercial crop pollination is a well-established agricultural practice, the migratory routes most frequently taken by beekeepers are becoming clearly defined [13, 19, 20]. Jabr [20] notes in this regard, "*After the almond bloom some beekeepers take their honeybees to cherry, plum and avocado orchards in California and apple and cherry orchards in Washington State. Come summer time, many beekeepers head east to fields of alfalfa, sunflowers and clover in North and South Dakota, where the bees produce the bulk of their honey for the year. Other beekeepers visit squashes in Texas, clementines and tangerines in Florida, cranberries in Wisconsin and blueberries in Michigan and Maine. All along the east coast migratory beekeepers pollinate apples, cherries, pumpkins, cranberries and various vegetables. By November, beekeepers begin moving their colonies to warm locales to wait out the winter: California, Texas, Florida and even temperature-controlled potato cellars in Idaho. The bees stay inside their hives, eating the honey they made in the summer and fall*." Migratory beekeepers need, therefore, to sequence their honey bee colony movements from one forage site to another; in other words, they must plan the migratory route they will follow during the year. To this end, they have to evaluate a range of forage sites in different locations [21, 22] and with different botanical and economic characteristics. The question that arises here is how can migratory beekeepers sequence the movements of their honey bee colonies and how can they determine the best sequence?

The microeconomic model formalized in Section 3 solves this problem by sequencing the movements of honey bee colonies and drawing up a ranking of the most profitable sequences. The model is microeconomic in that it establishes revenues, costs, profit and the gross income to be drawn from the sequences of

sites that the beekeeper may follow assuming conditions of certainty; it also has operational capacity and can simulate the effect of variation in output prices on the sequence ranking [23].

Pollination of the forage site contributes to both the profitability and the sustainability of the sequence to which it belongs [24]. When the forage site is covered by spontaneous vegetation, in addition to producing honey, the colonies also provide an ecosystem pollination service that helps maintain the sustainability of the local ecosystem by propagating numerous spontaneous/wild plant species [2]. In Section 4, the sustainability of the sequence will be correlated with the characteristics of the forage sites, their agro-environmental context, the honey bee well-being, the timing and duration of the placement period of the honey bee colonies on the site.

2. Methods

This chapter has an exclusively theoretical profile; in the absence of materials to comment, the focus will be on the methodological aspects.

The decision of the migratory beekeeper in order to sequence the movements of the honey bee colonies over the year can be assessed in terms of both profitability and sustainability. The interface between these two evaluations consists of the sequence of sites implemented by the migratory beekeeper. In other words, the sequence of sites represents the innovative methodological tool for correlating profitability and sustainability.

The evaluation of the profitability of the sequence is based on the microeconomic model of the migratory beekeeper. The model is formalized in stages: (a) for each site, the model calculates the revenue multiplying the quantities of honey and commercial pollination services produced on the site for the corresponding prices; (b) the variable costs of the site correspond to the sum of the costs of the variable factors applied on the site itself; (c) the revenue of the sequence is obtained by adding the revenues of the sites that form it; (d) the variable cost of the sequence is obtained by adding the variable costs of the sites that form it; (e) all costs that do not depend on the composition of the sequences are considered fixed; (f) the profit of the sequence is calculated by subtracting the variable cost and the fixed cost from the revenue; and (g) the gross income of the sequence is obtained by adding the gross incomes of the sites that form it.

In the composition of the sequences, the migratory beekeeper must respect an inviolable temporal constraint: the sum of the durations of the periods of colony stationing on the sites (placement periods) must not exceed the duration (365 days) of the annual biological cycle of the bee colonies. This is an innovative methodological aspect of the migratory beekeeper model.

The sequencing of sites is based on the start and the end dates of honeybee colony placement periods on forage sites. Forage sites with overlapping placement periods are alternative; otherwise, the sites are complementary. The inclusion of a site in the sequence involves the exclusion of all those alternatives to it. The model of the migratory beekeeper formalizes this condition by assigning the value 1 or 0 to a dummy variable that establishes the inclusion of the site in the sequence or its exclusion. The sequence is therefore composed only of sites that are complementary to each other from a chronological point of view.

The composition of the sequences is done by applying a recursive procedure to the complete set of sites that the migratory beekeeper can visit. After having numbered the sites in chronological order, according to the start date of the placement period, the method starts from the first site and ends in the overwintering

site, which is the base site. All the sequences that the migratory beekeeper can implement are identified proceeding recursively. In order to reduce the number of sequences to be computed, the recursive procedure may be subject to compliance with certain efficiency conditions. Finally, the ranking of the sequences is established by the level of gross income they could reach.

Conducting the evaluation of the sustainability of all the sequences that the migratory beekeeper can implement would be extremely dispersive and wasteful. It is necessary to limit the evaluation to the sequences that reach a satisfactory level of profitability and therefore appear in the first places of the ranking. Not having a case study to deal with, the chapter can only outline the link between sustainability and the explanatory variables of sites and sequences.

The relationship between the sustainability of the sequence and the sustainability of the sites that compose it is not however simple and additive because the conditions of sustainability on the sites are not independent of each other. For example, the winter mortality rate of the honeybee colonies is not only explained by the conditions observed on the wintering site but also may derive from other causes that change with the composition of the sequence. In general, the consistency of the population of a honeybee colony observed on a site critically depends on the characteristics of the previous sites. In some situations, the contamination of honey bees with harmful substances on a site could exert its effects on subsequent sites. The assessment of the sustainability of the sites therefore also presupposes the identification of the complete sequence. The evaluation of the sustainability of a sequence must also take into account the ecosystem service that the honey bees of the migratory beekeeper can perform at each site. The possibility of interaction with the spontaneous flora present in the various sites must therefore also be considered. Innovative from the methodological point of view is also the evaluation of the aspects concerning the biological and genetic peculiarities of the honeybee (*Apis mellifera* Linnaeus, 1758). The sustainability of the entire sequences must then take into account the ecological and ethological requirements of honey bees. In this regard, the number of sites per sequence is an aspect that significantly influences the overall sustainability of the sequences. Following these simple criteria, completely new in the discussions on the sustainability of beekeeping practices, some sequences (even for only one site) could be unsustainable because they cause serious damage to honey bee conservation and therefore to the conservation of biodiversity.

3. Sequencing profitability: The microeconomic model

The microeconomic model formalized in this section assumes that the migratory beekeeper has already selected feasible forage sites. In order to sequence the movements of the honey bee colonies over the year, the migratory beekeeper must first draw up a the list of forage sites compatible with the array of available fixed factors, in particular the means of transport and labor. It is essential that the list of sites is drawn up before the start of migration because an agreement has to be made with the owner of the forage site, sometimes with the help of a bee broker [7], before the bee colonies can be placed there.

The microeconomic model formalizes the process of chronological accumulation of the revenues, variable costs and profit (gross income) of the sequences. The technical unit that is moved sequentially from one forage site to another is an apiary, managed in a nomadic way and formed of a number of honey bee colonies that is, by assumption, invariant during their annual biological cycle. The productive scale of the beekeeping activity is therefore settled in advance. Simplified, honey bee

colonies have two market outputs: honey and the commercial pollination service. These outputs are differentiated by the type of vegetation found on the forage sites; by the same token, the prices of the outputs obtained on the forage sites also differ. Of course, honey bee colonies also produce pollen, propolis, royal jelly, wax, bee venom and bees. For the sake of simplicity, the model takes into account only the most important physical output in terms of income generation: the honey.

3.1 The forage site

We define a forage site as field covered by vegetation from which honey bees may collect nectar. The most important characteristics of the foraging site from a beekeeping perspective are (a) its location, (b) its size, (c) the vegetation covering it and (d) the flowering period.

Regarding the location of the forage site, the distance between it and the preceding one in the sequence is of great importance because it affects the time and cost of transporting the honey bee colonies. Regarding the vegetation, it is assumed that the forage sites are covered either by a crop or by spontaneous vegetation. Within each of these two basic types of vegetation, there are differences in terms of their botanical characteristics. All forage sites are by assumption monofloral; as a consequence, each forage site has a single flowering period and cannot appear more than once in the sequence. Each forage site is associated with a period during which the honey bee colonies are placed there, delimited by start and end dates. In addition to the time during which nectar is collected from the flowers, the placement period also includes the time taken to transport the honey bee colonies to the site from the one preceding it in the sequence.

3.1.1 Revenues from the site

The revenue generated by the honey bee colonies on a forage site jth belonging to the sequence has two components: the revenue from honey and the revenue from the commercial pollination service. The revenue of each component is calculated by multiplying the quantities produced for the respective prices:

$$R_{ji} = RH_{ji} + RS_{ji} = PH_j \cdot QH_{ji} + PS_j \cdot QS_{ji} \quad (1)$$

where R_{ji} = revenue from the jth site in the sequence ith; RH_{ji}, RS_{ji} = revenue from honey and commercial pollination service on the jth site in the sequence ith; QH_{ji}, QS_{ji} = honey and commercial pollination service produced on the jth site in the sequence ith; PH_j, PS_j = price of the honey and commercial pollination service on the jth site; $j = 1, 2, \ldots s$ = sites; and $i = 1, 2, \ldots n$ = sequences of sites.

The honey bee colony's annual biological cycle is divided into two phases: the first is the productive phase, which takes place on the forage sites $j = 1, 2, \ldots s-1$; the second is the wintering phase of the honey bee colony, which takes place on the site $j = s$; the honey bee colonies produce neither honey nor commercial pollination services on the latter site. The base site where the honey bee colonies overwinter is not strictly speaking a forage site because there does not need to be any vegetation.

All the honey bee colony placement periods on the forage sites $j = 1, 2, \ldots s-1$ are, for assumption, fixed and are independent of the sequences, while placement on the base site $j = s$ begins at the end of the placement period of the honey bee colonies on the penultimate forage site of the sequence, which varies with the sequences. The prices PH_j, PS_j of the honey and the commercial pollination service are assumed to be exogenous or independent of the quantities produced by the beekeeper. The

pollination fee per colony (hive) is the price of commercial pollination service. Output prices change with the type of vegetation on the forage site but are independent of the sequence to which the site belongs. We assume that the honey bee colonies produce on each site a quantity of extractable honey. The amount of commercial pollination service produced on the site is equal to the number of colonies used in the pollination of crop. The quantities of outputs obtained on a given site may vary with the sequence to which the site belongs.

3.1.2 The variable costs of the site

Instantaneous production models make a distinction between fixed and variable costs, which relate to the effects of variations in the quantity produced. In the model of the migratory beekeeper who conforms to the sequential production, step-by-step production during the year [23], fixed and variable costs are instead classified on the basis of the effects caused by replacing a forage site within the sequence. For example, the costs relating to monitoring the health of the honey bee colonies are variable as they vary from site to site depending on how long the honey bee colonies remain there and hence with the sequences. The costs involved in providing the honey bee colonies with supplementary feed are also variable because it is only needed on some forage sites. Health treatments for honey bee colonies are classified as fixed costs as they must be carried out on specific dates regardless of the forage site on which the honey bee colonies are located. Costs relating to the rates of depreciation of the buildings, mechanical devices and equipment used by the beekeeper are also, as is usually the case, fixed. Ultimately, all costs that remain constant, regardless of any changes in the composition of the sequences, are fixed. Fixed costs are therefore the same for all sequences.

The variable cost VC_{ji} of the jth site in the sequence ith is obtained by summing the costs vc_{jik} of the k = 1, 2,...m variable production factors used on the site itself:

$$VC_{ji} = \sum_{k=1}^{m} vc_{jik} \qquad (2)$$

The variable costs of a given site may vary with the sequence to which the site belongs. The base site $j = s$ also generates variable costs, even though it neither produces honey nor provides a commercial pollination service.

3.2 The sequence of forage sites

A sequence is defined as a series of sites that are chronologically complementary. Each sequence differs from another in at least one of the forage sites that form it. All the sequences begin at the base site of the previous biological cycle of the honey bees and end at the base site $j = s$. In order to assemble sequences, forage sites are classified as either alternative or complementary. Forage sites with overlapping placement periods are alternative; otherwise, they are complementary. The inclusion of a forage site in a sequence implies the exclusion of all those that alternate with it. Each sequence is therefore made up only of sites that are chronologically complementary.

3.2.1 The revenues from the sequence

The revenue generated by a sequence is calculated by summing the revenues of the forage sites that form it:

$$R_i = \sum_{j=1}^{s} (RH_{ji} + RS_{ji}) \cdot D_{ji} = \sum_{j=1}^{s} (PH_j \cdot QH_{ji} + PS_j \cdot QH_{ji}) \cdot D_{ji} \qquad (3)$$

where R_i = revenue from the ith sequence; RH_{ji}, RS_{ji} = revenue from the honey and commercial pollination service on the jth site in the ith sequence; and i = 1, 2,...n = sequences of forage sites.

The jth forage site is established as belonging to the ith sequence by the value assigned to the dummy variable: D_{ji} = 1 if the site jth belongs to the ith sequence; D_{ji} = 0 otherwise.

3.2.2 Variable and fixed costs of the sequence

The variable cost of the sequence VC_i is calculated by summing the variable costs of the sites VC_{ji} that comprise it. The fixed production cost is, as mentioned above, the same for all the sequences: $FCi = FC \ \forall i$th sequence.

The total production cost C_i of each ith sequence corresponds to the sum of the fixed cost FC_i and the variable cost VC_i.

$$C_i = FC_i + VC_i = FC + \sum_{j=1}^{s} VC_{ji} \cdot D_{ji} \tag{4}$$

3.3 The profitability of the sequence

The profit that the beekeeper draws by following the ith sequence will be:

$$\pi_i = R_i - C_i = \sum_{j=1}^{s} \left(PH_j \cdot QH_{ji} + PS_j \cdot QH_{ji}\right) \cdot D_{ji} - FC - \sum_{j=1}^{s} VC_{ji} \cdot D_{ji} \tag{5}$$

where π_i = profit from the ith sequence.

Shifting the fixed cost to the first member of Eq. (5), we get:

$$GI_i - \pi_i + FC = \sum_{j=1}^{s} \left[\left(PH_j \cdot QH_{ji} + PS_j \cdot QH_{ji}\right) - VC_{ji}\right] \cdot D_{ji} = \sum_{j=1}^{s} gi_{ji} \cdot D_{ji} \tag{6}$$

where GI_i = gross income from the ith sequence; gi_{ji} = gross income from the jth site of the ith sequence; and $gi_{ji} = R_{ji} - VC_{ji}$.

The gross income from each sequence is obtained by summing the gross incomes obtained from each of the sites that comprise it.

The ranking of the sequences remains unchanged regardless of whether it is drawn up on the basis of profit or gross income. The latter is obtained by adding a constant to the profit drawn from the sequence. Drawing up a ranking of sequences based on gross income has, however, an obvious operational advantage because it does not require migratory beekeepers to know their fixed costs, which they often do not record in their business accounts.

3.4 The constraint of the time

In microeconomic models of the farm, the allocative constraint is typically constituted by the amount of the land [25, 26]. The total amount of this input is fixed, but it may be allocated among the crops. The constraint requires the sum of the areas allocated to crops, all rivals, to be less than or equal to the total amount of land on the farm. In the case of the migratory beekeeper, the total area of the forage sites does not constitute an operational constraint because each sequence includes only some forage sites with complementary placement periods. In the case of the migratory beekeeper, which is similar to that of sea fishing [27], the main allocative constraint is constituted by the time that may be allocated to each

sequence of sites. At each site is associated a placement period of the honey bee colonies characterized by the start and end dates, as well as a duration (number of days). For any sequence of sites, the sum of the durations of the honey bee colonies' placement periods must be equal to or less than the amount of time available. The sum of the placement periods of any sequence must therefore be equal to or less than 365 days.

$$\begin{aligned} &\sum_{j=1}^{s} z_j \cdot D_{ji} \le 365 \quad \forall\ i\ \ th \text{ sequence} \\ &D_{ji} = 1 \quad \text{if the site } j-th \in i-th \text{ sequence} \\ &D_{ji} = 0 \quad \text{otherwise} \end{aligned} \tag{7}$$

where z_j = placement period (number of days) of the honey bee colonies on the site jth.

Between the end of the honey bee colonies' period of placement on one site and the beginning of the placement on the next site, there may be an empty period, a phase when the honey bee colonies are inactive. The beekeeper may decide to transfer them to an emergency site or keep them on the site after the end of flowering or move them earlier to the next site. The occurrence of an unproductive phase means that the sum of the periods of time that the honey bee colonies spend on the sites in a given sequence may be less than the annual amount of time. The variable costs that the honey bee colonies incur during the unproductive period of time are to be attributed to the entire sequence. Operationally, these variable costs are associated with the base site $j = s$. The same goes for those variable costs due to any delays that may arise in starting the sequence.

3.5 The complete model

The microeconomic model of the migratory beekeeping may be specified in the following form:

$$\begin{aligned} &GI_i - \sum_{j=1}^{s} (PH_j \cdot QH_j + PS_j \cdot QH_j - CV_{ji}) \cdot D_{ji} - \sum_{j=1}^{s} gi_j \cdot D_{ji} \\ &\text{s.t.} \sum_{j=1}^{s} z_j \cdot D_{ji} \le 365 \\ &D_{ji} = 1 \quad \text{if the site } j-th \subset i-th \text{ sequence} \\ &D_{ji} = 0 \quad \text{otherwise} \end{aligned} \tag{8}$$

The exogenous variables of model (8) in conditions of certainty are the prices, the quantities of outputs, the variable cost of each site and the placement periods of each forage sites. The value of the *dummy* variable D_{ji} is defined on the basis of the start and end dates of the placement periods. The ranking of the sequences in terms of gross income obtained can be determined on the basis of model (8).

The migratory beekeeper who keeps regular business accounts has the database needed to calculate: the revenues, variable costs and placement periods for each site. The model can therefore be applied to a database to calculate the gross income of the sequences and to verify *ex post* the position that the sequence adopted by the migratory beekeeper has in the ranking.

3.6 The recursive procedure of sequencing and the ranking

In order to sequence the forage sites and rank the sequences, the sites must first be numbered in chronological order according to the start date of the placement period. If two or more sites have the same start date, priority is given to the one with the nearest end date.

To assemble the first sequence, one begins by the forage site that comes first chronologically. the inclusion in the sequence of the forage site assigned no. 1 in the chronology implies the exclusion of all the sites alternative to it. Having completed this first step, a new forage site is added to the sequence, chronologically complementary to the first one; the inclusion of the new forage site in the sequence again implies the exclusion of all the sites alternative to it; having completed the second step, a new site, the third, chronologically complementary to the previous one, is added to the sequence. The procedure continues in the same way until the base site is reached, where the sequence ends.

Once the first sequence is completed, one goes back to site no. 1 in the chronology; all the alternative sites are excluded, and the second site in the sequence previously completed is replaced with a new site subsequent and chronologically complementary to site no. 1. The second sequence is completed by repeating the procedure described above, as are all the other sequences that begin with the forage site in the first chronological position. Having assembled all the sequences that begin with site no. 1 in the chronology, the sequences beginning with site no. 2 are completed by proceeding recursively and so on to assemble all the other sequences. Two conditions may be imposed in order to reduce the number of sequences to be computed: the recursive procedure (a) is halted when the placement period of the honey bee colonies on the forage site begins beyond a set date limit and (b) excludes all the sequences that contain one or more sites in less than others, all the other sites contained in the sequence being equal.

These two conditions are justified by the fact that when honey bee colonies are inactive, the variable costs rise but there is no increase in revenues. Each sequence is therefore a selection from the complete series of sites, where the placement periods of the honey bee colonies on these same sites do not coincide.

Once the recursive procedure has been applied, the gross income of each sequence can be calculated by summing the gross incomes of the sites that comprise it (Eq. (6)) and the sequences can be ranked on the basis of gross income.

4. Sequencing sustainability

The concept of sustainability is defined, according to the *Encyclopedia Britannica* [28], as "the long-term viability of a community, set of social institutions, or societal practice". The idea of sustainability rose to prominence with the modern environmental movement, which rebuked the unsustainable character of contemporary societies where patterns of resource use, growth, and consumption threatened the integrity of ecosystems and the well-being of future generations. Sustainability is presented as an alternative to short-term, myopic, and wasteful behavior. The concept of sustainability is nowadays closely linked to that of biodiversity and in the case of nomadic beekeeping biodiversity must consider both the biodiversity of the environment as well that concerning honey bee, which will be explained in detail below.

The aim of this paragraph is to associate to each site a level of sustainability related to the presence of honey bee colonies and to evaluate overall the sustainability of the sequences of sites already identified with the recursive sequencing

method described in Section 3. The evaluation is done with regard to the effects that managed honey bee colonies can generate and undergo. Associating a level of sustainability to each sequence of sites would allow drawing up a new and further ranking to be compared to that established based on profitability. The sustainability of the activity of honey bee colonies referred to the single site and even more to the sequence is not easily assessed because the factors involved are manifold, complex and difficult to identify and measure.

A preliminary issue concerns the relationship between the overall sustainability level of the sequence and the levels of sustainability of the sites that compose it. The relationship is not simple and additive because the sustainability conditions on the sites are not independent of each other. For example, the mortality rate of overwintering honey bee colonies is not only explained by the conditions observed on the overwintering site but also may be derived from other causes detectable in the composition of the sequence. In other words, the mortality of honey bee colonies in overwintering sites could be derived in part from problems related to one or more of the previous sites (in relation also to the time of positioning in a given site) but also to the negative effects of the number of movements and therefore from the number of sites in the entire sequence. The consistency of the honey bee colony arrived at a site depends partly also on the characteristics of the previous sites. The assessment of the sustainability of sites also presupposes the identification of the complete sequence, since the effect of a site starts from the removal from the previous site. Not having a case study to deal with is therefore possible only to tentatively define the link between sustainability and the explanatory variables of sites and sequences.

Before actually entering into the topic of this paragraph, however, we must make a fundamental premise. All bees, whether they are solitary, gregarious or living in temporary or permanent societies (such as *Apis mellifera*), are sedentary organisms that base their survival on the perfect adaptation to the climate and vegetation of the habitats in which they live and where they play their role of pollinators [29]. The connection between bees and environment, effective on different spatial scales in species with different social structure [30], influenced both the evolutionary path of bees as well as deeply determined the vegetation structure and therefore the whole biodiversity at the local level. The close link between these insects and the reproduction of a very high number of plants means that the plants that can best attract the most efficient, abundant and well-distributed local pollinators are also those that will have a greater reproductive success in the same environment. The pollinating insects and firstly the bees, which base all their existence and prosperity on the presence and abundance of pollen and nectar, are decisive in the floristic composition of many terrestrial ecosystems. Starting from this fundamental and preliminary consideration, in terms of sustainability, this obviously decreases, *ceteris paribus*, to the increasing of the distance from site to site.

Consequently, the sustainability of the presence of honey bee colonies in a single site can be interpreted and evaluated on the basis of four drivers: (1) vegetation present on the forage site; (2) agro-environmental and animal context in which the forage site is inserted; (3) well-being of both managed and wild honey bees; and (4) timing and duration of the placement period on the site.

4.1 Site vegetation

The honey bee colonies managed by beekeepers cannot live without adequate sources of pollen, nectar and possibly honeydew, which they collect from the vegetation and mostly on the flowers of angiosperm plants. The activity of honey bees therefore always involves the pollination of a huge number of flowers.

Bee pollination is considered a pollination service when the pollinated flowers produce seeds, fruit or, after germination, other plant products, destined to be harvested by humans for their feeding and for that of their own livestock or however for precise human purposes (plants for industrial use). Honey bee pollination is defined as an eco-systemic service when seeds, fruits or, after germination, other plant products feed the wildlife and therefore support the entire biodiversity. The role of pollination in crop production (both food and nonfood) has been assessed in many ways, from the point of view of both quantitative [1] and qualitative results [16]. The strong reduction in pollinating insects naturally present in intensely cultivated areas, determined both by landscape changes [31] and by the serious impact of the use of crop protection products [32], in recent years has led to a high demand in Europe for honey bee colonies for pollination service on a growing number of crops, also cultivated for nonfood purposes. This increase in demand for pollination services in agriculture is offset by an inadequate number of honey bee colonies managed by European beekeepers [33]. The fundamental driver of a site is its vegetation, and the sustainability of a site is therefore closely linked to the type and structure of the vegetation that covers it. A site can be completely covered by one or more crops or by wild vegetation, or by a puzzle of crops and wild vegetation. Crops can be classified as annual, poliannual or permanent. Annual crops requiring insect pollination, such as sunflower, rapeseed, buckwheat and many other herbaceous crops, often offer an interesting yield of nectar and pollen and therefore the beekeeper greatly benefits both from the production of honey and from the beneficial effect on bees (well-being and development of colonies). For this reason, annual crops are usually pollinated by beekeepers for free, and indeed, among beekeepers, there may be some competition to grab these flowering surfaces. These plants generally have a long flowering period and therefore it is not easy to foresee two or more close pollination cycles/sites on one of these crops. The same scheme can be applied to poliannual crops, mostly belonging to the group of fodder plants. Only in cases where the annual or poliannual insect-pollinated crop is implemented to produce seeds, the need for abundant pollination can make the farmers willing to pay for the pollination service offered by migrant beekeepers. In the case of permanent crops, like orchards, the blooms are usually concentrated and not very profitable for the beekeeper from the point of view of the honey harvest. It is the case with apples, pears and most of the drupaceous (cherry, peach, apricot, etc.) orchards. In addition to the poor honey harvest by quantity and quality, permanent crops are generally characterized by the short but very precise period when the pollinators are desired to stay in the site. The constant and generalized need for the use of crop-protection products in these crops is a deterrent to beekeepers who in fact prefer farms that adopt sustainable cultural practices oriented to the preservation of honey bees and other pollinating insects. As far as vegetation is concerned, in some cases, usually limited and circumscribed, managed honey bees as well as other pollinators can produce eco-systemic "disservices" and reduce the level of sustainability of their presence, contributing to spread on the site some orchard diseases transmissible also at flower level. This is the case, for example, with *Erwinia amylovora* [34], mainly on pear and apple trees, *Pseudomonas syringae*, on Actinidia [35] and *Colletotrichum acutatum* and *C. gloeosporioides* on citrus trees [36].

4.2 The agro-environmental and animal context

The wide and specialized agro-environmental context of modern agriculture, in many cases, does not allow the survival of permanent populations of wild pollinators nor their arrival from nearby areas as these insects generally have a home range of a few hundred meters or even less. Landscape composition, determined by

cultivated, natural and anthropogenic areas, has a decisive role on biodiversity [37] and significantly determining the presence and abundance of permanent pollinators (managed or not), due to the necessity or not of specialized pollination services. The actions aimed to diversify the bloom potential in the agricultural context, such as the so-called flower strips [38], have a positive effect on the consistency of local populations of wild bees, with a clear enhancement of the pollination service to neighboring crops [39]. In some cases, the presence of wild vegetation near the site can be negatively evaluated by the farmer, who paying for a pollination service fears, sometimes rightly, that honey bees could be distracted by other plants and neglect the flowers for which they are requested. The beekeeper, on the other hand, can in some cases obtain an extra well-being for his honey bees and reduce the risk of poisoning and contamination by crop-protection products if, in addition to the flowers for which he brought his bees in the site, there are other blooms not reached by crop-protection products in the surrounding areas.

In the agro-environmental context in which the site is inserted, there must also be factors that can make the pollination service more risky. Sites located near industrial areas or infrastructure such as power lines or repeaters [40] are not very appealing to migratory beekeepers.

4.2.1 Animal context

In context assessment, it should be borne in mind that honey bees are part of indigenous biodiversity in most of Europe, throughout Africa, the Middle East and some small areas of Central Asia. The fact that honey bees are wild organisms linked to their environment of origin, and that beekeeping is not a true form of animal husbandry but something very special [41], explains why migratory beekeeping with transfers of honey bee colonies on a large scale for crop pollination has recently been identified as one of the main causes of the phenomenon known as bee decline [4, 42]. Indeed, this factor would be the one responsible for making other causes even more serious. The fact that *Apis mellifera* is divided into 31 subspecies [43–45], each indigenous and well adapted to a specific geographic area, should set very precise boundaries to the movement of honey bee colonies in order to ensure sustainability to the pollination service on a precise forage site. This problem was clearly stated in a consensus paper drafted by the major Italian entomologists involved in honey bee research and officially presented on Jun 12, 2018, at the Edmund Mach Foundation in San Michele all'Adige (Trento, Italy): the San Michele all'Adige Declaration [46], or Appeal for Biodiversity Protection of Native Honeybee Subspecies of *Apis mellifera* Linnaeus, 1758 in Italy. The movement of honey bee colonies through the different areas of origin of the indigenous subspecies, the sale on a large scale of selected queens and the loss of most of the feral populations of *Apis mellifera* due to the parasitic mite *Varroa destructor* have led to a serious deterioration of the local honey bee populations, up to the possibility of extinction of some subspecies, for example, *Apis mellifera siciliana* [47]. This does not mean that the natural populations (subspecies and ecotypes) of *Apis mellifera* should be considered irremediably lost. Due to the effect of resilience, eliminating the perturbation factors, in this case the introduction of nonindigenous honey bees, local populations can most probably be restored due to the greater adaptation of the latter compared to the introduced ones [48].

Even the massive temporary transfer of managed honey bees in already impoverished areas with regard to the fauna of native pollinating insects (commonly called wild bees) could further impact negatively on the sustainability of the presence of bee colonies on forage sites. In recent years, several scientific papers have highlighted the risk of a possible interaction between honey bees managed by beekeepers in very large apiaries and wild bees, highlighting a possible

contraposition between the safeguarding of agricultural and apiculture productions on one hand and conservation of biodiversity on the other. *A. mellifera* could act, in some contexts, as an invasive species with a great impact on biodiversity, especially in the newly introduced areas (Oceania and the Americas). However, although the honey bee has become widespread in nature and has established wild populations in these "New Continents," the extent to which the introduced honey bees alter local biodiversity and have negative effects on the composition and density of indigenous pollinating faunas remains controversial [49, 50].

Finally, the context can have negative effects on the sustainability of beekeeping due to the presence of bears or other organisms potentially harmful to honey bees up to the phenomenon of thefts of hives.

4.3 Honey bees' well-being

First of all, it must be emphasized that the movement of the hives puts the honey bees under stress due to loading and unloading operations and the forced enclosure along the way from one site to another. Transport and unloading can in many ways affect the welfare of worker bees, brood and queen bees and therefore cause serious damage to the beekeeper in terms of loss of colonies [51]. These damages to honey bees' well-being are quite evident when the journey from one site to another is very long. The permanence of the colonies in the forage sites covered by spontaneous vegetation has very positive effects on the health of the honey bees, deriving mainly from the variety of botanical species that they can visit and therefore from different kinds of pollen, their primary source of food [52], that bees can collect.

Migratory beekeeping, especially if aimed to provide commercial pollination services to farmers, can produce a large-scale transfer of pathogens and parasites of honey bees and there are many known cases in this regard. Transfers can affect both migratory and sedentary beekeepers' bees [53]; migratory beekeepers' bees can receive pathogens and parasites at a given site but can also bring new diseases and parasites to the permanent beekeepers' bees. In both cases, the pathogens or the parasites will then be carried by the migratory beekeepers' bees also on the sites that follow along the sequence.

Another problem to the health of the migratory beekeepers' bees is the proximity between the pollination site and other forage sites covered by spontaneous and cultivated vegetation. During the stop on a forage site, the treatments with agrochemicals on the contiguous vegetation, due to the drift, can in fact cause damage to the health of honey bees and often also a contamination of the bee products, which would in some cases be unsellable. Honey bees generally fly within a radius of 1–3 km but can go much further, up to 10 km and more, in search of pollen. This feature makes honey bees able to come into contact with risk factors not strictly related to the pollinating service site. To prevent these risks, it is important to know very well the agro-environmental context and to take appropriate countermeasures.

Another critical aspect for the well-being of honey bees can be derived from the interference that can be created between the genetic pulls of the honey bees managed by the migratory beekeeper, those managed by sedentary beekeepers and also the feral colonies present on the site. Feral honey bee colonies have dramatically reduced in the last decades [54], coinciding with the advent of the parasitic mite *Varroa destructor*. The migration between the areas of origin of the different subspecies of *Apis mellifera* causes genetic pollution and, in the case of queen bee farmers (but obviously not only), a serious damage for local beekeepers, who try to preserve the native honey bees as they are perfectly adapted to the environment. Even the genetic pulls of the honey bees managed by the migratory beekeepers can be genetically contaminated, if the colonies transferred have queens in fertilization, which could mate with drones of a different subspecies or ecotype.

4.4 Timing and duration of the placement period on the site

The site is a place where honey bee colonies stay for a defined period of time depending on the duration of the flowering or from the requirements of the pollination service. The timing of the placement period on the site is defined by the flowering phenological phase of the plants on which honey bees must perform their pollination/foraging activity.

Timing and duration of placement period of the honey bee colonies on the forage site greatly vary depending not only on the species to be pollinated but also on the basis of the purpose of the stay on the site. In some cases, honey bee colonies must stop only for a time much shorter than the actual duration of the flowering, for example, to avoid running into the scheduled treatments with crop-protection products. In other cases, however, especially in crops cultivated to produce seeds of fodder or oleaginous plants for the production of alimentary oil or biofuels, honey bees must stand on the site for the entire flowering period.

In forage sites covered by wild vegetation, in the case of a prolonged placement period on the site, the pollinating and foraging effects are greater, since honey bees succeed, through their cognitive abilities, to better exploit the resources of a site that they "learn" to know and manage [55, 56]. The prolonged stay in the sites, especially if not in correspondence with a conspicuous bloom (producing monofloral honey) also improves the value of the ecosystemic service. From the point of view of sustainability, it is essential that bees manage to pollinate a broader spectrum of plants, creating the benefit of pollination to a large number of plant species and contributing substantially to ensure the conservation of the plant and overall biodiversity.

Timing can affect the level of sustainability of beekeeping as early blooms occur often when honey bee colonies may not yet be well developed but, on the other hand, these early blooms may allow honey bee colonies to complete their development in view of the transfer on further sites of the sequence or of their multiplication. Late blooms can bring another big advantage to the beekeeper, allowing the honey bees to breed winter bees in the presence of abundant food sources (pollen is the limiting factor in this regard) and at the same time to store significant stocks of honey, with a large saving of sugary foods that the beekeeper should provide to bees in the absence of such flowering. Autumnal blooms, however, can affect the survival of honey bees since the life span of a working bee depends on its more or less intense foraging activity. Pollination and the consequent production of honey of buckwheat (*Fagopyrum esculentum*) in the Alpine areas until the 1950s are interesting in this sense. The late flowering of this crop forced local beekeepers to ward off most of their colonies. Honey bees that were left on the buckwheat harvested abundant honey but were destined in large part to succumb by the end of winter, brittle from the intense harvest but without being then replaced, for the arrival of winter, by other new and strong bees. The migratory beekeeper in the selection of the site had to evaluate the advantages obtained with the production of honey in relation to the risks of widespread winter colony losses.

5. Conclusions

The microeconomic model of the migratory beekeeper formalized in this chapter allows calculating revenues, variable costs and gross income per each site and each sequence of sites. The sequence with the highest gross income, identified by applying the recursive procedure to the data provided by the migratory beekeeper, can be compared ex post with the one it has actually implemented to verify which

divergences exist in the visited sites. Ex ante, during the planning of the migration itinerary, the sequences with a gross income equal or lower by a predetermined percentage of the maximum can be submitted to the migratory beekeeper for the choice of the one to be implemented. The comparison between the sequences of sites is in fact an important decision factor because their composition provides the migratory beekeeper information on the possible variability of the gross income that the microeconomic model has not considered.

Migratory beekeeping is currently a necessity for the supply of pollination services to the growers and for the production of honey, especially monofloral honey, and other honey bee products. The migration between the sites, however, should occur respecting both the environmental and honey bee biodiversity, *Apis mellifera* indigenous subspecies and their relative local ecotypes.

A certain level of sustainability corresponds to each forage site belonging to a given sequence. The analysis conducted on the sustainability drivers of migratory beekeeping has identified some critical issues that should be carefully considered in the definition of best management practices for crop pollination [57]. In particular, the high number of movements in the sequence and the possible impairment of the genetic pool of honey bees at the sites are highly detrimental to the sustainability of the sequence of forage sites.

It is therefore necessary to update the policies to support professional beekeeping but also those relating to the management of agricultural environments, by encouraging sequences characterized by higher levels of sustainability and by protecting in a more concrete way the conservation of the genetic biodiversity of bees.

The integration between profitability and sustainability of the sequences of forage sites discussed in this chapter raises useful premises for the implementation of a pollinator habitat policy [58], which could effectively orientate migratory beekeeping toward higher levels of sustainability. The challenge is therefore to identify a path of environmental sustainability [59] that does not compromise but reconciles the profitability and sustainability of migratory beekeeping.

Conflict of interest

No conflict of interest.

Author details

Luciano Pilati[1*] and Paolo Fontana[2]

1 Department of Economics and Management, University of Trento, Italy

2 Edmund Mach Foundation of San Michele all'Adige, Italy

*Address all correspondence to: luciano.pilati@unitn.it

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Chapter 3

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

Sandra Rosa Fuselli, Pablo Gimenez Martinez, Giselle Fuentes, Rosa María Alonso-Salces and Matías Maggi

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.

Author details

Sandra Rosa Fuselli[1,2], Pablo Gimenez Martinez[1,3]*, Giselle Fuentes[1], Rosa María Alonso-Salces[1,4] and Matías Maggi[1,4]

1 Facultad de Ciencias Exactas y Naturales, Centro de Investigación en Abejas Sociales (IIPROSAM) , Universidad Nacional de Mar del Plata, Mar del Plata, Argentina

2 Comisión Investigaciones Científicas de la Provincia de Buenos Aires (CIC), La Plata, Argentina

3 Agencia Nacional de Promoción Científica y Tecnológica, Buenos Aires, Argentina

4 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

*Address all correspondence to: beedarian@gmail.com

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Chapter 4

Virulence of *Varroa destructor* in Colonies of Honey Bee *Apis mellifera*

Zahra Naeef Ayoub

Abstract

Although *Varroa* mite infestation of honeybee is widespread throughout the world, data about the level of infestation in the bees of our area are inadequate. *Varroa* mite infestation was first detected in Iraq in the mid-1980s. A large investigation was carried out to determine *Varroa* infestation level in the apiaries of Duhok Province, Northern Iraq. Otherwise, this study aimed to clarify the parasitic effect of the mite *Varroa destructor* on the mandibular and hypopharyngeal glands of *Apis mellifera.* A total of 1000 samples of adult workers of different ages from more than 20 separated apiaries were collected from August to the end of October 2013. Caped brood, drone and worker brood, from five apiaries were examined. A total of 450 newly emerged workers from three separated apiaries of the same area were collected during the late summer 2013. Effects of *Varroa* infestation on mandibular glands and hypopharyngeal glands of newly emerged workers of honeybees were investigated. High level of the infestation was found in all apiaries of Dohuk region and may act as a risk factor to the bee health. Results showed significant differences in the size of hypopharyngeal gland acini of newly emerged workers infested with one to three mites compared to noninfested newly emerged workers, while only newly emerged workers infested with three mites showed significant differences in the size of mandibular glands as compared to noninfested newly emerged workers. Management strategies of the mid- and late summer treatment are necessary to keep the mite population at low levels before and during the period when the winter bees emerge. Considerable numbers of *Varroa* mites can be controlled inside bee colonies without chemicals by removal of drone pupae or sometimes if necessary removing generations of worker pupae before emergence as adults. Using movable screened bottom boards in the opposite side of the hive entrance for the observation and counting naturally felled down *Varroa* mites were very beneficial in this area during hot summer periods.

Keywords: *Varroa* mite, parasite, honeybees, mandibular glands, hypopharyngeal glands

1. Introduction

Honeybees are proving to be excellent indicators of the state of an ecosystem, in addition to their products that contribute significant economic values to humans. The loss of natural pollinators as a result of habitat loss and pesticide application

has forced farmers to depend on domesticated honeybees for pollination. While natural habitats can provide full pollinator services, conventional agriculture clears these lands and adds pesticide amendments for crop production, greatly reducing habitat availability for pollinators and killing beneficial insects [1].

Honeybees are the most efficient pollinators of 80% of the crops worldwide, and farmers prefer their services because they greatly improve crop yields and can be transported when pesticides are applied. The decline of pollinators in recent decades is threatening the structure and function of natural and agricultural ecosystems. Pollinators provide essential ecosystem services by aiding plant and tree reproduction that require pollination assistance. Large-scale production of food crops in agricultural systems is, in many cases, only possible with the assistance of pollinators, primarily honeybees [2].

Recent declines in many of these pollinators have been blamed on land-use changes, diseases, chemicals, and climate change [3]. A typical established honeybee colony is made up of one mated queen, 0–300 male drones, and 20–200,000 small sterile female workers. The drones' primary function is mating with virgin queens. On the other hand, workers clean and rebuild the hive, cap/uncap, and tend larvae cells, gather nectar, pollen, and propolis, make honey, and defend the colony. The queen can lay around 1500–2000 eggs/day and can last up to 2 years. The queen communicates with colony members through pheromone secretions that, among other functions, signal attacks on the colony or promote swarming. Through their egg laying patterns, queens can indicate colony strength and therefore adequate honey production and pollination services [2, 3].

The life cycle of honeybees is different for each caste. The metamorphic process of worker bees takes 21 days and begins with fertilized eggs deposited at the bottom of a cell. After 3 days, the egg hatches into a first-instar larva and is fed by nurse bees. The first-instar larva grows through successive molting stages. The brood is then sealed by the nurse bees to allow pupa growth. The adult worker bee chews its way out its cell after spending 1 day as an unemerged adult. Complete metamorphosis in drones is similar to workers; however, it takes 3 days longer to fully develop due to the larger size of the cell. Queens take only 16 days to develop from egg to adult as a result of the nutritious royal jelly feeding [4].

Recent huge losses of honeybee colonies are causing overall population declines in the large parts of our planet. This occurrence is threatening the apicultural industry, while causing economic and ecological pressures on agricultural crop production and ecosystem services. Recent reports point to the spread of honeybee diseases and parasites as an explanation for these colony losses [5]. The ectoparasitic mite, *Varroa destructor* [6], is at the core of colony losses worldwide and has been responsible for the nearly complete eradication of wild and feral honeybee populations in Europe and North America since it was introduced to this new honeybee host species [7].

The apicultural industry depends heavily on chemical *Varroa* control treatments to keep managed colonies alive. These chemical controls can leave residues in hive products, have negative impacts on honeybee health, and remove selective pressures that would be required for host or parasite adaptations [4]. *Varroa destructor*, as a major pest of *Apis mellifera*, under different agricultural settings, in organic and conventional farms, has caused massive economic losses and expense for beekeepers. Different treatments were applied in colonies to determine impacts on mite loss that may influence colony health, life span, and individual health that affect honey yield and other products. *Varroa* mites remain the number one management problem for beekeepers and scientists alike. The onset of resistance to the treatments available, and the potential impact of secondary infections, will make controlling

the mite more difficult in the future. Knowledge of bee biology and both the biology and the pathology of *Varroa* mites are essential for understanding possible tolerance mechanisms in the honeybee host. Other factors that encourage spread of many bee infections in this area including *Varroa* mites are using traditional style of bee hives, depending mainly on natural swarming to obtain new colonies, poor knowledge of the beekeepers on using pesticides, and illegal entrance of bee colonies from the neighboring countries. The discrepancies in the rates of *Varroa* mite infestation levels in the different countries or even in the same region of the country could be attributed to many factors such as temperature, humidity, availability of pollen, numbers of apiaries, and density of honeybee colonies.

In spite of widespread of *Varroa* mite in the bees of the Northern Iraq but the data are poor about the level of infestation in the apiaries of the region, the aim of the present study is to determine the level of *Varroa* mite infestation in the apiaries of Duhok Province, Northern Iraq. Otherwise, several research studies worldwide have demonstrated distinct levels of virulence of the mite and the increased colony mortality rates due to its infestation; however, only a few studies report the mite's effects on specific tissues, glands, or other organs in bees. Therefore, this study aimed to clarify the parasitic effect of the mite *Varroa destructor* on the mandibular and hypopharyngeal glands of *Apis mellifera.*

2. *Varroa* mite infestation in honeybee colonies

The mite *V. destructor* Anderson is the species of ectoparasitic mites (Acari: Varroidae) that causes serious disease (Varroosis) of larvae, pupae, and adults of honeybee *Apis mellifera* L. The mite is also known to transfer pathogenic viruses into the bee [8] and is suspected to be one of the agents causing colony collapse disorder [9]. Currently, the disease represents one of the most important problems of the world beekeeping and is attributed by the International Epizootic Bureau to the list "B" of quarantine diseases of bees along with American foulbrood and Acariosis. Therefore, Varroosis must be regularly controlled to predict colony loss [10]. The hemophagous honeybee mite *V. destructor* is still the greatest threat for apiculture. No other pathogen has had a comparable impact on both beekeeping and honeybee research during the long history of apiculture. The mites have negatively affected the apiculture industry in every country that it has been introduced. Accurate estimates of the effect of V. mites on the apiculture industry are hard to find, but it is safe to assume that the mites have killed thousands of colonies worldwide; V. mites also have affected the feral (wild) population of bees in many areas. Since feral colonies were not managed for V. mites and the colonies were left unprotected, the loss of feral colonies quickly resulted as V. mites continued to spread. This external parasite feeds on the hemolymph of adult bees, larvae, and pupae. Heavy parasitism results in heavy bee mortality and subsequent weakening of the colony and can lead to colony death [11]. High levels of *Varroa* mite infestation was found in apiaries of the Dohuk region, northern Iraq [12].

The mite *V. destructor* is an ectoparasite that feeds on the hemolymph of adult bees and their brood in the postcapping stage [13]. Its reproductive potential and virulence are multifactorial and might vary according to the region of occurrence and bee race; *V. destructor* damages may lead to its complete death of a colony [14, 15]. The mite acts as a vector for viruses that may cause problems, such as bees growing with defective wings and high bee mortality rate. In addition, adult bees originating from parasitized pupae will have lower body weight, orientation problems, and lower life spans [13, 15, 16].

2.1 Origin and distribution of *Varroa* mites

V. jacobsoni Oudemans was first described as a natural ectoparasitic mite of the Eastern honeybee *A. cerana* in Java [17] and has a wide distribution on this bee throughout Asia [18]. The ectoparasitic honeybee mite *Varroa destructor* was originally confined to the Eastern honeybee *Apis cerana*. After a shift to the new host *Apis mellifera* during the first half of the last century, the parasite dispersed worldwide and is currently considered the major threat for apiculture. The mite that is responsible for the clinical symptoms of "Varroosis" in *A. mellifera* belongs to the species *Varroa destructor*, which was assumed to be *Varroa jacobsoni* until the year 2000 [6].

Prior to recent studies, *Varroa jacobsoni* a species of mite that parasitizes *Apis cerana* (Asian honeybees), was considered homogeneous. The more damaging *Varroa destructor* was previously included under the name *Varroa jacobsoni*, but the two species can be separated on the basis of the mitochondrial DNA (mtDNA) sequence.

Although *Varroa* mites from different populations are physically alike, their virulence toward *A. mellifera* is not uniform. The greatest variation is associated with *V. jacobsoni* of Javanese origin, these mites completely lack the ability to reproduce on *A. mellifera* [19, 20] and their mitochondrial DNA (mtDNA) cytochrome oxidase I (CO-I) gene sequences differ from those of phenotypically similar mites that reproduce on *A. mellifera* in Europe [21].

The *Varroa* mite did not receive much attention by scientists until a host shift occurred and it became a pest on *A. mellifera* in Europe. The mite was first found in Europe in 1977 and in North and South America in 1977 and 1971, respectively [22]. Since then, it has spread throughout the world with the help of honeybee importations [23, 24]. Today, only Australia [6, 15], Northern Scandinavia [25], and some extremely isolated island populations [26] remain free of *Varroa*. *Varroa destructor* [6] is the only identified *Varroa* species parasitizing European honeybees; it is an exotic and relatively recent invasive species to parasitize the European honeybee (*Apis mellifera*).

Varroa mites are widely considered the biggest honeybee health problem worldwide. Until recently, *Varroa jacobsoni* has been found to live and reproduce only in Asian honeybee (*Apis cerana*) colonies, while *V. destructor* successfully reproduces in both *Apis cerana* and *Apis mellifera* colonies [27]. The mite affecting honeybee *Apis mellifera* now has been officially renamed to *Varroa destructor*.

The only mite of economic importance is *Varroa destructor*, after which successfully shifted from the original host, *Apis cerana* to the western honeybee, *A. mellifera*. The new host lacks features that obviously established a stable host-parasite relationship in *Apis cerana* during a long period of coevolution [28].

The details of the host shift are unclear. Most likely, this shift occurred when *Apis mellifera* colonies were transported to Eastern Russia or the Far East in the first half of the past century, which led to a sympatric distribution of both honeybee species [23], and might have allowed the parasite to infest the new host. *Varroa* mites are widely considered the biggest honeybee health problem worldwide. Until recently, *Varroa jacobsoni* has been found to live and reproduce only in Asian honeybee (*Apis cerana*) colonies, while *Varroa destructor* successfully reproduces in both *A. cerana* and *A. mellifera* colonies [27].

2.2 Morphology of the mite

Varroa mites show a distinct sexual dimorphism [29], with many morphological adaptations to their host. A common feature of both sexes is the division of the body into two well-defined parts, the idiosoma and the gnathosoma. The idiosoma

comprises the larger part and one dorsal shield and different ventral shields. The female mites have a flattened, ellipsoidal idiosoma with greater width than length. The legs of the female are short and strong and show specialized structures, the apoteles, for adherence to the host. The dorsal and ventral shields are highly sclerotized and show a reddish-brown coloration. Thin and flexible membranes between the shields enable the mite to dilate during feeding and egg formation. The male body is pear shaped and shows only weak sclerotization, which is mainly present in the legs and the dorsal shield. Males are clearly smaller than females in all developmental stages. The legs of the males are longer in relation to the body size than the legs of females [30].

2.3 Mite biology and behavior

Varroa destructor is closely linked to its honeybee host and lacks a free living stage. There are two distinct phases in the life cycle of *V. destructor* females: a phoretic phase (transport phase) as a parasite on adult bees and a reproductive phase within the sealed drone and worker brood cells. Males and nymphal stages of the mite are short lived and can only be found within the sealed brood cells. On the adult bees, the *Varroa* females are transported to brood cells for their reproduction or spread by foraging and swarming bees [31]. On the adult bees, the *Varroa* mite female usually is hidden under the sternites of the bee [32]. The mites suck substantial amounts of hemolymph from both the adult bees and the preimaginal host stages within the sealed brood cells. Generally, mites are significantly more often found in brood cells than on adult bees, with up to 90% of the colony's mites found within the brood [33, 34]. The mites can feed and survive on both adult bees and their brood. The mites feed on their host's hemolymph (blood) through punctures made in the body wall with their sharp mouthparts.

The host finding and reproductive behavior of *Varroa destructor* is essential for understanding the population dynamics of the parasite, but it is also of particular significance for the beekeeping practice. Certain cues for the orientation of the mites could be used for development of biological control methods such as traps, repellents, or mating disruption by certain pheromones. The control of reproduction of a parasite is a crucial point for the stability of a host-parasite relationship [35]. The mites feed on both adult and brood of bees, weakening them and spreading harmful pathogens such as bee viruses. Infested colonies eventually die out unless control measures are regularly applied. *Varroa* mites cannot be completely eradicated, but beekeepers can successfully keep productive bees despite the presence of the mite. *Varroa* can be controlled by monitoring the infestation in their colonies and the use of appropriate control methods to keep mite numbers below levels that are harmful. The mites reproduce inside the sealed brood cells; to breed, a gravid (egg-carrying) female mite enters occupied brood cells just before the cell is capped over, where she remains in the brood food under the larva until the cell is sealed. She breathes through a respiratory organ, common in mites. Five hours after cell capping, the bee larva consumes the rest of the food. Mites prefer to breed in drone brood but will also breed in worker brood. About 4 hours after capping, the mite starts feeding on the immature bee and establishes a feeding site on this host that her offspring can feed from as they develop.

The life expectancy of *Varroa* mites depends on the presence of brood and will vary from 27 days to about 5 months. During the summer, *Varroa* mites live for about 2–3 months during which time, providing brood is available, they can complete three to four breeding cycles. In winter, when brood rearing is restricted, mites overwinter solely on the bodies of the adult bees within the cluster, until brood rearing commences the following spring [4]. *Varroa* mites are mobile and can readily move between bees and within the hive, to travel between colonies they

depend upon adult bees for transport—through the natural processes of drifting, robbing, and swarming. *Varroa* can spread slowly over long distances in this way.

Both wild and managed colonies may exchange mites via another mechanism that has received remarkably little attention or study, floral transmission. Recent study tested the ability of mites to infest foragers at feeders or flowers. They found that *Varroa destructor* mites are highly capable of phoretically infesting foraging honeybees; this details the mechanisms and maneuvers and describes mite behaviors postinfestation [36]. However, the movement of infested colonies by beekeepers as well as the natural swarming of feral colonies is the principal means of spread over long distances.

3. Recent developments for *Varroa* management

Many beekeepers and farmers are familiar with the *Varroa destructor*—the parasitic mite that essentially sucks the life out of honeybees, transmits diseases, and is considered a major contributor to colony collapse around the world. Several potential solutions have been in the works in the last few years, and last year, Bayer Bee Care registered a new tool that may give beekeepers in Europe a simple way to prevent the *Varroa* mite from spreading using an already-existing active ingredient; this new tool, called the "*Varroa* gate." Development of methods for administering existing varroacides, particularly organic acids, to allow safer and more effective controls with fewer adverse effects is most likely to be based on active ingredients of existing veterinary medicines and pesticides reformulated for *Varroa* control. Novel varroacides based on other (often naturally occurring) active ingredients are also under development. The development of synthetic pheromones (natural chemical messages) to provide control over V. mites, for instance, by inhibiting their feeding or reproduction.

Results indicated that double application of oxalic acid (OA) is worthwhile to beekeepers in *Varroa* management. It is not harmful to colonies and it reduces *Varroa* populations to such an extent that seven to eight doublings, which would take more than 1 year, are needed to build back to the original level [37].

The acaricide toxicity to the *Varroa* mites was consistent in both the caged adult honeybees and workers in the queen-right colonies; two types of acaricides, coumaphos at the highest doses and hop acids, were comparatively more toxic to the worker bees. Results of the same study show that various acaricides are variably effective for *Varroa* suppression when the mite populations are rising and brood is present [38]. Research continues to identify naturally occurring fungi and bacteria that could kill *Varroa* mites within the bee colony and to develop these into practical control methods.

A large number of essential oils and plant extracts were successfully used to minimize mite populations.

Biotechnical applications to minimize V. mite populations as a part of colony management (drone brood trapping) were used against *Varroa destructor*. Development of more sophisticated and effective biotechnical controls is likely to become part of routine colony management. Some beekeepers in many developed countries can easily apply methods for removing considerable numbers of *Varroa* mites from their colonies without chemicals by removal of drone pupae or sometimes if necessary they can remove generations of worker pupae before emergence as adults. These techniques can be performed after queen confinement on empty combs inside especially plastic bags made from queen excluder, although this technique will cause worker loss but at the same time will minimize mite populations

inside colonies; therefore, this method is effective especially during the beginning of the brood rearing period [39].

Recent study estimated *Varroa* sensitive hygiene (VSH) by calculating the removal rates of parasitized brood, which is sometimes found to correlate with the removal of dead brood, either freeze-killed or pin-killed brood. They used an artificial mite introduction method; they introduced female adult mites in recently capped brood cells and assessed the removal rates. They could not conclude that *Varroa* sensitive hygiene only or preferentially targets reproducing mites, leaving nonreproducing mites undisturbed; they concluded that more than one mechanism of resistance may evolve in response to the selection pressure by *Varroa* mites [40].

4. Materials and methods

Sample collection and investigating the virulence of *Varroa* mites in the area were carried out in Duhok province, Northern Iraq, during August, September, and October 2013.

4.1 Adult worker collection

Adult worker samples included a large number of newly emerged workers before feeding, in addition to other group of adult workers those presented in brood chamber.

4.1.1 Adult worker samples of different ages

In order to obtain the level of infestation of *Varroa* mites in apiaries of this area, a total of 1000 samples (adult workers of different ages) from 20 separated apiaries in Duhok province, Northern Iraq, were collected from August to the end of October 2013. Adult bees (samples) were taken from both sides of three uncapped brood combs of five colonies in each apiary. Collected bee samples were kept individually in eppendorf tubes containing 30% ethanol, and then the number of mites on the individual worker was counted.

4.1.2 Samples of newly emerged adult workers

A total of 450 samples (newly emerged workers) from 30 colonies in three separated apiaries of the Duhok Province; Northern Iraq, were collected during the late summer 2013. Note that the egg laying ends by middle of October in this area. Effects of V. mite infestation on mandibular glands and hypopharyngeal glands of newly emerged workers of honeybees were investigated using three colonies of *Apis mellifera* from each apiary. The tested colonies headed with young active queens. The combs containing sealed brood with emerging workers were transferred into suitable room at 32–34°C; and 50 newly emerged bees were collected from each colony. Collected bee samples were individually kept in eppendorf tubes containing 30% alcohol and carefully examined under a dissecting microscope, and then the number of mites on the individual newly emerged workers was counted. The samples of each apiary were separately grouped into noninfested newly emerged workers and infested with one mite (1M), two mites (2M), and three mites (3M). Collected bees were stored in a freezer until dissection.

4.2 Direct examination of brood (brood samples)

4.2.1 Worker brood

A total of 300 worker pupae from 30 colonies in three separated apiaries of the Duhok Province during August, September, and October were collected. In each apiary, parts of the comb containing sealed worker brood in the center of brood chamber were transferred to the laboratory. Cells of pupae recently capped were scratched, and then all stages of *Varroa* female mites in each cell were counted, and the walls of the cells and the removed caps were also examined as the mite frequently hides there.

All worker brood samples were individually kept in eppendorf tubes containing 30% alcohol and then carefully examined under binocular microscope. Empty brood cells (after brood removing) were observed using an appropriate source of light, and the numbers of mites were counted. It is worth to mention that honeybee brood production in our area starts from the beginning of April to the end of October.

4.2.2 Drone brood

A total of 300 drone pupae from 30 colonies in three separated apiaries of the Duhok Province in early summer were collected.

To obtain the level of infection, these drone brood were examined, the same was done as in worker brood collection; parts of the comb containing capped drone pupae were scratched, and then all stages of *Varroa* female mites in each cell were counted.

4.3 Examination of hive debris for mites on the bottom boards

Naturally felled down *Varroa* mites were also recorded for 6 weeks using sticky boards with 15 colonies in three separated apiaries. The distance among these apiaries was not less than 15 km. In each apiary, five untreated colonies were tested weekly for 6 weeks starting from the last week of September. A sticky board is placed below a screen mesh (3 mm × 3 mm) allowing for daily or weekly counting of *Varroa* mite. The number of dropped mites was recorded daily, using movable plastic sheets coated with vaseline placed on the bottom board of the tested hives; this board can be pulled from the opposite side of the hive entrance for the observation without irritating the foragers and guard bees in front of the hive. This method of the counting is very beneficial in this area because during hot summer days, it is impossible to open the hive for observations when temperature reaches 45°C. The total number of dropped mites during 1 week was considered the main indicator of the infestation level in each colony during the study.

4.4 Dissecting

The samples of each apiary those separately grouped into noninfested newly emerged workers and infested with one mite (1M), two mites (2M), and three mites (3M) were tested under binocular microscope to determine the effect of mite infestation on the mandibular glands and hypopharyngeal glands of these newly emerged workers.

Frozen samples were thawed at room temperature and immediately dissected to prevent tissue deterioration. The bees were dissected under a stereomicroscope at ×40 magnification. The size of mandibular glands and size of acini in hypopharyngeal glands of all workers from three groups (apiaries) was recorded.

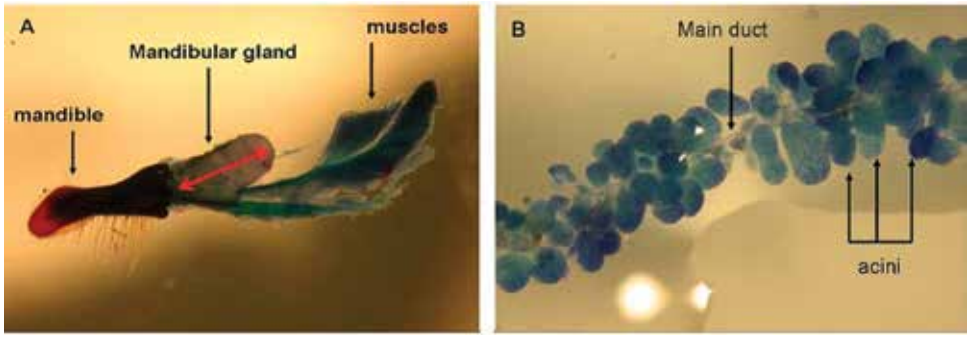

Figure 1.
(A) The mandibular gland of a honeybee worker; (B) part of the hypopharyngeal gland of a honeybee worker.

In each dissected worker, mandibular glands from both sides of the head were dissected (the mandible with the gland separated from the head), placed on the surface of a clean glass slide, stained by diluted Giemsa stain, and after few minutes washed by physiological saline. The longest and shortest dimensions of each gland were measured. The length and width of each gland were used to calculate the product for each gland (length × width). The average size of two glands for each dissected worker was calculated (**Figure 1A**).

A longitudinal incision was made in the top of the head. Then the hypopharyngeal glands were dissected on the surface of a clean glass slide, stained by diluted Giemsa stain, and washed by physiological saline. The longest diameter of 15 acini from each side (30 acini from each worker) of the head was measured (**Figure 1B**).

5. Impact of *Varroa* mite infestation on immature and adult bees

The total number of colonies reared in traditional beehives around Duhok city was about 100,000–150,000, while the total number of bee colonies reared in modern beehives was about 400,000–450,000 in this area.

Wide variations in the infestation level were observed between apiaries even among colonies of the same apiary. These variations in the infestation could be attributed to several factors such as colony population, availability of nutritional resources, using of supplemental diets, and differences in the age of queens of tested colonies, in addition to the beekeeper experience in the apiary management.

Other factors that encourage spread of many bee infections including *Varroa* mites are using traditional style of beehives, depending mainly on natural swarming to obtain new colonies, poor knowledge of the beekeepers on using pesticides, and illegal entrance of bee colonies from the neighboring countries. The discrepancies in the rates of *Varroa* mite infestation levels in the different countries or even in the same region of the country could be attributed to many factors such as temperature, humidity, availability of pollen, numbers of apiaries, and density of honeybee colonies.

The levels of infestation were higher in drone pupae compared to worker pupae. Female mites prefer to enter cells and oviposit on drone pupae over worker pupae.

Significant differences were found in the size of both mandibular and hypopharyngeal glands among noninfested newly emerged workers with *Varroa* mites and infested groups. A significant difference in the size of hypopharyngeal gland acini was found in bees infested with one, two, and three mites compared to noninfested newly emerged workers (**Figure 2**), while only bees infested with three mites showed significant differences in the size of mandibular glands compared

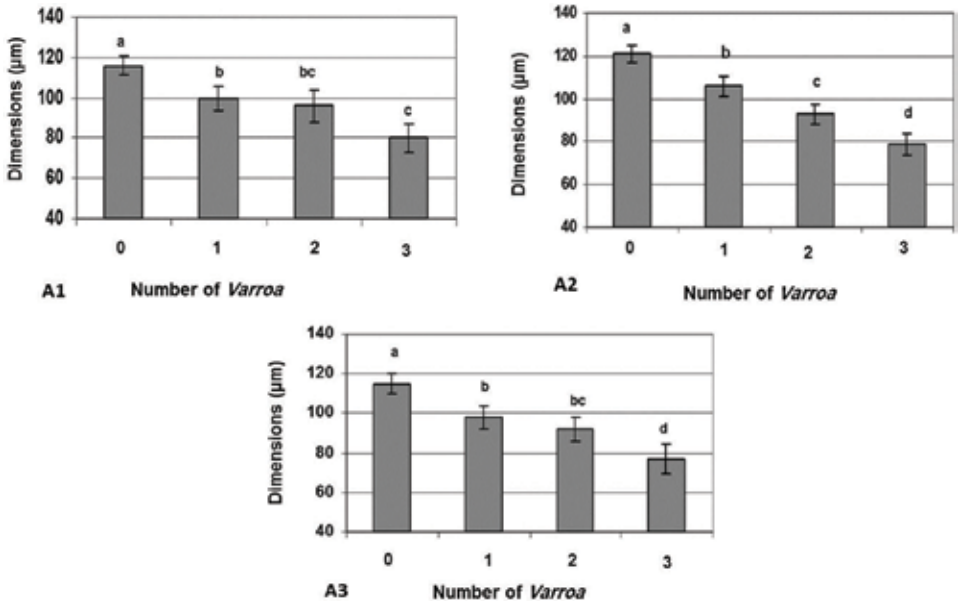

Figure 2.
Dimensions of hypopharyngeal gland's acini (mean ± SE) of noninfested newly emerged workers (0 Varroa) compared to infested groups with one Varroa (1), two Varroa (2), and three Varroa (3) in three apiaries: A1 (apiary 1), A2 (apiary 2), and A3 (apiary 3).

to noninfested bees (**Figure 3**). It seems that the hypopharyngeal glands are more affected by the mite infestation than mandibular glands. Deficiency of protein strongly affects hypopharyngeal glands because the development of these glands requires sufficient amount of protein, which is severely reduced by *Varroa* infestation. Depletion of protein level in the body of infested pupae is due to the reduction of hemolymph, which is consumed by direct feeding of the parasite as well as indirectly by improper feeding during larval stage, which reared by previously infested nurse bees in the colony.

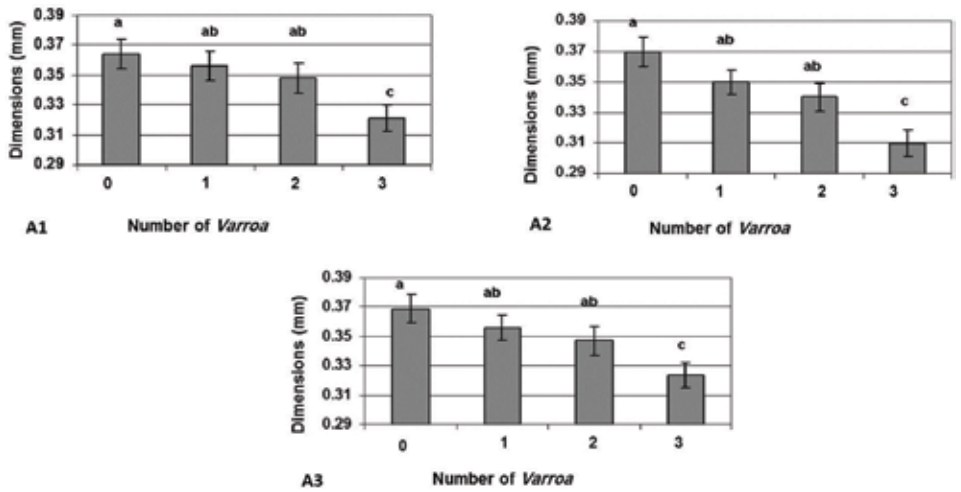

Figure 3.
Dimensions of mandibular glands (mean ± SE) of noninfested newly emerged workers (0 Varroa) compared to infested groups with one Varroa (1), two Varroa (2), and three Varroa (3) in three apiaries: A1 (apiary 1), A2 (apiary 2), and A3 (apiary 3).

Mandibular glands appeared less sensitive to the *Varroa* infestation compared to hypopharyngeal glands in which newly emerged workers resulted from infested pupae with one and two mites did not show significant differences in the size of mandibular glands compared to noninfested brood. This may be attributed to the earlier development of the hypopharyngeal glands than mandibular glands in both larval and adult stages.

Without periodic treatment, most of the honeybee colonies in temperate climates would collapse within a 2–3 year period. The parasite pierces the body wall of its host and then extracts the hemolymph. All *Varroa destructors* resident within the brood cell can repeatedly revisit this feeding site because it remains open for several days [41]. This unique ability of *Varroa destructor* to repeatedly feed on its bee host suggests that they probably secrete antiwound healing factors from their salivary glands [4]. One of the structures that may be directly affected by *Varroa* mite infestation is the hypopharyngeal gland [42], which is located in the head and produces a protein-based substance that is used to feed larvae, the queen, and the drones [43]. Another structure that can be affected by *Varroa* mite infestation is the mandibular gland [44]. The mandibular glands of *A. mellifera* are exocrine glands responsible for the production of pheromones, which play a direct role in communication among members of the colony [45]. The hypopharyngeal glands are more affected by the mite infestation than mandibular glands [46]. Several researches have demonstrated distinct levels of virulence of the mite and increased colony mortality rates due to its infestation; however, only a few studies reported the mite's effects on specific tissues, glands, or other organs in bees. Nevertheless, a little number of scientific papers had been published on the issue, glands, or morphological changes in other organs of the infected honeybee. After injuring the pupae's epicuticle, the mite feeds from its hemolymph [13]. This process may compromise the bee development due to disturbances of natural hormonal regulatory mechanisms, considering that the pupal stage is critical to its later development. One of the structures that may be directly affected is the hypopharyngeal gland [28], which is located in the head and produces a protein-based substance that is used to feed larvae, the queen, and the drones [43].

6. Lack of biosecurity is a risk to honeybee health in developing countries

Honeybee biosecurity is a set of measures designed to protect a beekeeper's honeybees from the entry and spread of pests. Honeybee biosecurity is the responsibility of every beekeeper and every person visiting or working in an apiary. Implementing honeybee biosecurity is essential for a beekeeper's business. If an exotic or endemic pest establishes in an apiary, business costs will increase (for monitoring, cultural practices, additional chemical use, and labor), productivity will decrease (yield and/or colony performance), and markets may be lost. The health of the honeybee industry also ensures the continued success of many other plant industries that rely on honeybees for pollination. Early detection and immediate reporting increases the chance of an effective and efficient eradication.

The biosecurity measures of an individual beekeeper can be enhanced by collaborating with others in a particular region. Through this collaborative approach, biosecurity threats to all apiaries in a region can be minimized. Promotion of honeybee biosecurity at the regional level can be enhanced through the engagement of the community and by understanding the area's vulnerability, and the potential source and nature of threats. Neighboring apiaries (managed or abandoned), feral colonies, and/or unregistered hives are examples of potential biosecurity threats. Regional biosecurity efforts are strengthened by identifying what resources and

expertise are available and by having a commitment from stakeholders to implement biosecurity measures and surveillance programs. Implementation of honeybee biosecurity strategies underpins regional biosecurity, which in turn underpins national biosecurity.

7. Prospects for the development of new methods to deal with V. mites

Over the coming years, we can expect new developments that will change the way we control infestation with V. mites. Breeding programs in many countries are aiming to select and develop bees that are more tolerant of *Varroa*. These bees either may be able to naturally maintain better control over the mite population or may be more tolerant to the presence of the mites and their associated pathogens.

Helping honeybees help themselves: breeding *Varroa*-resistant bee populations is a long-term solution for the mite problem. A number of honeybee colonies are showing the first signs of resistance—honeybees with a behavioral trait called *Varroa*-sensitive hygiene can detect the *Varroa* mites in the closed brood cell. These bees pull out the infested pupae and thus stop the *Varroa* mite from multiplying in the colony. This behavior was originally only known to occur among Asian honeybees. On the basis of these observations, researchers intend to strengthen this defensive capability by practicing selective breeding among European bee populations and thus create long-lasting protection against the parasites; but several more years of study are required. It is therefore still necessary that researchers and beekeepers continue to work on developing new methods to combat the *Varroa* mite.

It is especially important to know the enemy better through intensive monitoring of the *Varroa* mite. In long-term observations, researchers gain significant facts and figures about the mites' population and the efficacy of current countermeasures. These results help them optimize and complement *Varroa* treatments. So far, they were even able to identify sexual attractants called pheromones that can help to specifically develop new natural or synthetic varroacides in the future. Currently, experts are examining and testing the pheromones in the laboratory. Other treatments are used, for example, the sterilization of male and female mites or heat treatment, to protect the bee colonies from the *Varroa* mite.

8. Conclusions

Bee population in northern Iraq witnessed dramatic decreases at the late 1980s due to the wide spread of *Varroa* mite infestation according to the interviews made with local beekeepers. Moreover, war conditions led to the migration from the rural areas to the urban areas and economic sanction imposed by United Nation on Iraq; all these participated in the heavy loss of bee industry in the area.

Based on the results obtained throughout investigating a large number of colonies in Northern Iraq, high level of *Varroa* mite infestation found in all apiaries of the region and may act as a risk factor on the health of bees. Beekeepers lost most of their colonies, particularly those with traditional hives. This kind of hives are made of simple wood baskets covered with clay; they cannot be inspected and should be destroyed for honey harvesting; therefore, this type of hives should be neglected and it is necessary to encourage local beekeepers to use modern bee hives instead of these undeveloped hives.

Despite extensive using of acaricides by beekeepers, the parasite remains threat to the bee hives of the area including feral colonies. The apiculture sector was destroyed in Iraq after the gulf war; at that time, only feral colonies existed in the mountains.

Beekeeping process started again after 1991, and infested honeybee colonies were illegally entered the area from neighboring countries; Therefore, *Varroa* mite infestation is widely spread in apiaries of the Dohuk region, northern Iraq.

The simple method of counting mites in hive debris is a useful parameter for monitoring the population development of *Varroa* in colonies with hatching brood. Using movable screened bottom boards placed in the opposite side of the hive entrance for the observation and counting naturally felled down *Varroa* mites were very beneficial in this area during hot summer periods.

Varroa mite infestation strongly affects colony health in two ways: directly, when the mites feed on the hemolymph of the developing and adult bees, and indirectly affecting the population growth of the colony. This leads to shortage of pollen and nectar gathering by foragers as well as insufficient quantities of royal jelly secreted by nurse bees to provide the developing bees, because both larval and adult nutrition have direct effects on the honeybee body growth.

The reduction in the size of hypopharyngeal glands has a potential adverse effect on the production and quality of royal jelly that causes abnormal development of the brood. Mandibular glands appeared less sensitive to the *Varroa* infestation compared to hypopharyngeal glands; this may be attributed to the earlier development of the hypopharyngeal glands than mandibular glands in both larval and adult stages. *Varroa* mite can act as a vector for several viruses, which have tropism to specific structure of the body. Workers infested by *Varroa destructor* as pupae fail to develop key physiological characteristics of normally developed winter bees. The winter bees that develop as larvae in the presence of highly infested nurse bees in the late autumn will be less likely to survive until spring or result in colonies containing a large number of workers with underdeveloped hypopharyngeal and mandibular glands. If a considerable fraction of the wintering bee population is infested during the pupal stage, definitely these bees will affect the spring population of newly emerged workers and thereby the overall colony survival in the next season. Beekeepers in this area should therefore combine the late autumn management strategies with the mid- and late summer treatment protocols to keep mite population at low levels before and during the period when the winter bees emerge. This compilation of present-day knowledge on *Varroa* honeybee interactions emphasizes that we are still far from a solution for *Varroa* infestation and that, therefore, further research on mite biology, tolerance breeding, and *Varroa* treatment is urgently needed.

Author details

Zahra Naeef Ayoub
Department of Plant Protection, College of Agriculture, University of Duhok, Kurdistan Region, Iraq

*Address all correspondence to: shamdin_hb@yahoo.com

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Chapter 5

Beekeeping in Brazil: A Bibliographic Review

Karuane Saturnino da Silva Araújo, Bruno Machado Araújo, Diego Carvalho Viana, Jailson Honorato, Virlane Kelly Lima Hunaldo, Ila Raquel Mello Cardoso, Dark Luzia dos Santos Neto, Leonardo Moreira de Oliveira, Eline Pacheco, Cláudia Lima Dias, Ivaneide de Oliveira Nascimento, Djany Souza Silva, Gerbeli de Mattos Salgado, José de Ribamar Macedo Costa, Jaisane Santos Melo Lobato and Sandra Maria Botelho Mariano

Abstract

Brazil presents favorable conditions for beekeeping, having a suitable climate; native flowering plants with great potential for the production of honey, pollen, propolis, and royal jelly; and bees adapted to our conditions, tolerant to the main apicultural diseases and highly productive. Through the meliponiculture, the conservation of mainly native forest areas is allowed; therefore, they are the best environments for the creation of native bees and production of by-products of the beehive with quality. The stingless bees are very sensitive to any disturbance due to anthropogenic action. A systematic bibliographical review was carried out in different electronic databases, through descriptors referring to beekeeping in Brazil. The identification of articles and their inclusion occurred between January 2018 and April 2018. The bibliographic research was conducted in the following electronic databases: (1) Scientific Electronic Library Online (SciELO), (2) Public Library of Science (PLOS) Biology, and (3) ScienceDirect. In order to help in the process of standardization of bee products and traceability of the production chain, it was possible to draw a profile of the main bioactive substances of the beehive products of Brazil. It was also possible to relate the benefits of an adequate management of beekeeping and meliponiculture in Brazil.

Keywords: beekeeping management, meliponiculture, beehive products, propolis

1. Introduction

Bee products such as honey, beeswax, pollen, royal jelly, and propolis have a wide range of use. In addition to human nutrition, bee products can be used in traditional medicine [1].

Brazil has favorable conditions for bees and has a great variety of vegetation from which bees can collect resins, which cause a great chemical diversity among bee

products collected in different regions and different seasons [2]. The chemical diversity aspect of bee products demonstrates that prior to their use they must be chemically standardized to ensure quality, efficacy, and safety, and thus it is possible to correlate the type of product with its therapeutic application [3].

Several studies point to health-related benefits in propolis use. In the Brazilian Northeast, studies demonstrate, the benefits of the use of red propolis in renal lesions include beneficial alterations in the histopathological aspect of the renal tissue and potential clinical benefits in the use of this propolis to protect the kidneys against ischemic acute renal failure [4]. *Tetragonisca angustula* is a stingless bee distributed widely in Brazil and Mexico. The biological activity of *Tetragonisca angustula* honey, particularly its antimicrobial activity, has been well documented and studied, demonstrating good antimicrobial activity against *Staphylococcus aureus* bacteria [5, 6]. Honey produced in the Eastern Amazon, Brazil, was reported with the total content of phenolic compounds higher than those already reported in the literature, as well as high antioxidant activity [7].

Brazilian vegetation offers a large amount of chemical compounds through its diversity in which products of apicultural origin can offer great potential in the quality of hive products produced in the country, since Brazil has some of the largest biomes in terms of biodiversity and total area. In the neotropics, the flora through the biodiversity existing in them is incomparable in relation to the other existing biomes, being the flora existing in Brazil extremely rich with diverse floral morphology which attracts a great amount of pollinators. The Amazon stands out as being the main maintainer of the planet's greatest diversity [8].

Most conservation decisions occur at national or regional spatial scales, where information becomes useful at such decision-making scales, which is essential to guide conservation practice. Conservation practices such as meliponiculture tend to aid the conservationist effect of the sustainable use of Brazilian vegetation. It is also worth noting that Brazil is one of the most biodiverse countries in the world and, consequently, use and conservation decisions have impacts on these local populations [9].

Through the meliponiculture the conservation of mainly native forest areas is allowed; therefore, they are the best environments for the creation of native bees and production of by-products of the beehive with quality. The stingless bees are very sensitive to any disturbance due to anthropogenic action.

The Management of beekeeping identifies measures against the specific risks in this activity is very important for increasing productivity, income, and production and promoting beekeeping [10]. In this context, a systematic bibliographical review was carried out in different electronic databases, through descriptors referring to beekeeping in Brazil.

2. Body: research methods

Study design: the study of systematic bibliographic review in different scientific electronic databases is through descriptors referring to beekeeping in Brazil. The identification of articles and their inclusion occurred between January and April 2018.

Electronic databases: the bibliographic research was conducted in the following electronic databases: (1) Scientific Electronic Library Online (SciELO), (2) Public Library of Science (PLOS) Biology, and (3) ScienceDirect. Additional information was obtained from the manual search based on the references listed in the articles included in the review.

Search strategy: the searches were conducted using descriptors cataloged in the Health Sciences Descriptors (DeCS) and in the Medical Subject Headings (MeSH), in Portuguese and English contained in the title or in the summaries of the studies. The combination of terms used together or separately in the respective databases (SciELO, PLOS Biology, ScienceDirect) were *gestão de apicultura* (beekeeping

management), *meliponicultura* (meliponiculture), *produtos da colméia* (products of the hive), and *própolis* (propolis).

Selection and analysis of publications: for the selection of articles, a personal study form was prepared with the following information: author and year, title, study development period, federative unit, city and area of research, study design, descriptor used to locate the publication, objective, and main results. An inclusion criterion was used in which the selected articles had to be original, published in international or national journals, in English or Portuguese, between 2013 and 2018, from the subareas of health sciences, medicine and health sciences, research articles, and open articles accessed and indexed in one of the bases previously mentioned. Papers containing data on beekeeping in Brazil were selected for review.

Sampling: a total of 10,645 scientific papers on apiculture were identified in Brazil and in the world, searched in the databases and with the descriptors mentioned above. Of the total of articles published between the years 2013 and 2018, 4149 works were related to the subject of beekeeping. Articles were repealed because they were repeated in other languages, and products from Brazil were not evaluated. For the present revision of the scientific literature, 25 papers were selected.

3. Results

Only articles containing beekeeping studies in Brazil between the years 2013 and 2018 were selected for review. As can be seen in **Figure 1**, the number of published papers on beekeeping is relevant. However, we can see below the index

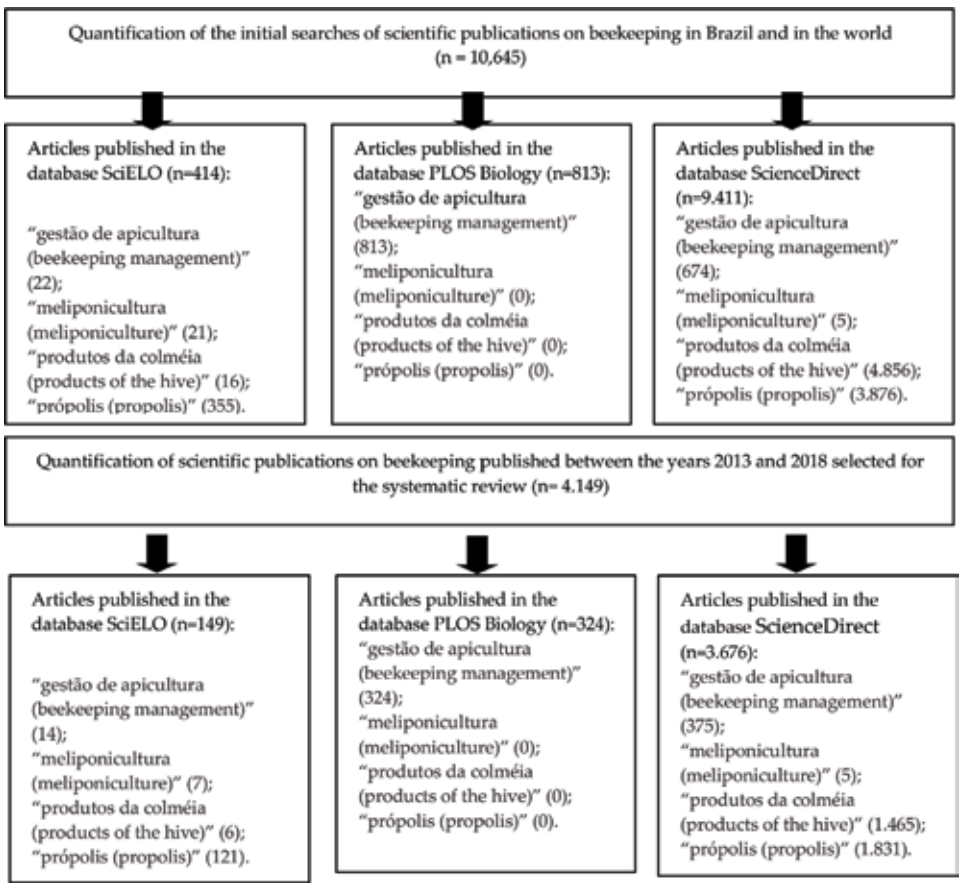

Figure 1.
Logical framework of the systematic review, studies on beekeeping in Brazil between the years 2013 and 2018.

of works on this subject in Brazil, mainly in the base PLOS Biology. The results obtained with the application of the described search strategy are presented in the logical framework of the study (**Figure 1**).

Table 1 summarizes the papers evaluated for this study.

N°	Bibliographic reference	Conclusions
01	Canhos et al. [11]	By 2014, more than 95% of SpeciesLink Network users were from Brazil. In the last decade, the open-access movement has boosted the development of many web platforms for data sharing. Adequate policies unfortunately have not followed the same pace, and now many initiatives may perish. Part of the problem facing electronic infrastructures is that agencies prioritize innovative projects and rarely fund ongoing and long-term initiatives
02	Faita et al. [12]	In terms of their defensive behavior, bees in the southern region of the state were not influenced by bees of the European origin
03	Ponciano et al. [13]	It was verified that the educational level positively influenced the development of beekeepers. The level of illiteracy was null for beekeepers in these municipalities, and most of them have finished at least high school. Other characteristics that contributed to raise this index in the mentioned municipalities were technical assistance, management of the queen exchange, and the practice of migratory beekeeping. Thus, the modernization of beekeepers in order to improve the technological level, expand productivity, and diversify their production necessarily passes through the level of knowledge of the beekeeper and socioeconomic situation
04	Alves et al. [14]	The increase in temperature and the decrease of humidity resulted in increased frequency of bees foraging activity, accounting for 46.9% of activity in *A. mellifera*
05	Camargo et al. [15]	In the multivariate analysis of the grouping, five groups were formed, due to the similarity of management, indicating the highest average production in hives in the most populous area of Santa Helena and lowest average production in the most populous of Marechal Cândido Rondon. The clustering of hives, differences in honey production, and floristic survey indicated that these differences could be associated with management and climatic differences recorded during the production period in the studied areas
06	Salis et al. [16]	The melliferous species evaluated in the region of the Urucum Massif, on the western border of the Pantanal, flourish throughout the year, with the highest number of species in summer (January to March) and lowest in winter (July to September). The interaction between the growth habit of melliferous plants and the climate influences the flowering season and causes herbs and lianas to flower mainly in summer and autumn (January to June) and trees and shrubs in the spring (late September to December). There is availability of nectar and pollen throughout the year, with supply decreasing in the winter months (July to September)
07	Souza et al. [17]	Both the quantitative and qualitative analyses of the pollen types found in the propolis of *Scaptotrigona* aff. were valuable for their characterization. The results obtained provide support to know the possible species suppliers of resin for Tubi, increasing the scientific knowledge about the propolis of stingless bees that are still incipient, and may also contribute to the protection of this species of bee in the elaboration of conservation programs and replanting of their areas of occupation
08	Araújo et al. [18]	Propolis samples collected in the two regions of Tocantins presented concentrations of moisture, ash, and wax that fall under Brazilian legislation for propolis quality. They also evidence a high concentration of phenol compounds and good antioxidant capacity. The variety of phenol compounds identified in propolis samples collected for this study, compared to the diverse biological functions described in literature for these compounds, indicates that there is a great pharmacological potential in this raw material

N°	Bibliographic reference	Conclusions
09	Siqueira et al. [19]	The red propolis, collected in the region of Brejo Grande-SE, presents characteristics of identity and quality compatible with the parameters established by the Ministry of Agriculture and has proven effective against *E. faecalis*, being able, with more studies, to become a valuable endodontic treatment
10	Pimenta et al. [20]	The present study demonstrated that, although medications based on brown propolis with or without calcium hydroxide have limitations inherent to an in vitro study, they are effective against *E. faecalis*
11	Siqueira et al. [21]	The results of this study show that, similar to chlorhexidine, red propolis alcoholic extract has good fungistatic and fungicidal activity against most samples of *Candida* species. This antifungal activity may hold a promise for future applications as an alternative treatment for infections caused by these fungi. Further investigation into the use of red propolis for the prevention and treatment of periodontal diseases is required, including microbiological, randomized controlled trials and longitudinal studies
12	Toledo et al. [22]	For the first time, films could be successfully obtained using the residue obtained from the preparation of propolis extracts, which is normally discarded. Density, water vapor permeability, moisture uptake capacity, and the mechanical properties of the films were adversely affected by the addition of polymeric adjuvants with different affinities for water. The thermal stability of films was observed, and using the technique of FT-IR, it was proven that while the adjuvant played a role, BP is the main component responsible for the characteristics of the films. Therefore, considering the results obtained and the principles of sustainability (reduce, reuse, and recycle), this work could contribute to the utilization of BP to obtain films for use in the food and pharmaceutical industries. However, further research is needed to gain a better understanding of the properties and applications of these films
13	Pagliarin et al. [23]	The results of this study suggest that propolis paste may be a feasible substitute for triple antibiotic paste in the disinfection of root canals of immature teeth with necrotic pulps, with the advantage of not causing changes in tooth color. The new tissues formed inside the root canal showed characteristics similar to the cementum, bone, and periodontal ligament
14	Valones et al. [24]	The present study demonstrated that a dentifrice containing exclusively rosemary extract has the capability of inhibiting the growth of the studied bacteria. However, for the inhibition of *L. rhamnosus*, a propolis-based commercial dentifrice was more effective than the dentifrice containing rosemary extract
15	De Luca et al. [25]	All rats gained weight and remained apparently healthy and active throughout the experiment. The PV (typ. 12 propolis from Southeastern Brazil—propolis ethanolic extract, 15%, w/v) reduced the development of smooth enamel caries as compared to the untreated group, without significant difference of GS (gold standard). GS significantly reduced the severity of sulcus lesions, affecting dentin. No macroscopic abnormality or abnormality of the tissue was observed in the oral cavity of the animals throughout the experiment
16	Monteiro et al. [26]	The level of schooling, the number of hives populated, and the participation and/or knowledge about some type of program or specific actions for the apicultural segment promoted by the federal government exerted positive effects on the index of innovation and learning of beekeepers in all evaluated quantile, while the variable time of operation of the beekeeping company only influenced the IIA (innovation and learning index) in the lower part of the distribution
17	Potrich et al. [27]	*B. bassiana* reduced the survival of *A. mellifera* workers in the four bioassays tested (spraying on *A. mellifera*, contact on a smooth surface, contact on soy leaves, and mixed with candy paste) in the laboratory. The entomopathogens *B. bassiana*, *B. thuringiensis*, and *M. anisopliae* did not cause morphometric changes in the midgut of *A. mellifera* fed with candy paste

N°	Bibliographic reference	Conclusions
18	Sattler et al. [28]	The results of the chemical analysis showed that the samples were in accordance with the relevant regulations. The composition of vitamins and pollen types varied among the samples. Some bee pollen could be classified as a source of a particular vitamin in a standard dose (25 g). Lipid and protein content from Rio Grande do Sul presented higher mean values (p b 0.05) compared with the other two states. Some correlations between chemical composition and botanical taxon were observed. Principal component analysis showed that the samples from the states of Rio Grande do Sul and Paraná presented similarities in terms of composition for each location. HCA (hierarchical cluster analysis) and PLSDA (partial least squares discriminant analysis) were not able to classify the samples based on the chemical markers used. The analysis of vitamins confirmed that BP from this region can be a good source of antioxidant vitamins and that it can provide important nutritional information to food researchers and bioactive compounds for consumers
19	Neves et al. [29]	The botanical origin of propolis samples is difficult to ascertain on the basis of palynological analysis only, and a more definitive confirmation depends on analysis comparing the chemical profile of the samples with the chemical profile of resins and extracts from the plants found in close vicinity of the bee's hives. It should be stressed that red propolis has been suggested to be the only propolis type derived from a plant from the Leguminosae family (*D. ecastaphyllum*), rich in isoflavones such as genistein and formononetin. Although flavonoids exhibit pleiotropic activity affecting several different targets, and synergistic effects cannot be ruled out, our results suggest that the isoflavone formononetin is responsible at least partially for the antimicrobial activity of red propolis
20	Bittencourt et al. [30]	Twenty-nine metabolites were identified along with 34 other metabolites that were classified into the following classes: triterpenoids (12), acetyltriterpenoids (3), sesquiterpenes (6), steroids (4), and hydrocarbons (9). The antioxidant capacity (IC50) ranged from 21.50 to 78.77 μg/mL, whereas the content of total phenolic compounds ranged from 31.88 to 204.30 mg GAE/g of dry weight. Total phenolic compounds and methyl retinoate showed a positive correlation with the antioxidant capacity, whereas tetradecanal, γ-palmitolactone, and ethyl hydrocinnamate showed a negative correlation. Different sets of metabolites are shown to correlate with the antibacterial activity of the extracts, which is largely dependent on the type of microorganism. This innovative approach allowed us to identify likely bioactive compounds in the extracts, although the mechanisms underlying antibacterial activity encompass a complex trait, which might involve synergistic effects
21	Almeida et al. [31]	HPLC/UV and LC/ESI/FTMS/Orbitrap identified different secondary metabolite classes such as isoflavones, chalcones, pterocarpans, flavonones, flavones, phenolic acids, terpenes, and guttiferones in the Brazilian red propolis tinctures. LC/ESI/FTMS/Orbitrap was a useful tool in the confirmation of different chemical markers of the red propolis and demonstrated the complexity of this apiceutical product. The tincture and microcapsules of the red propolis presented high flavonoid quantities especially for the spray-dried microcapsules. The tinctures and spray-dried microcapsules presented similar antioxidant activity and were better than the freeze-dried microcapsules. The tinctures and microcapsules proved to be bioactive against Gram-positive and Gram-negative bacteria; moreover, the Gram-positive bacteria were more sensible than the Gram-negative bacteria. The LC/ESI/FTMS/Orbitrap and microbiological methods were sensitive and could distinguish the quality of the tinctures and the microcapsule compositions. Thus, the tinctures and microcapsules of the red propolis have a potential application for nutraceutical products
22	Araújo et al. [32]	In conclusion, propolis was more efficient in inhibiting mycelia growth of *Pythium insidiosum*, while geopropolis showed a fungistatic effect. This effect

N°	Bibliographic reference	Conclusions
		may be due to the propolis chemical composition, which has more active compounds than geopropolis. Since propolis exhibited a better response, further experiments should be carried out both in vitro and in vivo, as treatment with conventional antifungal agents is still problematic
23	Silva et al. [33]	The acute study revealed no lethal effects at 300 mg/kg of HERP, but toxic signs were observed, as HERP had na LD50 of more than 300 mg/kg, indicating a warning. The most toxic signals in subacute studies were observed in males at a dose of 200 mg/kg HERP. These results suggest estrogen-like activity, possibly from the isoflavones in HERP
24	Rodrigues et al. [34]	The results of the analyses show the main components (inorganic and organic components) of the "caba-leão" wasp nests (*Sceliphron* sp., *Sphecidae*) used by "caboclos" as a topical medication to treat mumps and earaches. The ethnopharmacological data collection consisted of samples of wasp nests and soil, as a source of inorganic elements, from the Jaú and Unini Rivers, in the River Negro basin, Amazon, Brazil. The inorganic components are formed by minerals (quartz, kaolinite, illite, and gibbsite), identified by X-ray diffraction and infrared spectroscopy, which are common in the soil of the region. The analyses by X-ray fluorescence indicate that the most common oxides are SiO2, Al2O3, and Fe2O3 within minerals
25	Silva et al. [35]	Twenty-two pollen types were identified. The total phenolic content ranged from 17 to 66 mg GAE/g of extract; the highest contents were found in honeys produced from pollen types such as *Clidemia* and *Myrcia*. The antioxidant activity was higher in the samples that contained higher quantities of phenolic compounds. In relation to the antibacterial activity, samples CAD3, CAD4, and SAD3 presented the best results. Fourteen phenolic compounds were determined. Among them, we identified the flavonoid taxifolin, which has not previously been described in honeys from stingless bees, and we report the identification of catechol in Brazilian honey samples for the first time

Table 1.
Summary of the papers of beekeeping in Brazil between the years 2013 and 2018.

4. Discussion

It was possible to observe a large number of articles published on this topic, but it was necessary to compile the data obtained by all these studies. The products obtained by beekeeping are honey, beeswax, pollen, and royal jelly, which are used in both human nutrition and conventional and traditional medicine [1]. For the commercialization of these products, there are requirements in the Brazilian legislation for inspection, with the objective of evaluating the zoosanitary requirements and the certificates for the importation of queen bees and bee products [36]. The beekeeping activity in Brazil has a great challenge related to the absence of laboratories and official techniques to analyze the absence of pathogens, except for the microbiological analysis to identify spores of *Paenibacillus larvae* [37].Brazil is the ninth largest honey producer in the world and the second largest producer in Latin America behind only Argentina. In Brazil, the amount of honey produced was 37.82 thousand tons in 2015, representing a reduction of 1.7% in relation to the previous year. The last drop had occurred in 2012, when honey production was heavily affected by the scarcity of rainfall in the major producing regions [38].

It is interesting to discuss bee products and aspects of health in Brazil. Therapeutic activities of Brazilian propolis and honey were reported in the works highlighted in the present literature review (**Table 1**). The identified compounds

are described as important in defense of oxidative damage of biological molecules and capable of assisting in the treatment of various pathologies.

Propolis is a water-insoluble resinous mixture of bees' saliva and vegetable resin with a wide range of important biological and therapeutic properties (antioxidant, anti-inflammatory, antitumor, immunomodulatory, antibacterial, antiviral, autobiofilm, antifungal, analgesic, anesthetic), and several studies have shown that propolis has bactericidal and bacteriostatic activity against several Gram-positive bacteria, such as *S. aureus*, *S. mutans*, and *B. subtilis*, and Gram-negative bacteria, including *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. In addition, a synergistic inhibitory effect of propolis and antibiotics has also been reported on growth of *S. aureus*. The relevance of such antimicrobial activity of propolis is increased if one considers the growing appearance of antibiotic-resistant microorganisms in hospitals and in the community. Notably, this situation is exacerbated by the inadequate use and prescription of antibiotics and the scarcity of new medicines [39–42].Torres et al. [43] performed the chemical characterization and antimicrobial activity of propolis obtained from the stingless bees *Melipona quadrifasciata quadrifasciata* and *Tetragonisca angustula*, and verified that their ethanolic extracts of propolis (EEPs) of *M. quadrifasciata quadrifasciata* and *T. angustula* presented antimicrobial activity against Gram-positive and Gram-negative bacteria and antioxidant activity. The chemical analysis of EEPs revealed the presence of terpenoids, flavonoids, and polyphenols, which were more abundant in EEP from *M. quadrifasciata quadrifasciata*.

The use of propolis has also been associated with the treatment of lung cancer, since its therapeutic potential for the treatment of several diseases is well studied and elucidated [44]. Szliszka et al. [45] performed some epidemiological and preclinical studies and suggested that polyphenols and flavonoids in propolis have direct antitumor and chemopreventive effects. Propolis was used as a dietary supplement, and it was observed that the anticarcinogenic activity of the flavonoids in it can suppress the growth of the lung tumor, inhibiting angiogenesis and inducing apoptosis of tumor cells [46]. It has also been reported that the protective effect of propolis against various types of cancer, including lung cancer, can occur by directly inhibiting the growth of tumor cells and inducing apoptosis [47] or indirectly by capturing ROS and increasing the activities of antioxidant enzymes [48].

Brihoum et al. [49] investigated the antitumor effect of Algerian propolis on a human lung adenocarcinoma cell line and its chemopreventive effect on experimentally induced lung cancer and found results suggesting that Algerian propolis may act against lung cancer and may lead to the potential use of these natural compounds for the treatment and prevention of lung cancer.

According to [35], which evaluated the propolis of the region of Amazonas state in Brazil, the antioxidant activity was higher in the samples that contained larger amounts of phenolic compounds. The papers highlighted in the present review identified the following substances of antioxidant capacity: gallic acid, ellagic acid, catechin, gallocatechin, hesperidin, kaempferol, luteolin, morin, naringin, naringenin, and rutin. Honey analysis also confirmed the presence of antioxidant substances, highlighting phenolic compounds.

The meliponiculture has a great importance in Brazil and in the world. Most of the published works were carried out in the South [50], Southeast [51], and Northeast regions [52]. These three regions also have the highest honey production in Brazil, which in 2013 was 35,365 tons: the South contributed 50.2%, the Southeast 21.5%, and the Northeast 21.3%. The Central-West region is one of the least-producing honey regions in the country, with only 4.4% participation in the national production, despite the rich native flora found mainly in the Cerrado and Pantanal biomes [16]. Conservation practices such as meliponiculture tend to support the conservationist effect of the sustainable use of Brazilian vegetation [9].

In order for beekeeping to offer greater economic benefits to the producer, it is necessary to associate this activity with other agricultural activities. This activity requires relatively low investment and continuous cash flow for agricultural depots [10].

According to [12], the productivity of apiculture products is directly linked to the appropriate management techniques applied in the apiary. In the study by [13], it was noticed that the technological level of beekeepers is relatively low, which can exert a strong influence on the management of beekeeping in Brazil. Apiculture, in order to be competitive, requires the adoption of good management practices, adequate equipment, specific knowledge of technologies, management, marketing, and organization to add value to products [13].

5. Conclusion: key results

In order to help the process of standardization of bee products and traceability of the production chain, it was possible to draw a profile of the main bioactive substances of the beehive products of Brazil. It was also possible to relate the benefits of an adequate management of beekeeping and meliponiculture in Brazil. Standardization is not yet observed in scientific research and legislation in Brazil. The available information on the honey flora in Brazil is still empirical and restricted to some regions of the country. The low amount of research on the subject in Brazil reflects on the technological level of apiaries and consequently on the management of beekeeping in Brazil. In this context, it is necessary to carry out more research and public policies that help in the improvement of beekeeping and meliponiculture in Brazil.

Author details

Karuane Saturnino da Silva Araújo[1*], Bruno Machado Araújo[2], Diego Carvalho Viana[1], Jailson Honorato[1], Virlane Kelly Lima Hunaldo[3], Ila Raquel Mello Cardoso[4], Dark Luzia dos Santos Neto[4], Leonardo Moreira de Oliveira[1], Eline Pacheco[1], Cláudia Lima Dias[1], Ivaneide de Oliveira Nascimento[1], Djany Souza Silva[3], Gerbeli de Mattos Salgado[1], José de Ribamar Macedo Costa[3], Jaisane Santos Melo Lobato[3] and Sandra Maria Botelho Mariano[4]

1 State University of the Tocantina Region of Maranhão (UEMASUL), Imperatriz, MA, Brazil

2 State University of Maranhão (UEMA), Balsas, MA, Brazil

3 Federal University of Maranhão (UFMA), Imperatriz, MA, Brazil

4 Federal University of Tocantins (UFT), Brazil

*Address all correspondence to: karuane@gmail.com

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This book comes to fill a void in beekeeping research worldwide since it addresses a series of issues of great contingency such as the problem and control of varroa, the management of the American foulbrood, management of hives to perform an adequate transhumance, and the way of handling Brazilian beekeeping. It is a text that is aimed at scientists, producers, undergraduate and graduate students, companies, and the general public who handle hives at a professional or amateur level that have from one to many hives.The book corresponds to the authors' experience of many years who with their contributions will improve the productive activity of beekeeping in the world concert.

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