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Edited by Muhammad Asif Aziz

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Meet the editor

Dr. M. Asif Aziz is an Associate Professor of Entomology in charge of the Bee Research Unit and an additional director at the Pak Korea Capacity Building Centre at Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi (PMAS-AAUR) Pakistan. With a rich academic tenure exceeding 15 years, he played a pivotal role in the Apiculture Committee of Pakistan's Ministry of Climate Change. Dr. Asif's research focuses on mite management, honeybee health, and royal jelly production. Widely recognized for delivering influential lectures on enhancing rural livelihoods through apiculture, he has conducted numerous training workshops. A prolific author, Dr. Asif has more than forty-four 44 peer-reviewed articles to his credit. He earned his Ph.D. in Entomology from the University of Agriculture, Faisalabad, Pakistan.

Contents

Preface

Welcome to the captivating realm of *Melittology – New Advances*, a literary voyage that delves into the intricate world of bees, unraveling their profound impact on science, culture, and health. This anthology is meticulously curated for inquisitive minds and enthusiasts eager to uncover the latest breakthroughs in the field of bee studies. Within these pages, our esteemed authors embark on a journey, dedicating themselves to providing you with a comprehensive understanding of various facets related to bees and their remarkable products. Each chapter is a testament to their dedication and profound expertise.

Our exploration commences with a thoughtful exploration into the antimicrobial wonders of natural honey, reviewing its chemical composition, antimicrobial and antibacterial activities, antifungal effects, and even its antiviral properties. Venturing beyond the scientific domain, we intricately examine the intimate connection between honey and humanity. The authors weave a narrative that encompasses the historical, sacred, and holistic dimensions of honey, shedding light on how honey transcends its scientific attributes to become a cultural and spiritual entity. A groundbreaking chapter unfolds as meticulous palynological and physicochemical analyses reveal the distinctive characteristics of honey from *Butia yatay* palm savannas in Argentina, marking the inaugural report on producing and characterizing honey from this botanical and geographic origin.

Further chapters delve into the diversity and distribution of stingless bees, shedding light on their nesting and foraging behavior, with a particular emphasis on meliponiculture in Indonesia and the imperative need for conservation efforts. Recognizing the critical role of honeybee populations in maintaining ecosystem health, a chapter on diseases in honeybees provides a comprehensive examination of symptoms, diagnosis, and management strategies for various afflictions. Biological control methods are explored, offering sustainable solutions to combat bacterial, fungal, protozoan, and viral diseases, as well as various pests of honeybees.

The therapeutic potential of propolis in dentistry is laid bare, detailing its multifaceted applications and emphasizing its analgesic and biocidal properties in diverse dental contexts. Concluding our exploration is a captivating chapter on propolis obtained through meliponiculture, touching upon sociocultural importance, historical uses, and the chemical composition of geopropolis. This chapter serves as a bridge from ancient wisdom to the modern medicinal applications of bee products.

As we embark on this enriching journey through *Melittology – New Advances*, we express heartfelt gratitude to our esteemed co-authors and their dedicated teams, whose expertise and passion have breathed life into this volume. The realization of this work has been a collaborative effort, and special acknowledgment is extended

to Publishing Process Manager Mateo Belir at IntechOpen, whose dedication and meticulous attention to detail have steered the project with precision. We also extend gratitude to Commissioning Editor Jelena Germuth, whose keen insights and editorial expertise have shaped the content of this book to meet the highest standards.

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Chapter 1

Antimicrobial Properties of Natural Honey

Fatiha Abdellah

Abstract

In present time, drug resistance in microbes is a very serious problem. The consequences of antibiotic resistance are significant. It can lead to the persistence of infections, increased healthcare costs, prolonged hospital stays and higher mortality rates. The research to obtain new antimicrobial compounds is vitally important. Hence, natural products are considered as safe alternatives to synthetic drugs. Honey is highly regarded for its nutritional value and therapeutic properties it has been used in traditional medicine in many countries for thousands of years. Its effectiveness as an antimicrobial agent is primarily due to its unique chemical composition natural hydrogen peroxide content, low water activity and acidic pH. The antimicrobial activity of honey can vary depending on factors such as floral source, geographical origin and processing methods. Honey has a strong antimicrobial effect and it may be an alternative natural source of medicine to prevent and treat many diseases caused by pathogenic microorganisms.

Keywords: honey, antibiotic resistance, antibacterial activity, antifungal activity, antiviral activity

1. Introduction

Microbial resistance to modern antimicrobial drugs is a serious problem worldwide. It has a significant consequences and leads to the persistence of infections and increased healthcare costs. It's important to solve this problem by developing new drugs to which microbes have little or no resistance [1]. Honey has been known for its antimicrobial properties for thousands of years [2]. It is a complex mixture, its chemical composition and biological properties vary according to its geographical and botanical origins [3]. The effectiveness of honey as an antimicrobial agent is associated with many factors such as low pH, high osmolarity, hydrogen peroxide content, presence of methylglyoxal (MGO) and defensin-1. Furthermore, flavonoids and phenolic compounds contribute to the antibacterial effect of honey [4]. Honey's antimicrobial activity can vary depending on factors such as flower source, geographic origin and processing methods [5]. In addition, honey showed no side effects and was inexpensive, which is an additional advantage when used for medicinal purposes [6]. Honey has an important antimicrobial activity and it can be used as an alternative natural agent in a number of medicines.

2. Chemical composition of honey

Honey consists mainly of sugar (approx. 80%) and water (approx. 17%), in addition to many secondary components (approx. 3% in total), such as proteins, amino acids and organic acids, vitamins, minerals, polyphenols and volatile compounds [7, 8]. The principal sugars in honey are fructose and glucose, although very small amounts of other mono, dior-oligosaccharides (maltose, sucrose, nigerose, isomaltose, furanose and maltose) have also been identified (see **Table 1**) [9]. The chemical composition of honey varies fundamentally depending on the flower source, but seasons, environmental factors and processing conditions are also important.

2.1 Carbohydrates (sugars)

Honey contains three principal types of sugar. These are fructose, which is one of the highest at 41%, glucose, which has around 34% and sucrose, which is between 1 and 2% [10]. The ratio of one type of sugar to another depends on the flower source and the level of the enzyme invertase, which breaks down sucrose into glucose and fructose. This enzyme is found in the flower from which the bees collect nectar, but it is also found in the bee's body [11].

Substance	Content (%)	
Water		17.2
Sugars	Levulose (d-fructose)	38.19
	Dextrose (d-glucose)	31.28
	Sucrose (saccharose)	1.31
	Maltose and other reducing disaccharides	7.31
	Higher sugars	1.50
	Total sugars	79.59
Acids	(Gluconic, citric, malic, succinic, formic, etc.); total acids calculated as gluconic acid	0.57
Proteins	(Amino acids: glutamic acid, alanine, arginine, glycine, leucine, isoleucine, aspartic acid, valine, histidine and lycine)	0.26
Ashes	(Minerals: potassium, sodium, magnesium, calcium, phosphorus, iron, manganese, copper, etc.)	0.17
Minor component	Mainly comprising pigments, aromatic substances, sugar alcohols, tannins, enzymes and diastases including amylase, peroxidase, succindehydrogenase, phosphatase and invertases, vitamins including thiamine, riboflavin, acid nicotinic acid, vitamin K, folic acid, biotin, pyridoxine and pantothenic acid	2.21

Table 1.
The main components of honey.

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2.2 Amino acids and proteins

The content of amino acids and proteins in honey is relatively low, at most 0.7%. Proteins are found in honey from nectar and pollen as an essential part of plants. Proteins in honey can be found in simple or in a very complex forms [12]. Honey contains approximately all physiologically essential amino acids. The main amino acid proline is a measure of honey ripeness. In normal honey the proline content should be more than 200 mg/kg. Values less than 180 mg/kg mean that the honey is probably adulterated by added sugar [13].

2.3 Aroma compounds and phenolics

The most volatile compounds found in honey probably come from the plant, but some of them are added by bees. Phenolic acids and polyphenols are secondary plant metabolites. They have been suggested as possible markers for determining the botanical origin of honey [14]. Dark honey is reported to contain more phenolic acid derivatives but fewer flavonoids than light honey [15]. According to many studies the most important phenolic compounds found in honey are: caffeic acid vanillic acid, þ-coumaric acid, syringic acid, quercetin, ferulic acid, myricetin, kaempferol pinocembrin, inobanksin, ellagic acid, chrysin, 3- hydroxybenzoic acid, galangin, chlorogenic acid, 4-hydroxybenzoic acid, gallic acid, rosmarinic acid, hesperetin, benzoic acid and others [16, 17].

2.4 Minerals and trace elements

Honey contains different amounts of minerals. Potassium is the main mineral element, averaging about a third of the total, but there is a wide variety of trace elements. Several studies have shown that the trace element content of honey mainly depends on the botanical origin of the honey. Minerals have about 3.68%. Although this portion of honey does not account for a large amount, the minerals contained in honey add to honey's value for human consumption. Honey contains most of the minerals: potassium, chlorine, sulphur, calcium, sodium, phosphorus, magnesium, silicon, iron, manganese and copper [18]. At the observed mean, dark honeys are richer in minerals than light ones. Of course, singles can find darker species poorer than lighter species [18].

3. Antimicrobial activity of honey

Honey is characterised by strong antimicrobial activity against both pathogenic and non-pathogenic microorganisms (bacteria, yeasts and fungi), even those that have developed resistance to many antibiotics [18].

3.1 Antibacterial activity of honey

Honey has a proven antibacterial effect against a broad spectrum of bacterial species including aerobes and anaerobes, Gram positives, and Gram negatives (**Table 2**) [19].

Bacterial species	Disease caused
Bacillus anthracis	Anthrax
Corynebacterium diphtheriae	Diphtheria
Escherichia coli	Diarrhoea, septicaemia, urinary infections, wound infections
Haemophilus influenzae	Ear infections, meningitus, respiratory infections, sinusitis
Klebsiella pneumoniae	Pneumonia
Mycobacterium tuberculosis	Tuberculosis
Proteus spp.	Septicaemia, urinary infections
Pseudomonas aeruginosa	Urinary infections, wound infections
Salmonella spp.	Diarrhoea
Salmonella choleraesuis	Septicaemia
Salmonella typhi	Typhoid
Salmonella typhimurium	Wound infections
Serrata marcescens	Septicaemia, wound infections
Shigella spp.	Dysentery
Staphylococcus aureus	Abscesses, boils, carbuncles, impetigo, wound infections
Streptococcus faecalis	Urinary infections
Streptococcus mutans	Dental caries
Streptococcus pneumoniae	Ear infections, meningitis, pneumonia, sinusitis
Streptococcus pyogenes	Ear infections, impetigo, puerperal fever, rheumatic fever, scarlet fever, sore throat, wound infections

Table 2.
List of bacterial species sensitive to honey and diseases they cause [19].

3.1.1 Factors responsible for the antibacterial activity of honey

Honey's antibacterial activity is attributed to many factors such as: low water content, high viscosity, acidity, hydrogen peroxide content and non-peroxide components, especially the presence of MGO (**Figure 1**) [20].

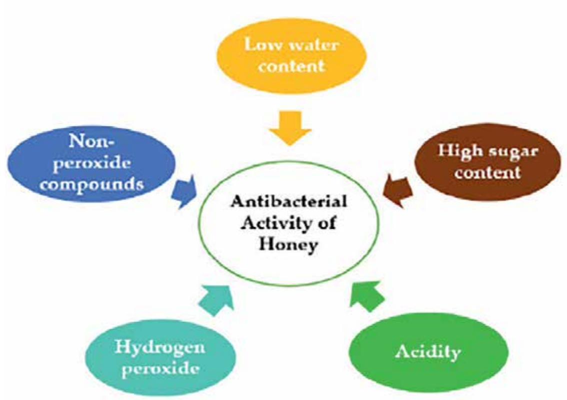


Figure 1.
Schematic diagram presenting the parameters contribute to the antimicrobial activity of honey.

3.1.1.1 Low water content

Water activity is defined as the unbound water molecules in a sample which have a proportional relationship with bacterial contamination. The normal range of water activity (aw) of honey is between 0.562 and 0.62. This is less than the range shown to completely inhibit bacterial growth (0.94–0.99). Pure honey therefore has a very low water content to promote the growth of microorganisms [20].

3.1.1.2 High sugar content

The high sugar content of honey makes the water inaccessible to microorganisms. It creates a hypertonic environment that draws water out of bacterial cells through osmosis, causing them to shrink and eventually die [21].

3.1.1.3 Acidity

Acidity is one of the most important parameters that contribute to the antibacterial activity of honey. It is attributed to the presence of certain acids, in particular gluconic acid (approx. 0.5% w/v). The acidity of honey (pH 3.24.5) is significantly lower than the favourable pH (6.5–7.5) for the growth of most bacteria [22].

3.1.1.4 Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is one of the most essential factors responsible for the antibacterial activity of honey. In honey, hydrogen peroxide is produced by an enzymatic reaction during the transformation of nectar into honey by gluco-oxidase under aerobic conditions [23].

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Glucose Acid} + H_2O_2 \qquad (1)$$

3.1.1.5 Methylglyoxal (MGO)

Methylglyoxal is one of the dicarbonyl components resulting from the Maillard reaction which takes place in all products very rich in sugars, such as nectar. This molecule has a powerful bactericidal power and its content varies according to the geographical and floral origin of the honey and it has a strong correlation with its antibacterial effect [24].

3.1.1.6 Defensin-1

Bee defensin-1 is a small peptide with molecular weights ranging from 3.5 to 6 kDa made by the hypopharyngeal and mandibular glands of bees, and possesses a strong antibacterial activity but only against Gram-positive bacteria including *B. cereus,* S. aureus and Paenibacillus larvae [2].

3.1.1.7 Phenolic compounds and flavonoid

Honey contains various phenolic and flavonoid compounds that play a role in its antibacterial effects and inhibit bacterial growth [22].

3.1.2 Mechanisms of the antibacterial effect of honey

Several mechanisms have been proposed for the antibacterial action of honey. Some examples are as follows:

- The antibacterial activity of several honeys is due to their ability to degrade bacterial DNA.

- Honey's high sugar content creates an osmotic effect, drawing water out of bacterial cells and dehydrating them, which inhibits their growth. Also, the high sugar content of honey leads to the denaturation of bacterial proteins, disrupting their function and inhibiting bacterial growth.

- Honey's thick consistency can create a physical barrier that prevents bacteria from accessing nutrients and adhering to surfaces.

- The bactericidal effect of honey was based on the disruption of cell division. These effect was also associated with extensive cell destruction (loss of structural integrity), lysis and changes in cell form and surface area of pathogenic bacteria.

- Honey has been found to block bacterial attachment to tissues (an important step to initiate infection) and inhibit biofilm formation (which protects bacteria from antibiotics) [25].

3.2 Antifungal activity of natural honey

The incidence of fungal infections is increasing and has emerged as a leading cause of illness and death. Most clinically used antifungal drugs have various drawbacks in terms of toxicity, efficacy and cost. The resistance of pathogenic fungal strains to commonly used antifungal drugs has necessitated the search for new types of antifungal drugs. Honey is a natural product that has been used for its antifungal activity and can be used as an alternative for the treatment of severe fungal infections [26]. Several *in vitro* studies have demonstrated the antifungal properties of honey.

In a study done by Abdellah et al. [27] they reported that Daucus carota honey has an antifungal effect against Candida albicans.

Anyanwu [28] has demonstrated that honey samples used in his study showed different levels of antimycotic activity against the tested fungal isolates, namely, *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum, Microsporum gypseum,* Candida albicans and *Saccharomyces* sp.

Estevinho et al. [29] reported that lavender honey inhibited the growth of the pathogenic yeasts Candida albicans, Candida krusei and Cryptococcus neoformans.

Alzahrani et al. [26] indicated that four varieties of honey from different botanical and geographical origins (Manuka, Acacia, Lavender and Wild carrot) were effective against Candida albicans.

In a study done in Australia by Irish et al. [30] they reported that four varieties of honey inhibit the growth of *Candida* species (*C. albicans*, *C. glabrata* and *C. dubliniensis*).

The result of a study done by Koc et al. [31] demonstrated the capacity of honey samples from different floral sources to inhibit the growth of four yeast strains (Candida albicans, C. krusei, C. glabrata and *Trichosoporon* spp.).

The antifungal properties of honey can vary depending on factors such as the type of honey, its origin and the specific fungal strain.

3.2.1 Mechanism of antifungal effect of honey

Honey has been exhibiting antifungal properties through various mechanisms of action. Here are a few examples:

Osmotic effect: honey has a high sugar content, mainly fructose and glucose. This high sugar concentration creates an osmotic effect that draws water out of fungal cells, effectively dehydrating and killing them.

Hydrogen peroxide production: some varieties of honey contain an enzyme called glucose oxidase, which produces hydrogen peroxide when honey comes into contact with bodily fluids. Hydrogen peroxide has antifungal properties and can help to inhibit the growth of fungi.

Acidity: the low pH of honey (around 3–4.5) creates an acidic environment that is unfavourable for the growth of many fungi.

Phytochemicals and polyphenols: honey contains various phytochemicals and polyphenols that have been shown to possess antifungal properties. These compounds can disrupt the fungal cell membrane, interfere with cell division and inhibit fungal enzymes and fungal growth.

Immune system modulation: honey's immunomodulatory effects can indirectly contribute to its antifungal activity. It can help to enhance the immune response, which in turn can better combat fungal infections.

Release antimicrobial peptides: honey contains certain peptides that have antimicrobial properties. These peptides are released when honey comes into contact with body fluids, and they can inhibit the growth of fungi.

3.3 Antiviral proprieties of honey

Since antiquity, many diseases have been treated by honey. It is an important therapy against respiratory pathogens, including viruses that cause coughing. Several studies have demonstrated the antiviral activity of honey against a range variety of viruses [32]. Its effectiveness can vary depending on the type of honey and the specific virus involved.

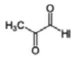
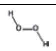
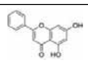
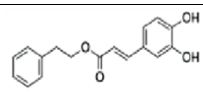
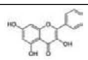
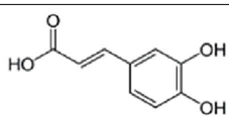
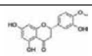
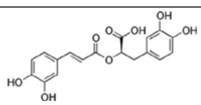
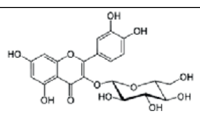
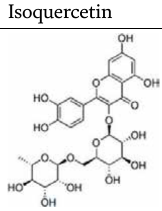
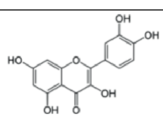
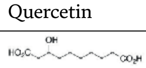
In 2014 Behbahani [33] reported that Iranian monofloral honey had a potent anti-HIV-1 effect.

The antiviral effect of manuka honey against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been reported by Elbashir et al. [34].

The results of a study done by Ghapanchi et al. [35] demonstrated that honey has an inhibitory effect against herpes simplex virus type 1.

3.3.1 The components responsible for the antiviral effect of honey

The antiviral properties of honey are attributed to its natural compounds including hydrogen peroxide and other bioactive compounds (**Table 3**).

Compound	Discretion	Mechanisms of antiviral activities	Reference
 Methylglyoxal	Dicarbonyl resulted from the conversion of DHA during the ripening of honey	Blocks formation of virion assembly and maturation	[33]
 Hydrogen peroxide	Produced mainly during glucose oxidation	Viral inactivation	[36]
 Chrysin	Flavonoid	Inhibition of viral protease enzymes	[37]
 CAPE	Polyphenolic ester	Inhibition of viral protease enzymes	[37]
 Galangin	Flavonoid	Inhibition of viral protease enzymes	[37]
 Caffeic acid	Flavonoid	Inhibition of viral protease enzymes	[37]
 Hesperidin	Flavonoid	Inhibition of viral protease enzymes - Binding to S-RBD and then blocking the interaction with ACE2	[38]
 Rosmarinic acid	Polyphenolic hydroxycinnamic acid	Inhibition of viral protease enzymes	[38]
 Isoquercetin	Flavonoid	Reduction of viral load	[38]
 Rutin	Flavonoid	Reduction of viral load	[39]
 Quercetin	Flavonoid	Reduction of viral load	[39]
 3-hydroxy-sebacic acid	Fatty acid	Unknown	[40]

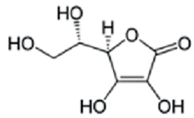
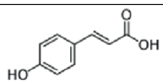
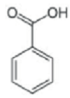
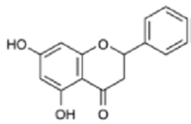
Compound	Discretion	Mechanisms of antiviral activities	Reference
 Ascorbic acid	Sugar acid	Activation of antiviral immune responses	[41]
 *p*-Coumaric acid	Phenolic acid	Unknown	[42]
 Benzoic acid	Aromatic carboxylic acid	Unknown	[42]
 Pinocembrin	Flavonoid	Unknown	[42]

Table 3.
Bioactive compounds in honey that could have antiviral activities.

3.3.1.1 Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is one of the most important components in honey reportedly responsible for its antiviral effect. The result of the study done by Mentel et al. [36] showed that H_2O_2 strongly inactivated human coronavirus 229E (HCoV-229E) and influenza viruses (A and B). Another study reported that H_2O_2 has an inhibitory effect against the avian viruses: H5N1, IBV and Newcastle Disease Virus (NDV) [43]. A recent study indicates the virucidal effect of H_2O_2 against feline calicivirus (FCV), which infects domestic cats [44].

3.3.1.2 Ascorbic acid

Ascorbic acid (vitamin C) is a widely used antioxidant that has demonstrated antiviral immune responses, particularly against the influenza virus [41].

3.3.1.3 Acidity

The low pH of honey creates an unfavourable environment for viruses to thrive.

3.3.1.4 High sugar content

Honey's high sugar content can create a dehydrating environment that limits the virus's ability to thrive.

3.3.1.5 Fatty acid

The antiviral effect of honey could also be due to the fatty acid 10-hydroxy-2-decenoic acid (10-HAD); which induces adhesion of leukocytes to viruses, leading to their eradication [1].

4. Conclusion

The antimicrobial effect of honey has been well-established through several studies. Honey contains natural compounds like hydrogen peroxide, low water activity and high acidity that create an unfavourable environment for bacteria and other microorganisms. Additionally, certain types of honey contain unique components such as methylglyoxal (MGO), which further enhance their antimicrobial properties. While honey can be effective against a range of bacteria, yeasts and fungi, its potency may vary depending on factors such as honey type, source and processing. Overall, honey's antimicrobial properties make it a promising natural alternative for various applications, including wound healing and preservation.

Conflict of interest

The author confirms that this chapter's content has no conflict of interest.

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Chapter 2

Honey beyond Science

Nicoleta Ciocîrlie

Abstract

The approach in this chapter is based on the interconnections expressed in Nature by Honey, Sacred Geometry, and Humanity. I come here with a holistic approach of Honey. (*Holistic = characterized by the belief that the parts of something are interconnected and can be explained only by reference to the whole.)* The role assigned and fulfilled by honey in relation to man is one of food (superfood), natural remedy, therapeutic agent, elixir, cosmetic ingredient, and many other roles, completed with the vital role of bees, as pollinators. Bees produce honey through impeccable teamwork, discipline, and commitment, and honey itself is a gift offered by all the flowers, harvested in honeycombs with a sacred geometry pattern, sealed with wax, and used by bees, other wild animals, and humans. The microbiological and physio-chemical characteristics of honey confirm its complexity and antimicrobial efficiency and highlight the uniqueness and perfection of honey and its benefit to humanity. Science also helps us detect fakes and the adulteration of honey, which can be identified through laboratory analysis. The benefit of honey is undeniable and can be explored from different angles: scientific or spiritual, food or medicine, resource or elixir, inspiration or delight.

Keywords: holistic honey, honey microbiology, honey physio-chemistry, honeycomb sacred geometry, hive sonic geometry

1. Introduction

Interconnection is a reality that we are not fully aware of. Even there are sayings *that if a butterfly flaps its wings in the southern hemisphere, it influences the wind breezes in the northern hemisphere* [1, 2] showing that the Planet Earth is our home and we, humans, are living in the same place in the Universe, we are not aware all the time about the hidden meanings, messages, and roles which everyone has for the whole and for each separately.

In the same approach, the Honey is more than we perceive with our senses and it's more than we can classify and categorize based on our knowledge. If we take a look at the Maslow Pyramid of needs, at the bottom of it there are the basic needs of humans, the ones without life itself cannot exist, and there we can find Honey in the role of food. The Honey finds her place on the higher levels of the Maslow Pyramid when it is connected with Health and even on the highest level, through the connection with spiritual awareness.

We find the Honey in all the ancient books of knowledge and wisdom and in all the sacred texts of major world religions. The Honey was used as food, medication, gift, or traded as merchandise from the very ancient times [3–5].

Let us think from a different angle. Let us find out if there are reasons to consider that the shape influences the properties. The geometrical shape used by the bees for the storage of honey can influence somehow the "Power of Honey"- the beneficial properties. It could be. There are a lot of new advanced research tools that can help us to prove that. The shape, the sound, and the vibrations have an impact on matter. It is already known that honey demonstrates qualities that cannot be reduced to the sum of its components. There is something more, beyond the molecules and compounds identified by laboratory analytical methods. When humanity is ready, the results of more daring research, which come out of the strict patterns of science until now, also appear. This is what happened with the study of water and with the astonishing results obtained by Masaru Emoto's research [6]. Is humanity ready to apply the same approach to Honey?

2. Honey and humans

The unitary recognition by all kinds of specialists all over the world, from all times, of the benefits of honey for humans based on ancient wisdom, completed with its inclusion in certain ancient rituals, can make redundant any attempt to say more than is known but also it can make from any new approach another chance to discover new meanings, new angles of analysis, new ways to confirm what is written in the sacred texts of the world, how that honey is the nectar of the Gods and that it is the Elixir of abundance and the sweetness of Heaven.

All this exploration resembles an effort to take the necessary steps to validate or deny a hypothesis. It is like closing a circle confirming the hypotheses from the beginning: the ancient wisdom knew and wrote about honey and all the scientific research made in the meantime sought to verify and to demonstrate what the ancients knew. As scientific research tools become more and more sophisticated, more precise, and more efficient, honey is further studied, more carefully, more precisely, and more complex and the new generations receive new evidence that the ancient wisdom is valid and authentic.

2.1 Honey and sacred writings

In the ancient resources connected with the most important sacred texts of the world, the wisdom treasures of the mystic knowledge and the beliefs of the ancient were honored, preserved, hidden, and protected in different forms (symbols, texts, and ancient records), for thousands of years. Monks and scribes, from all major religions like Taoism, Buddhism, Islam, Hinduism, Judaism, and Christianity, made their contribution with humility and sacrifice so that we have access to today's versions [7, 8].

In the Bible—the Holy book of Christianity, Honey is associated with wealth and symbolizes prosperity. Honey is an image of the Promised Land that shows God's love and care for his people and is also connected with the perception of luxury and delicacy. The Bible uses the sweetness of honey as a metaphor for delight.

The Quran mentions honey that is a "healing for mankind," in Surah 16:68–69, where The Quran describes the Honey-producing process by the Bees and the medical values of all the Bees's products, including Honey. The Surah 47:15 shows how Honey maintains its sacred value in the afterlife being mentioned as a gift to the righteous who believe in Allah.

In Traditional Chinese Medicine, there are five elements of acupuncture which represent the quality of Qi energy (the five elements are: metal, wood, water, fire, and earth). Honey is considered a major component of Earth and is linked to the spleen meridians of the human body [9].

2.2 Honey and its place in the hierarchy of human needs and the scale of levels of consciousness

In order to better understand the behavior, psychology, and evolution of humanity, we have brand specialists who managed to launch valid theories beyond their time. Among them are Abraham Maslow [10] and Dr. David Hawkins, who formulated an analytical and hierarchical approach to people's needs in correlation with their evolution and also a scale of levels of consciousness. Of course, there are many other tools that calibrate the degree of fulfillment and well-being of man, but I will limit myself to these two: the hierarchy of human needs (Maslow) and the scale of levels of consciousness (Hawkins) [11].

Maslow's theory of human needs includes a hierarchy of them, starting with the basic needs that ensure the survival of the human body (the need for food and water) and continuing progressively, step by step, with physiological needs (that ensure health) then continue with needs for safety, love, self-esteem, and culminating with the need for self-actualization which is at the top of the pyramid. In this algorithm, honey takes place on the level of basic needs but even higher, in the group of needs for optimal physiological functioning, ensuring health. Honey is mainly used as a food, but recent research intervenes with evidence regarding its beneficial role in health. Another step in the hierarchy of needs can use honey, which is also helpful to man and to fulfilling his need for love, esthetics, beauty, and integration, because it is included in numerous cosmetic products that contribute to maintaining the beauty and cleanliness of the body. Maslow's hierarchy of needs can still be applicable in today's culture. The system of values appreciated by each human being influences the way they set their own priorities. There are extremely many variables that determine the way an individual relates to the whole, how he identifies his needs, and how he sets and fulfills his goals. All this complex mechanism is reflected in the originality of each of us [12]. The highest level in the hierarchy of needs refers to self-actualization. As everything is dynamic, the problem arises of updating Maslow's approach. Thus, it is considered that in the process of becoming the Self and in the journey toward the higher levels of the pyramid of needs, one goes through successive stages that belong to the individual with the valences of intimacy, and autonomy that lead to interactions and relationships, through the processes culminating in Affiliative Relationships. On this progressive upward dynamic of awakened and conscious humanity, the need immediately following the fulfillment of the current one, as individuals, communities, and indeed as a species, is Interdependent Actualization [13].

Interdependency is the concept that involves a balance of self and others recognizing the importance of approaching meaningful and appropriate ways to meet each other's needs. Bees have always worked in groups. If human evolution has reached the moment when we realize that the meaning of being together brings added value, the bees already apply this behavior from the beginning.

The Map of Consciousness was created due to Dr. Hawkins. Conventional physicochemical analysis findings that the perception systems and the value systems to which the human being relates determine the quality of life, physical and mental health, emotional balance, and value in society through results and harmony in relationships with others. Bees in the process of honey production are acting on high levels of consciousness and offer humanity examples and models of harmonious interactions and beneficial results for the environment in which they live. Depending on the place occupied on the scale of levels of consciousness, people observe and apply the model of bees, or not. This Scale of Consciousness ranges from low to high, the lower levels are shame, guilt, apathy, grief, fear, desire, anger, and pride, and then it comes to a threshold that makes the difference between the involuted state of beings and marks the step toward evolution. This threshold is the state of Courage, after which are evolutionary states of consciousness. Beyond Courage there are steps of consciousness that lead to advanced states of consciousness and make a difference for the better in society, starting with neutrality, then willingness, acceptance, and reason, arriving at the consciousness of love, joy, and peace, and reaching enlightenment [11].

Can a correlation be made between the consumption of honey and the level of human consciousness? If we choose to pay attention to honey in correlation with the way it is produced by bees and if we observe the bees and the way they are organized in the hive, the raw material from which honey is obtained, the nutritional quality of honey, we surely find the meanings behind the appearances. But for that, it takes openness and availability to think and perceive outside of material limitations and restrictions. The scale of levels of consciousness can be applied in any situation and actually reflects the way in which we, humans, relate to a situation. The more we act from a higher level of consciousness, the more we manifest wisdom, love, joy, and peace and bring harmony. We are like the Honey of God, as the sacred texts wrote.

3. Holistic honey

In terms of the Simple Truth, Honey is produced by bees from bloomed flowers. The season when this happens is in the warm and bright seasons, when the plants and trees are blooming, when Nature shows many colors, and when the cycle of life on the planet is expanding, prosperity, flourishing, joyful, and optimistic time. We cannot talk about honey without including bees and flower nectar or other sources from which bees produce honey, the blooming Nature, the Sun, Life, the existence on Earth.

Holistic Honey is about interconnection. Everything is connected with everything, as the word is explained in the dictionary: *Holistic = characterized by the belief that the parts of something are interconnected and can be explained only by reference to the whole* [14].

And there is the word and its meaning. Since Honey has been valued since ancient times, we can start our approach with the word. Applying the ancient Essene Numerology system to the word שבד= debhash (Honey in Hebrew language—the sacred language from some of the sacred texts) we have to find the number for the word. In this system, each letter has a corresponding number. If we add up the numbers corresponding to each letter that forms the word, we get the number 9. The meaning of this number according to this ancient system of Essene wisdom is: "*Completion and Wisdom, Companion, Perfection. The number 9 is connected to the power of action… 9 sets processes in motion and is a catalyst for movement… 9 is involved in the great cycle…is good in assessing wholeness…*". [15]. Every word is correct for the bees and for the Honey.

The Honey is stored by the bees in honeycombs whose shape is hexagonal, with angles of 120 degrees between the sides. We can think about many possible answers to many "how" or "why" questions about this shape. How can be understood the influence of this shape on the beneficial quality of Honey? How can manage the bees to build these honeycombs with such perfect precision? Why hexagons and no other shape?

If we explain the honeycomb hexagons from mathematical and logical perspectives, we can fall into the trap where the Logic prevents us from seeing the big picture. The engineering approach to understanding how bees build honeycombs can direct us toward minimizing and limiting the perspective. Miracles do not really happen in the precise world of exact sciences. "*The interpretation of the honeycomb architecture as the result of blind physical forces rather than biological engineering can lead to state that there does not seem to be much room left for the honey bees 'engineering prowess'*" [16].

There is a mathematical truth behind the geometric shape of the honeycombs, with no gaps or small spaces between the units, and they do not need additional wax for patching. The scientists proved that "there are only three geometrical figures with equal sides that can fit together on a flat surface without leaving gaps: equilateral triangles, squares, and hexagons" [17–19]. The "conjecture"—and that's what it was, a mathematical guess—proposed that a structure built from hexagons is probably a wee bit more compact than a structure built from squares or triangles. A hexagonal honeycomb would have "the smallest total perimeter." The antic author of this theory could not prove it mathematically, but that's what he thought [20]. The geometrical pattern of honeycombs is impressively symmetrical. Compactness matters. Wax is expensive. A bee must consume eight ounces of honey to produce a single ounce of wax. The hexagon is the shape that most efficiently breaks flat space up into little units making honeycombs that hold the most amount of honey while using the least amount of wax. Darwin agreed that "The Honeycomb is absolutely perfect in economizing labour and wax." Thomas Hales, a mathematician at the University of Michigan, produced in 1999 the mathematical demonstration of Varro's theory: a hexagonal structure is indeed more compact [21].

The hexagons are also studied in interior design, analyzing the impact of the geometric shapes in the room on the mood of the person. It is considered that this naturally occurring shape, this geometric wonder induces a feeling of well-being, and an openness to collaboration and communication because at subliminal level our brain makes the association with bees and everything they represent (their teamwork the ability to work in teams, the cooperative nature). Not only our mind is stimulated by the sacred geometry of the honeycomb shape, but also our emotions are triggered in a good way and we can feel connected with the community, our creativity receives an activation impulse and a sense of balance becomes obvious, and all these are just a few of the feelings evoked by. This positive energy makes it an attractive fit for creative industries. They are combined with the shape of diamonds that symbolize clarity, knowledge, and quality. They were also originally said to be symbols of immortality and, to this day, still communicate excellence and sophistication. Using hexagons alongside diamonds can go a long way when bringing a space to life and serves as an attractive focal point of the room [22].

From the sacred geometry to the sonic geometry is just a step. The next step in the holistic approach to Honey. The research in Sonic geometry shows that "Geometry reveals Harmonics," considering that we can play the numbers of the geometry, the total sum of the angles, as frequency (120 degrees × 6 angles = 720). It seems that it is not easy to find these frequencies in other places than in Nature. The 720 Hz frequency (the sum of the angles from the geometric hexagonal shape in the honeycombs) helps to increase intuition and to see the interconnectedness of all things. The musical harmony of the hexagons is C# 216 Hz, 1 octave below 432 Hz [23].

There are research works on the sound frequency in a hive and this can be an important tool for assessing the status of the bees in the hive, the health, or the swarming period. The acoustic methods based on labeling the sound in the hive to predict the swarming was studied for the beekeepers who consider this as a honey loss. It was observed an increase in the power spectral density and approaching to swarm the sound augmented in amplitude and frequency to 300 Hz, with occasionally rapid changes to 500 Hz [24, 25]. Another team of researchers from Mexico found that the sound signal from a beehive with a queen shows a characteristic pattern around 400 Hz (it could be 432 Hz—the sacred sound of Nature) (**Table 1**) [26].

We need more research with accurate methods to identify the sound associated with a certain status of the beehive (varroa mite infections, size bee's population, pre-swarming behavior), to be able to identify by sound frequency the health status of a colony [27]. The medical research also studies the correlation between different frequencies of sounds with the activity of the cerebral cortex [28]. The harmonic geometry of sound is only at the beginning of scientific exploration, but it is already known that the frequency of the sound in the hive indicates the status of the bee colony (dynamics, health), and the frequency and harmony of the sounds in nature influence the human condition. The studies carried out on water clearly showed how its structure changes depending on the sound frequency [6].

The proof of the harmonic nature of life is all around. Honey as food, medicine, or remedy is more than we can touch and feel with our senses! It's connected with everything in many ways.

4. Science of honey

The necessary explanations for the logical understanding of the beneficial properties of honey are provided, according to the level reached, by science. Chemistry comes with a detailed approach, with in-depth analysis of the chemical compounds in different types of honey. The specialized scientific literature abounds in resources, results, research, and conclusions. For the most part, all these researches show results that converge toward a common conclusion, the same conclusion decreed thousands

Signal	Frequency range (Hz)	Signal pattern	Sender	Significance
Recruit	200–350	Pulse Sequence	Forager	Indicate the existence of a quality food source
Tooting	300–500	Pulse Sequence	Queen	Subset of piping. Prevents hatching of further queens
Quacking	300–350	Pulse Sequence	Queen	Subset of piping. Indicates viability of confined, mature queen
Worker piping	300–550+	Single pulse	Scout	Triggers colony hissing to prepare to swarm
Hissing	300–3600	Single pulse	Colony	General warning/defense signal. Occurs during swarming, hive attacks, and other adverse events

Table 1.
The acoustic signature of honeybee's colonies (from Amro Qandour & col, [25]).

of years before, in all ancient writings and sacred literature. The truth emerges, regardless of the angle from which we look at the situation.

The presence of honey in sacred writings but also the definitions from the national and international regulations (Codex Alimentarius), together with all available resources dedicated to honey as food or medicine, addresses aspects that are confirmed by science. The simple version of the conclusions of the chemistry research on honey is that honey is sweet and beneficial to health.

The scientific approach comes with the perception of complexity. Therefore, from simple to complicated in the study of honey we can analyze definitions and find out details. Having its roots in scientific expertise, the definitions for honey appear, both in dictionaries and in regulations applicable to food and medicines.

The European Council Directive relating to Honey gave this definition in Annex 1: "*Honey is the natural sweet substance produced by Apis mellifera bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature*" [29].

4.1 Honey chemistry

If we talk about the chemical composition of honey, the same European Regulation, in Annex 2 considers that "*Honey consists essentially of different sugars, predominantly fructose and glucose as well as other substances such as organic acids, enzymes and solid particles derived from honey collection*" [29–31]. According to Codex Alimentarius and EU Regulations, there are values established for each composition criteria for Honey: Moisture content, Sucrose content, sum of Glucose and Fructose content, water Insoluble Solids content, Hydroxy methyl furfural content, Acidity, Diastase Activity (Schade Scale).

The researchers agreed that Honey is a complex composition of sugars, minerals, vitamins, enzymes, proteins, amino acids, organic acids, and bioactive substances made by Bees in honeycombs, from the nectar of flowers. There are different variations between the types of honey and there are many factors that influence its chemical composition, but even so, about 80% of the physical and chemical composition is common. Changes in composition could result from the type of bee that produces the honey, the environmental conditions, and the floral source. Such variations would lead to different colors, viscosity, taste, and properties of the honey [32–34].

About 78–82% of the honey's composition is represented by carbohydrates, the bulk of which (75%) is made up of the monosaccharide glucose and fructose. Fructose is usually more abundant than glucose with some exceptions (honey from *Brassica napus* and *Taraxacum officinale)* [35–37]. Analyzing different samples of honey from different regions of the planet, it was identified more than 20 types of carbohydrates. [38] The principal carbohydrate that existed is fructose (28–40%) followed by glucose (20–35%), and the disaccharide and trisaccharide concentrations are around 5,2 and 1, 1%, respectively [37, 38]. The most identified disaccharides are sucrose, maltose, turanose, maltulose, and nigerose. Small amounts of a few trisaccharides were also identified such as isomaltotriose, ketose, psopanose, erlose, centose, and panose [34].

Honey contains also vitamin traces, among which are Riboflavin, Pyridoxine, Niacin, Pantothenic acid, Ascorbic acid, and Thiamin, as well as minerals such as Potassium, Magnesium, Calcium, Phosphorus, Copper, Chlorine, Sodium, Iron, Sulfur, selenium, arsenic, barium, silver, chromium and Manganese.

The mineral content influences the color of the honey, so dark honey has a mineral content of 0.2% compared to light honey, which has minerals in a proportion of only 0.04%. The most abundant mineral element in Honey is Potassium. In even smaller amounts, there are other macroelements and microelements that may also be present in honey: iodine, zinc, lithium, cobalt, nickel, and cadmium [39–41].

Honey can also contain proteins (0.5%) that are present both in the form of enzymes and in the form of amino acids. The most common enzymes in honey are diastase or amylase, invertase or sucrase or α-glucosidase, CAT, and glucose oxidase. The individual amino acids in Honey are proline (the most important one), arginine, glutamic acid, cysteine, and aspartic acid. Carbohydrate metabolism involves the activity of certain specific enzymes and the biochemical analysis of honey highlighted their presence in the analyzed honey samples such as diastase, glucose oxidase, glucosidase, invertase, and catalase. Diastase concentration may also be used as an indicator of honey quality, with higher-quality honey usually containing more diastase [42, 43].

Another important biochemical compound of honey is represented by organic acids that are present in smaller amounts (the average value identified in honey being 0.57%). The most abundant organic acid in honey is gluconic acid but there are also present aspartic, acetic, butyric, formic, citric, lactic, malic, propionic, gluconic, fumaric, galacturonic, butyric, glutamic, glyoxylic, succinic, glutaric, 2-hydroxybutyric, α-hydroxyglutaric, isocitric, α-ketoglutaric, malonic, methylmalonic, 2-oxopentanoic, pyruvic, quinic, shikimic, tartaric, oxalic, levulinic, and formic acids, among others.

The chemical groups responsible for the smell and aroma of honey include diverse volatile compounds, such as C_{13}-*nor*isoprenoid, benzene derivatives, monoterpenes, sesquiterpenes, and, to a lower content of esters, superior alcohols, aldehydes, ketones, and fatty acids. The polyphenols and volatile compounds in honey are connected with the beneficial health effects of honey, but more research is needed to demonstrate the mechanisms of these beneficial effects on the human body [44, 45].

The importance of honey for human health is also analyzed in detail. The chemical, enzymatic, polyphenolic, and volatile compounds of phytochemical substances in honey are connected with the anticancer, antioxidant, antimicrobial, and anti-inflammatory activities of honey [31].

The authenticity of the honey and its quality, the identification of adulterations, comes to the attention of the competent control authorities. The report of the JRC "Round Table" (European Commission) confirms analytical techniques to authenticate honey [46–48].

4.2 Honey microbiology

The physicochemical parameters of honey and the presence of natural antimicrobial substances are not favorable for the growth of microorganisms. Honey has a diverse microbiome, most of which originates from pollen, flowers, soil, air, dust, and the honeybee digestive tract. Additionally, some secondary microbial contaminants may be introduced into honey during human processing. Water activity (a_w) is one of the very important parameters that influence many biochemical interactions in the living world and also the growth of microorganisms. In general, microbial development occurs in the water activity range of 0.620–0.995, osmophilia yeasts develop at 0.60–0.65, xerophilic molds between 0.65 and 0.75, halophilic bacteria between 0.75 and 0.85, and all other bacteria between 0.91 and 1.00. The water activity parameter of honey is between 0.50 and 0.65. The chemical reaction is generally acidic, with pH ranging from 3 to 5 due to the presence of organic acids. All these physicochemical properties of honey are influencing microbial growth. In addition to the inhibitory effect on microbial development given by the low water activity and low pH, honey contains naturally antimicrobial components (including antimicrobial peptides, hydrogen peroxide, and antioxidants). The only organisms that can survive the osmotic stress of honey are some spore-forming bacteria and yeasts. Most of the bacterial species identified in honey were osmotolerant, xerotolerant, and acidotolerant [49].

The scientific evidences and advanced research that explained the mechanisms underlying the antimicrobial effects of honey, through various phytochemical factors or as a result of osmotic effect, acidity, and hydrogen peroxide, prompted a comprehensive review of numerous reports of the antimicrobial activities of honey. The antibacterial activity of honey was proved in some instances where antibiotics were ineffective. The bactericidal effect of pure honey has been shown against many pathogenic microorganisms including *Vibrio cholerae*, Shigella spp., Salmonella spp., other enteropathogens like *Escherichia coli*, and other Gram-negative and Gram-positive organisms [50].

All the research works done with the purpose of a better understanding of the antimicrobial capabilities of Honey have developed new areas of interest and made discoveries not initially intended, but which have proven extremely valuable for the knowledge of environmental effects on microbial biodiversity and ecosystems associated with different types of honey. All these investigations on the microbiome of honey and other bee products brought new information about Bee diseases that cause significant ecological and economic damage, such as Colony Collapse Disorder (CCD). Even though the presence of pathogenic microbial DNA may not directly correlate with honeybee diseases, using advanced metagenomic tools to determine the relative abundance of these pathogens can provide information on possible hive diseases and overall beehive health [49].

Future studies should continue the series of investigations that can limit until eradication the important bee diseases that are triggered by ecological imbalances or miscalibrations of the bee microbiome, with unwanted implications on honeybee diseases and overall bee colony health.

5. Conclusions

1. Science can prove things according to our ability to understand and our openness to look at from different perspectives. "Ninety- nine percent of who you are is invisible and untouchable" (R. Buckminster Fuller).

2. Science has advanced tools for the study of honey, both physical-chemical and microbiological.

3. The medical sciences deepen the research through which the beneficial effects of honey on health are understood (honey acts as medicine or intervenes in the prevention of the installation of pathological manifestations).

4. The research carried out on the microbiology of honey can clarify important aspects of the health of bee colonies.

5. The geometric shape of honeycombs, the hexagon—is studied both by scientists specialized in physics and mathematics as well as by specialists in music and sonic geometry, establishing the interrelationship of these parameters with human well-being.

6. Honey is identified in the ancient sacred writings and recognized before the development of science, as being valuable and beneficial for man and humanity. The application of an Essene numerological system in the analysis of the word named honey reveals qualities that correspond to both honey and bees.

7. Honey can be understood in a holistic context and finds its place on several levels of the hierarchy of human needs, the old texts show an association of honey with high states of consciousness (love, joy, peace).

The new trends to a holistic approach to our reality, correlated with all the advanced analytical techniques and with the Digitization of everything, the involvement of Artificial Intelligence in all areas of interest of humanity will gradually bring to the attention and consciousness of humanity the indisputable evidence of the interrelationship and non-separation between Nature, Divinity, and Humanity. Bees are exemplary masters and models of functioning at the highest levels of Consciousness. They lives in colonies, groups, and teams, maintaining harmony inside the colony and outside the hive, in peace and harmony with everything that surrounds them. They are our models of discipline, respect, and beneficence, and they have been available to humanity since ancient times, waiting with peace and patience for man to understand and apply in society, in communion, the sweet lesson of Honey Bees, conceived from the sun, flowers, sacred geometry, harmony, and unity with the divine and with the planet that hosts us generously.

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Chapter 3

Palynological and Physicochemical Characterization of Honey from *Butia yatay* Palm Savannas in Argentina

Lucía Brelis, Veronica Busch and Agustín Sanguinetti

Abstract

Butia yatay palm savannas (*palmares de yatay*) of Eastern Argentina constitute a unique natural and cultural landscape threatened by land conversion. Honey production, as a non-timber forest product, can become a conservation-through-use strategy for this landscape if shown to be a valuable product. Therefore, here we describe palynological and physicochemical parameters of honey obtained from hives situated in one of the remaining largest *Butia yatay* palm savannas in Entre Rios, Argentina, during the palms' blooming peak. Melissopalinological analysis showed that three pollen types (Myrtaceae type, *Butia yatay*, and *Eryngium horridum*) accounted for 88–96% of the total pollen counted. Palm pollen was consistently present in all the analyzed samples as secondary pollen regarding its frequency with an average of 33.5% of the total pollen counted. This honey presented high proline content, high conductivity, a color range from light amber to amber, significant polyphenol bioactivity, and rheologically behaved as a Newtonian fluid. This is the first instance of producing and characterizing honey from this peculiar botanical and geographical origin, thus contributing to Argentinian efforts to hierarchize regional and local honey types. It is also the first report of *Butia yatay* palms as a significant nectar source for honey production.

Keywords: Argentinian honey, Entre Ríos, palm grove, honey analysis, melissopalynology, botanical origin, rheology

1. Introduction

Butia Becc. (Arecaceae: Cocoeae) is a South American endemic palm genre comprising 24 species distributed in Argentina, Brazil, Paraguay, and Uruguay [1]. *Butia yatay* (Mart.) Becc. is one of the most widely distributed species occurring in three of the aforementioned countries (**Figure 1**). Adult palms of *B. yatay* can reach up to 12 meters high and bloom between the months of November and February during the austral summer. Individuals usually occur in dense and extensive spatial aggregations such as palm savannas where they are the dominant element on the arborescent

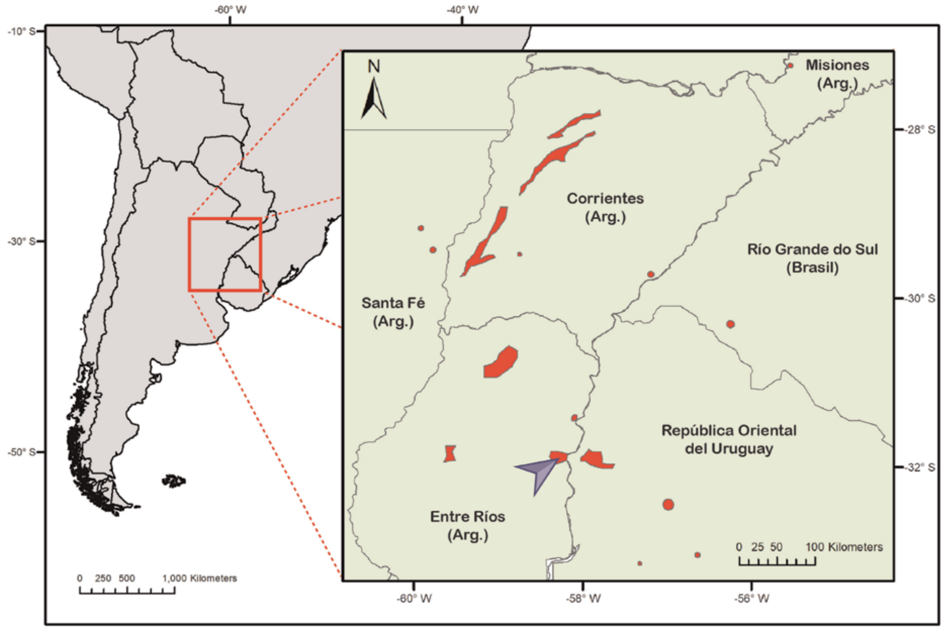

Figure 1.
(Left) Distribution map of remnant Butia yatay *palm savannas (adapted from Brazeiro et al. [2]); the arrowhead shows the apiary's location. (Right) Study site vegetation physiognomy where palms are the dominant arborescent element within an extensive scrubland.*

stratum (**Figure 1**). These savannas, called 'palmares' in Spanish ('palmar' in singular), have been suffering serious threats due to land conversion for agriculture and overgrazing by cattle raising. Consequently, the global conservation status of *B. yatay* has been proposed as vulnerable [2].

Products derived from native palm populations, without causing their destruction, are considered Non-Timber Forest Products (NTFPs) and possess enormous potential for both conserving the ecosystem and enhancing local economies through their utilization and commercialization [3]. There are numerous examples of conservation-through-use strategies specifically focusing on palms [3–6]. *Butia yatay* palm savannas constitute not only a valuable natural and cultural landscape but also a local economic resource due to its late valorization as a tourism attraction and as a food source for local gastronomic products [7]. Many studies show the contribution of several species of palms as an important part of the vegetation that nourishes bees and their hives [8–17]. Nonetheless, there are no records of *Butia yatay* as a resource for bees in the literature reviewed to date.

Argentina exports annually around 70 tons of honey for an amount of 240 million USD. This takes it among the top 3 honey exporters in the world together with New Zealand and China [18]. However, almost all these exports are made in bulk without specifying their regional or botanical origin. The physicochemical and sensory properties of honey largely depend on the nectar collected by bees. Therefore, a specific region with distinct flora and climate will produce a unique and unfamiliar variety of honey. In order to increase the value of these exports the Argentinian government has been promoting diverse strategies such as Protected Geographical Indication (PGI) and Protected Designation of Origin (PDO) starting with a regional map of honeys' identities [19].

As an effort to contribute to this national characterization of kinds of honey and to add value to *B. yatay*'s NTFPs, we hereby describe palynological and physicochemical parameters of honey obtained from hives situated in one of the remaining largest *Butia yatay* palm savannas.

2. Study site and studied honey samples

The apiary where samples were taken from is placed in a private natural park (*La Aurora del Palmar*; Entre Ríos province, Argentina, 31.82° S, 58.33° W) which is within one of the remaining most extensive *Butia yatay* populations (**Figure 1**). The site belongs to the Mesopotamian district of the Pampean phytogeographical province [20] and has a humid subtropical climate (Cfa) according to Koppen's classification. The local landscape is a mosaic of natural fields subjected to light cattle raising or preserved as a strict reserve, *Pinus* plantations, cultivated lots, and riparian vegetation associated with streams. Natural fields are grasslands or scrublands with a variable density of *B. yatay* individuals. A detailed description of the vegetation of these fields can be found in Batista et al. [21].

Six langstroth beehives each 100 m apart were marked, fed, and checked for their sanitary status during the winter of 2021. Honey supers with empty frames and freshly stamped wax were added at the beginning of October before the first palms started to bloom. At the end of December when the frames were already full (>90% cells capped) and the peak of palms' bloom had passed the supers were taken to an extraction room. Honey from each hive was extracted by centrifugation at room temperature, homogenized, and labeled. Samples had, in general, a fruity aroma and an exceptionally sweet taste and were preserved at 4° until analysis.

3. Melissopalinology

Honey samples were subjected to standard melissopalynological qualitative studies [22]. From each sample 10 g of honey was dissolved in 20 ml of warm distilled water, centrifuged at 600 g for 10 m, discarded the supernatant, resuspended in the same amount of water, and centrifuged again at the same speed. The remaining pollen sediment was then mounted in glycerine jelly with basic fuchsin and inspected under an optical microscope [23]. To estimate frequency classes, at least 1000 pollen grains were identified and counted per sample [22]. These classes are according to the percentage of grains counted: predominant pollen (D: ≥45%), secondary pollen (S: 45–16%), important minor pollen (M: 15–3%), minor pollen (m: 3–1%) and present (+: <1%). The samples in which one pollen type represented ≥45% were classified as monofloral, and those in which no pollen type reached this percentage were classified as multifloral [22].

Pollen types were identified by comparing them with a reference collection that was made from the plants in the study site. When possible, they were identified to species level, otherwise to the minimum taxonomic level. The surrounding vegetation was inspected regularly every 2 weeks to estimate the period in which species were in bloom and if they were actively visited by bees.

A total of 24 pollen types were recognized in the samples. Most of them appeared as very few grains in only one or two samples and were pooled as 'other trace types' (**Table 1**). The results show a predominance of three types in the pollen spectrum

Family	Pollen type	Sample [pollen frequency/frequency class]												
		1		2		3		4		5		6		Av %
Myrtaceae	t. *Myrtaceae*	4.1	M	21.9	S	53.2	D	38.8	S	29.1	S	48.5	D	32.6
Arecaceae	*Butia yatay*	38.0	S	34.4	S	22.7	S	39.6	S	33.4	S	33.0	S	33.5
Apiaceae	*Eryngium horridum*	54.0	D	37.1	S	12.4	M	15.5	M	30.1	S	11.9	M	26.8
Apiaceae	*t. Ammi*	3.6	M	2.0	m	1.1	m	1.1	m	3.5	M	2.1	m	2.3
Boraginaceae	*Echium plantagineum*	0.2	+	1.6	m	3.6	m	1.9	m	0.9	+	0.3	+	1.4
Salicaceae	*Salix* sp.	0.2	+	0.4	+	0.3	+	0.4	+	0.5	+	0.5	+	0.4
Alismataceae	*Hydrocleis nymphoides*			0.6	+	2.3	+	0.4	+			1.5	+	1.2
	other trace types			2.0		4.3		2.2		2.5		2.0		2.6

Table 1.
Main pollen types and their frequency and frequency classes in samples of honey from Butia yatay *palm savannas. Abbreviations: D, predominant pollen: (>45%); S, secondary pollen (45–15%); M, important minor pollen (15–3%); m, minor pollen (3–1%); +, present sporadic pollen (<1%).*

Figure 2.
Pollen types present in all the samples. A. Butia yatay. *B.* Eryngium horridum. *C. Type Myrtaceae. D. Type* Ammi. *E.* Echium plantagineum. *F.* Salix *sp. e: equatorial view. p: polar view.*

along the samples: Myrtaceae type (Myrtle family), *Butia yatay* palm pollen, and *Eryngium horridum* (Apiaceae) pollen (**Figure 2**). Together, the three predominant types accounted for 88–96% of the total pollen counted. The fraction of *B. yatay* pollen ranged from 22.7% to 39.6%, with an average of 33.5%. Instead, the fraction of Myrtaceae and *E. horridum* ranged from 4.1% to 53.2% in the former and 11.9% to 54.0% in the latter. These two types had an almost perfect negative correlation (−0.98), where samples enriched with Myrtaceae were poor in *E. horridum* and vice versa. Along with these predominant types always appeared a small proportion of pollen of *Ammi* type (bishop's weed, Apiaceae), *Echium plantagineum* (purple viper's-bugloss, Boraginaceae), and *Salix* species (willows, Salicaceae) (**Table 1** and **Figure 2**). In some samples, a very low proportion of pollen of *Hydrocleys nymphoides* (waterpoppy, Alismataceae) also appeared. Three samples have predominant pollen frequencies (≥45%) and can be regarded as monofloral according to literature [22] and to Argentinian legislation: sample 1 with 54.0% of *E. horridum* and samples 3 and 6 with 53.2% and 48.5% of Myrtaceae type. In all the samples *Butia yatay* consistently achieved a secondary pollen frequency (45–15%).

The predominant pollen types found are in accordance with the surrounding vegetation and the specific months when suppers were filled with honey. Myrtaceae species such as *Blepharocalyx salicifolius*, *Eugenia uniflora,* and some species of *Myrcianthes* and *Myrceugenia* are common components of local riparian vegetation that bloom in the late spring and early summer [24]. These, like many other Myrtaceae, are very nectariferous species and are expected to have a high pollen load per volume of nectar which usually overrepresents them in honey samples [25]. *Butia yatay* and *E. horridum* started blooming at the end of October and peaked during the second half of November and throughout December. Field inspections confirmed that bees actively visited inflorescences of these species which were the most conspicuous and abundant floral resource during this period in the grazed palm savanna where the hives were located. *Eryngium* species have previously been informed as an important palynological element of local regional honey types [14, 26–28], sometimes even surpassing the 45% threshold to be categorized as the dominant type and to define them as monofloral [14]. On the other hand, there are very few records of palm pollen in Argentinian honey samples [14, 15, 27, 29], but none of them are of *B. yatay*. To our knowledge, this is the first report of the presence of pollen of *Butia yatay* palm in honey in all its occurring area. Nonetheless, there are two reports [10, 11] regarding *Butia odorata*, a species restricted to Eastern Uruguay and Brazil, in which honey samples from hives placed in extensive palm savannas had up to 90% of *B. odorata* pollen.

4. Physicochemical analysis

Several physicochemical analyses could help to a comprehensive understanding of honey's quality and composition shedding light on how these factors influence its properties.

Among these factors, moisture content significantly impacts the properties of honey as it can affect its texture, stability, and susceptibility to spoilage. High humidity conditions during honey harvesting can be associated with the collection of immature or prematurely harvested honey, as well as poor beekeeping practices. Naturally, honey contains a certain amount of moisture, and this level plays a crucial role in determining its quality. Excessive moisture in honey can lead to undesirable consequences such as fermentation or the growth of harmful microorganisms. These issues can compromise the overall quality and shelf life of the honey. On the other hand, insufficient moisture content can cause honey to crystallize and alter its texture. To maintain the desirable properties of honey, it is essential to carefully manage its moisture content. This involves ensuring that the honey is harvested at the appropriate time, when it has reached the optimal moisture level. Additionally, proper manufacturing practices, including adequate storage and handling techniques, are crucial for preserving honey's quality and preventing moisture-related issues. By managing moisture levels effectively, beekeepers can ensure that honey retains its desired characteristics, such as its texture, stability, and extended shelf life [30, 31].

Another important factor is the hydroxymethylfurfural (HMF) that is generated as a result of the thermal degradation of sugars during its processing and storage. The formation of HMF is influenced by various factors, including temperature, time, pH, and sugar concentration. The presence of elevated levels of HMF in honey can serve as an indicative parameter of inadequate storage or processing practices, which may negatively impact the quality of the honey. High HMF content can lead to changes in the color, flavor, and aroma of honey, thereby diminishing its sensory appeal to consumers. Furthermore, the consumption of honey with excessive HMF content in significant quantities may potentially pose adverse health effects. Some studies have suggested that HMF can react with certain amino acids and proteins, forming compounds that could be detrimental to human health. It is important to highlight that honey produced in regions characterized by high ambient temperatures tends to exhibit higher levels of HMF. The elevated temperatures accelerate the rate of sugar degradation and consequently contribute to the formation of HMF during honey processing and storage. To ensure the maintenance of honey quality, it is crucial to adopt appropriate storage and processing techniques that minimize HMF formation. This entails proper temperature control, limiting exposure to heat, and utilizing efficient packaging materials that offer protection against external factors. Moreover, adherence to established quality standards and regulations is essential to ensure that honey maintains an acceptable level of HMF. By implementing these measures, honey producers and beekeepers can safeguard the desirable properties and nutritional integrity of honey, providing consumers with a high-quality and safe product. Continuous research and monitoring in the field of honey production are imperative to further enhance our understanding of HMF formation and its impact on honey quality and human health [32].

The acidity of honey contributes to its sensory properties, stability against microorganisms, improvement of chemical reactions, and antibacterial and antioxidant activities. It can be evaluated by determining pH and acidity parameters. The former provides information about ionized organic acids and inorganic ions such as phosphate and chloride, which are relevant to enzymatic activity, product texture, and inhibition of microbial growth. On the other hand, the acidity parameter is characterized by the presence of organic acids, mainly gluconic acid, resulting from the enzymatic action of glucose oxidase on glucose, and is in equilibrium with its corresponding lactones and aforementioned inorganic ions, which also contribute to acidity. The values of these parameters are influenced by the botanical source, harvesting time and conditions, freshness, and storage state of the honey. It is important to highlight that while acidity is a desirable characteristic in honey, excessively high acidity levels can indicate the presence of fungal and yeast growth. Measuring the pH and acidity parameters of honey provides valuable insights into its quality. The pH level indicates the presence of ionized organic acids and inorganic ions, which are essential for various chemical reactions and contribute to the stability of honey against microorganisms. However, it is crucial to maintain a balance, as very high levels of acidity indicate fungal and yeast growth, compromising the quality and safety of the honey. By monitoring and controlling acidity levels, honey producers can ensure the integrity and safety of their product [33, 34].

Among the amino acids present in honey, proline stands out as the most relevant as it is one of the parameters that can indicate the genuineness of honey. The presence and quantity of proline in honey thus can provide insights into whether it has been adulterated or mixed with other sugars or syrups. Pure and unadulterated honey typically exhibits higher levels of proline compared to honey that has been altered or diluted.

The proline content in honey depends on the time bees process the nectar and their maturity. Indirectly, the levels of this amino acid reflect the botanical origin of honey, fluctuating according to floral sources and being related to geographical origin [35–37].

Sugars constitute the predominant fraction in the composition of honey, with more than 22 types identified, wherein fructose and glucose are the most relevant reducing sugars. Their ratio and proportion serve as key indicators of the quality and crystallization capacity of honey, which vary according to the botanical diversity of the collected nectar, climatic conditions, and geographical location of the beehive. A higher fructose-to-glucose ratio is associated with a reduction in the glycemic index, which is of interest for both direct consumption and the development of functional products. Moreover, the detailed analysis of the specific sugar content enables the detection of adulterants and provides valuable information regarding the maturity level of honey [38, 39].

The mineral substances present in honey contribute to its nutritional value. The presence of approximately 37 macro and microelements has been discovered, with potassium (K) being the most abundant, followed by sodium (Na), magnesium (Mg), and calcium (Ca). The concentrations of these elements, both macro, and micro, vary in the mineral fraction of honey depending on the soil where the nectar-bearing plants grow. The mineral richness in honey can be estimated through the determination of ash, a parameter currently used to classify its origin and as a quality criterion [40]. It is important to note that the ash content of honey can serve as an indicator of environmental pollution, as high levels of toxic minerals pose a risk to human health. Furthermore, the mineral content of honey is related to other physicochemical parameters, such as color. In general, it is observed that light floral honey has lower ash content (0.2–0.3%) compared to darker honey (0.5–0.6%) [41–43].

It has been demonstrated that honey contains a wide variety of components with antioxidant activity, with phenolic acids and flavonoids cited as the main contributors to this activity due to their mechanisms of action and reactions based on the transfer of hydrogen atoms or simple electrons. This capacity, along with other parameters mentioned earlier, depends on the botanical origin, environmental and seasonal conditions of the harvest, as well as the processing of the honey. Furthermore, a positive correlation has been observed between antioxidant capacity and the color of honey, with darker varieties exhibiting higher antioxidant capacity compared to lighter ones. The interest in investigating the antioxidant potential of honey and analyzing its phenolic compounds and flavonoids has increased due to its potential as a food and functional ingredient, thanks to its bioactive properties [44, 45].

In the present work, physicochemical analyses were conducted on fresh honey samples from *Butia yatay* palm savannas. Moisture content was determined using refractometry, following the AOAC 969.38 B [46]. The acidity content was quantified through neutralization titration, and pH was measured using a pH meter, following the AOAC 962.19 [47] and Bogdanov and Marcazzan [48] methodologies, respectively. Additionally, total reducing sugar content was evaluated using spectrophotometric techniques, as described in Ávila Núñez et al. [49]. Glucose was determined following the method proposed by Goñi et al. [50], and total polyphenols were quantified according to Singleton et al. [51]. HMF was quantified using HPLC, following Rivero et al. [43]. Furthermore, the content of insoluble solids in water was determined using the validated V22 MAFF method from the J. Assoc. Public Analysts. The ash content of the honey samples was determined by combustion of the sample at 550° C until constant weight, following AOA 923.03. Additionally, proline analysis was conducted following the guidelines outlined in the IRAM N 15940-2.

Honey samples	1	2	3	4	5	6
Moisture (%)	17.0 ± 0.1[b]	16.3 ± 0.1[c]	16.5 ± 0.1[c]	17.6 ± 0.1[a]	17.0 ± 0.1[b]	16.5 ± 0.1[c]
HMF (mg/kg)	nd	nd	nd	nd	nd	nd
Acidity (meq/kg)	40.0 ± 0.1[a]	36.5 ± 4.7[ab]	36.3 ± 2.1[b]	35.5 ± 1.3[ab]	43.3 ± 2.0[c]	35.0 ± 0.7[b]
pH	4.3 ± 0.2[ab]	4.3 ± 0.1[b]	4.5 ± 0.2[a]	4.5 ± 0.2[a]	4.4 ± 0.4[a]	4.7 ± 0.2[a]
Water-insoluble solid (%)	0.03 ± 0.02[a]	0.05 ± 0.01[a]	0.02 ± 0.01[a]	0.05 ± 0.17[a]	0.02 ± 0.02[a]	0.04 ± 0.01[a]
Proline (mg/kg)	677 ± 21[a]	690 ± 101[a]	673 ± 77[a]	651 ± 61[a]	424 ± 43[a]	582 ± 45[a]
Reducing sugars (%)	71.5 ± 1.1[a]	79.8 ± 1.7[a]	71.9 ± 1.4[a]	68.1 ± 4.3[a]	75.7 ± 0.7[a]	74.7 ± 7.5[a]
Glucose (%)	24.2 ± 0.2[cd]	30.4 ± 0.4[a]	22.5 ± 0.2[ef]	21.2 ± 0.2[f]	28.0 ± 0.5[b]	24.9 ± 0.5[c]
Ash (%)	0.51 ± 0.01[ab]	0.50 ± 0.02[a]	0.57 ± 0.01[c]	0.59 ± 0.002[c]	0.53 ± 0.04[b]	0.53 ± 0.01[ab]
Total phenolic content (mg GA/100 g)	623 ± 3[a]	654 ± 36[a]	583 ± 1[a]	668 ± 36[a]	578 ± 33[a]	618 ± 20[a]

Table 2.
Physicochemical parameters of honey samples. The means ± standard deviation values are reported. Different letters in the same optimal powder indicates significant difference ($P < 0.05$, $n = 3$).

Table 2 shows the values obtained of the physicochemical parameters of the 6 honey samples analyzed in this study. The moisture content ranged from 16.3% to 17.6%, values that are below the maximum limit of 20.0% suggested by the Codex Alimentarius for unclassified honey. These results are consistent with those reported by Acquarone et al. [52] and Salgado [16] for Argentine honey samples, who obtained ranges of 15.3–19.0% and 15.4–20.0%, respectively. These values indicate that honey is mature, with no risk of fermentation and good stability. Additionally, the determination of hydroxymethylfurfural (HMF) by HPLC resulted in non-detectable amounts in the analyzed samples, which could be attributed to their freshness and the absence of prior thermal treatments before analysis.

Free acidity and pH are good indicators of quality, and according to the Codex Alimentarius, these parameters should not exceed 50.0 milliequivalents/kg and should be between 3.4 and 6.1, respectively. The palm savanna honey samples comply with these international regulations, presenting values of 35.0–43.3 milliequivalents/kg and 4.3–4.7, respectively. These results are expected in fresh honey samples with optimal moisture content and they are similar to those results reported by Salgado [16] of 8.0–68.0 milliequivalents/kg of honey and a pH of 3.2–6.4 for other Argentinian honey samples. Other similar results were obtained in a study conducted by Cabrera and Santander [53] on honey from the province of Formosa (Argentina), where a content of 28.9 milliequivalents/kg of honey and an average pH of 4.01 was found.

Regarding the total reducing sugars content, a range of 68.1% to 79.8% was observed, with glucose contents ranging from 21.2% to 30.4%. These values are similar to those reported by Ciappini et al. [54] of 63.2–70.6% for reducing sugars and 23.6–38% for glucose (*Trifolium* spp., *Medicago sativa*, *Eucalyptus* spp. and *Melilotus* spp. floral honey from Santa Fe province, Argentina). Maldonado et al. [55] also reported glucose values in the range of 23.7–38.2% (*Citrus limon* honey, Tucuman province, Argentina). These results comply with international regulations for genuine honey.

The ash content values obtained through gravimetry were below the upper limit established by the Codex (<0.6% for unidentified honey samples). However, the observed range for this parameter was from 0.50% to 0.59%, significantly higher than those reported by Baroni et al. [56] and Acquarone et al. [52] for Argentine honey samples. This is consistent with the values obtained for conductivity and color, suggesting that the samples analyzed here possess a substantial mineral content.

The amino acid proline is a quality criterion specified in the Codex Alimentarius standards, which recommends a minimum of 180 mg/kg for genuine honey. In these honey samples, a higher content of proline was found in the range of 424–690 mg/kg of honey. These values are also higher than the range of 50–499 mg/kg reported by Montenegro for honey samples from the province of Chaco (Argentina).

Regarding water-insoluble solids, they were found within the limits established by the Codex, with values of 0.02–0.05%. According to the regulations, honey obtained by centrifugation should not contain more than 0.1 g/100 g.

Finally, the total polyphenol content in these honey samples was 578–668 mg/100 g, indicating a higher antioxidant capacity compared to the values reported by Maldonado et al. [57] of 130–290 mg/100 g. These findings suggest that *Butia yatay* palm savanna honey may have potential health benefits for humans.

The overall physicochemical characteristics obtained for *Butia yatay* palm savanna honey indicate high quality, freshness, absence of thermal treatment, and production under good apicultural practices. Furthermore, the high polyphenols content and proline indicate high bioactivity and confirm its genuineness. These positive attributes contribute to the overall excellence of the honey and its potential health benefits for consumers.

5. Electrical conductivity

The conductivity of honey is an indicator of the amount of ions present in the liquid. Conductivity refers to the ability of a material to conduct electrical current. The conductivity of honey can vary depending on its composition and origin. Generally, a higher conductivity indicates a higher content of mineral salts and other dissolved compounds. This may be associated with factors such as the quality of the nectar used by bees and the processing of the honey. Besides, the conductivity of honey can indicate its authenticity and quality. For example, some adulterated or diluted honey may have lower conductivity due to the dilution of dissolved components.

Furthermore, it has been shown that the trace element content in honey is related to its botanical origin and color. Floral honey generally has a lower mineral content compared to dark honey types such as honeydew, chestnut, and heather honey [41]. However, this determination is not only useful for assessing the botanical origin of honey but also its geographical origin. In Acquarone et al. [52], statistically significant differences were observed in the maximum electrical conductivity values depending on the geographical origin of the analyzed samples. This is interesting because by studying the variation of conductivity concerning soluble solids, it is possible to obtain curves that differentiate honey from a particular region.

In the present work conductivity of honey was measured at different solid content by using a Milwaukee MW801 conductometer. The dependence of specific electrical conductivity (K) on honey concentration was characterized by a maximum at a C_{max}

value corresponding to a dry solids of honey concentration of 30–35% (w/w) [52]. The obtained results were fitted using a nonlinear regression with the following equation (Eq. (1)):

$$K = K_{max} * e^{\left(-0.5 * \frac{(C - C_{max})}{SD^2}\right)} \quad (1)$$

where K and K_{max} are the electrical conductivities at concentration C and at maximum (C_{max}), respectively, and SD is the amplitude of Gaussian curve.

Figure 3 presents the results of the dependence between electrical conductivity and honey concentration. It can be observed that when the solids concentration in honey was high (60%), the electrical conductivity values decreased due to the increased viscosity (high honey content). Towards the origin of the x-axis, as the solids concentration decreases, a peak corresponding to the maximum ion concentration is observed, followed by a decrease as these ions are diluted. The electrical conductivity values depend on the concentration and mobility of ions present in the honey solution.

Regarding the obtained values from the nonlinear regression, it was observed that all samples exhibited the same behavior. The used model predicts very well the experimental data as R-squared value greater than 0.9844. The peaks corresponding to the maximum conductivity (K_{max}) values for *Butia yatay* savanna honey samples ranged from 26.96 to 29.14% solids.

Other honey samples from Argentina showed lower curves for conductivity but similar C_{max}. These could be related to a lower mineral content and ash content [52].

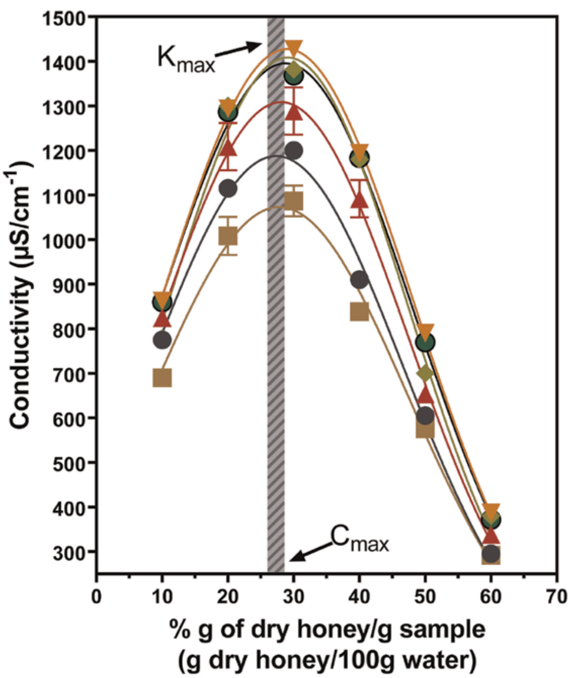

Figure 3.
Conductivity of Butia yatay *savanna honey samples at different solid concentrations (%). The gray zone indicates the solid concentration range (Cmax) for the maxima conductivity (Kmax). Sample 1 ● (black circles), sample 2 ■ (brown squares, sample 3 ▲ (red up triangles), sample 4 ▼ (orange down triangles), sample 5 ◆ (green diamonds), sample 6 ● (green circles).*

Similarly to the present *Butia yatay* palm savanna honey, other honey from Spain with different botanical origins showed similar high conductivity values. These samples showed 976 μS/cm for heather honey and 986 μS/cm for forest honey at 20% solids [58]. Samples from Argentina of *Condalia microphylla* also showed high conductivity values (841 μS/cm, 20% w/v solids). Considering these authors evaluated only a concentration, it is possible to say that those honey samples would have a higher conductivity at C_{max} (close to 30%) similar to those reported in the present work.

6. Rheology

Understanding and controlling the rheological properties of materials is vital for optimizing industrial processes across various sectors, including food and drink, cosmetics, pharmaceuticals, and oil and gas.

In industrial processes, changes in rheology can significantly impact pump performance as they must overcome the resistance and viscosity of the transported fluid. Higher viscosity fluids require more pumping force, leading to increased energy consumption or reduced throughput. The flow behavior of material also affects the efficiency of transport systems like pipelines, where pressure drops and flow rates depend on the rheological characteristics of the medium being transported.

Rheology is also crucial in the mixing process. In applications where different components or additives need to be uniformly dispersed or blended, the rheological properties of the materials involved influence mixing efficiency and final product quality. Understanding flow behavior and viscosity allows the selection of appropriate mixing equipment, optimization of mixing parameters, and prevention of issues such as uneven distribution or clumping.

In summary, rheology studies and understanding how materials respond to external forces are critical for optimizing industrial processes. By characterizing and controlling the rheological properties of materials, companies can enhance process efficiency, reduce energy consumption, improve product quality, and ensure the reliability of transportation systems.

The power law viscosity, also known as Ostwald's law of viscosity or the flow behavior index, is a rheological property that describes the relationship between shear rate and shear stress in fluids. According to the power law model, the viscosity (η) of a fluid can be represented by the equation (Eq. (2)) and if taking logarithm (Eq. (3)):

$$\eta = K * (\gamma)^{n} \tag{2}$$

$$\log\ (\eta) = n * \log\ (\gamma) \tag{3}$$

where: *η* represents the viscosity of the fluid, K is the consistency index or coefficient of consistency, γ is the shear rate (velocity gradient), and n is the flow index or consistency exponent.

The flow index (n) characterizes the flow properties of the fluid. For Newtonian fluids, which have a constant viscosity regardless of the shear rate, n is equal to 1. Non-Newtonian fluids, however, can exhibit different flow behavior indexes: If $n < 1$, the fluid is pseudoplastic or shear-thinning, meaning its viscosity decreases as the shear rate increases. If $n > 1$, the fluid is dilatant or shear-thickening, indicating its viscosity increases with increasing shear rate.

The rheological properties of honey can vary significantly based on botanical origin, moisture content, sugar content and composition, and processing. Temperature also modifies the viscosity, higher temperatures are related to lower viscosities. Another factor that may affect this is the glucose monohydrate crystallization process occurring during honey storage.

Once honey undergoes crystallization, it transforms into what is known as set honey. Set honey is a semi-solid, two-phase structure that differs significantly in its properties compared to its liquid state, which is referred to as strained honey. The crystallization process brings about significant alterations in the sensory properties of honey, specifically affecting its texture and water activity. Moreover, the rheological properties of crystallized honey undergo continuous modifications during storage as a consequence of the crystallization process [59].

In the current study, honey samples were subjected to a heating process at 40°C to dissolve all existing crystals, followed by incubation at 25°C for 30 minutes. The viscosity of the honey samples was then measured at various shear rates (0.3, 0.6, 1.5, 3.0, 6.0 rpm) while maintaining a temperature of 25°C ± 0.1°C, using a NDJ8s Digital Portable intelligent viscometer.

Figure 4 illustrates the logarithmic relationship between viscosity and shear rate (using Eq. (3)). The linear regression analysis of these curves determined the flow index of the honey samples, whose values were close to 1 for all samples at 25°C (n obtained values at **Figure 4**). Therefore, *Butia yatay* palm savanna honey exhibited a Newtonian behavior at 25°C while the differences among samples could be attributed to natural variations in composition (individual sugars and water content) [60].

Other authors showed that steady shear and dynamic rheological tests revealed Newtonian behavior with a viscosity Arrhenius model dependence on temperature: for Greek honey samples in the range of 20–60°C [60]; for Ethiopian honey samples

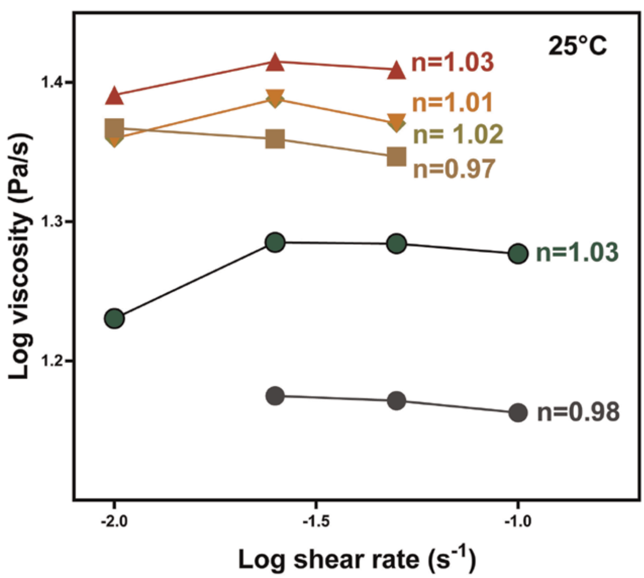

Figure 4.
Log viscosity vs. log shear rate of honey samples at 25°C. The law power flow index is indicated for each honey sample. Sample 1 ● (black circles), sample 2 ■ (brown squares, sample 3 ▲ (red up triangles), sample 4 ▼ (orange down triangles), sample 5 ◆ (green diamonds), sample 6 ● (green circles).

in the 25–45°C range [61]; and for Portuguese honey samples in the 30–70°C range [62]. Da Silva et al. [62] proposed a model combining both temperature and solid content to predict honey viscosity behavior with good results for 40 Brazilian honey samples in the 10–60°C range. Furthermore, honey samples from the northeast of Argentina also probed to be Newtonian with Arrhenius temperature dependence in the range of 10–50°C [57]. These authors have found that some differences in rheological properties between Argentina regions may be attributed to natural variations in honey composition related to climate and flora variation. Higher temperatures and environmental moisture might also influence the maturation of honey in the beehive, affecting its physicochemical properties and leading to differences in honey rheology [57].

7. Color

The color of honey exhibits a proportional variation based on the presence of pigments (carotenoids and xanthophylls), polyphenols (flavonoids), minerals, as well as the source of nectar (flowers or honeydew). Monofloral honey types possess distinctive and defined hues. Honey manifests a broad spectrum of colors, ranging from pale white to deep amber, including tinges of red, yellow, and green. However, lighter brown or amber tones dominate [63]. These discrepancies hold significance in studies concerning geographical and origin denominations, as well as commercial relevance, facilitating honey exports and allowing exporters to target favorable markets.

The color of honey is internationally standardized using the Pfund color scale, which provides a recognized and standardized technique for rapid, effortless, and cost-effective color measurement. This scale ranges from 0 to 140 Pfund millimeters, classifying honey into distinct categories such as water white, extra white, white, extra light amber, light amber, amber, and dark amber [64, 65].

For color analyses a portable Hanna Honey Colorimeter was used. This equipment (calibrated with glycerol) enables the assessment of light transmission through a clear honey sample into the cell, ensuring it is devoid of any residues or air bubbles that may impact the measurement. **Figure 5** shows the color of the analyzed sample expressed in Pfund millimeters. Meanwhile, **Table 3** shows the color categories. It can be observed that the analyzed samples corresponded to amber honey, with only sample 2 samples falling within the upper limit for light amber honey. These findings align with those obtained for total polyphenols and conductivity, signifying that *Butia yatay* palm savanna honey possesses higher mineral and total polyphenol contents compared to other lighter honey variants from Argentina. For example, other authors found that honey from central Argentina showed color ranging from water white to light amber categories, corresponding to a very low mineral and ash content [66]. On the other hand, Uruguayan hone samples with high pollen content (>50%) of the palm *Butia odorata* showed similar values of color (75 mm Pfund, light amber) to those obtained in the present study [11].

8. Conclusions

In this work, we characterize honey produced in the *Butia yatay p*alm savanna in the province of Entre Ríos, Argentina, during the palms' blooming peak. Palm pollen was consistently present in all the analyzed samples as secondary pollen regarding its

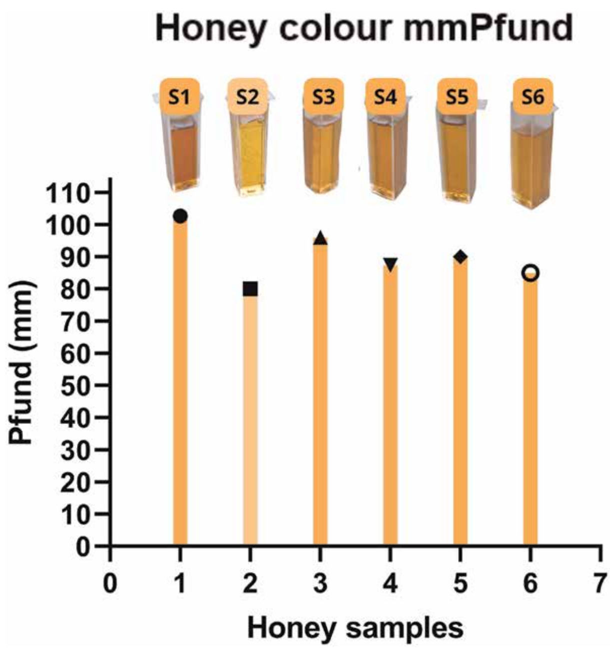

Figure 5.
Color of honey samples (mm Pfund). A photo of each sample is shown.

Honey color	Pfund range (mm)
Water white	x ≤ 8
Extra white	8 < x ≤ 17
White	17 < x ≤ 34
Extra light amber	34 < x ≤ 50
Light amber	50 < x ≤ 85
Amber	85 < x ≤ 114
Dark amber	x > 114

Table 3.
Color categories of honeys. The mm Pfund range is shown for each color category.

frequency with an average of 33.5% of the total pollen grains. Thus, the contribution of this species as a nectar source for honey production is significant and it is here informed for the first time. This honey also showed interesting characteristics such as high proline content, elevated conductivity, a color range from light amber to amber, and significant polyphenol bioactivity. These properties together with a fruity aroma and an exceptionally sweet taste distinguish this honey as an attractive non-timber forest product (better instead, palm savanna product) with potentially higher market values. This could contribute to local beekeepers' economy by improving this honey trading while contributing to the conservation of palm populations through their use as well.

This is the first instance of producing and characterizing honey from this peculiar botanical and geographical origin, thus contributing to Argentinian efforts to hierarchize regional and local honey types to add value to them in the market, as well as contributing to general mellitological knowledge. Consequently, we plan to carry on organoleptic and further physicochemical studies in the near future to enhance our knowledge of this palm honey in its entirety.

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Chapter 4

Diversity, Distribution, Nesting, and Foraging Behavior of Stingless Bees and Recent Meliponiculture in Indonesia

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Abstract

Stingless bees are one of the social bees that are spread across tropical and subtropical countries, including Indonesia. Indonesia has 46 species of 10 genera, and they are endemic in at least three distinct areas. Some species live in specific microclimates and environmental conditions; for example, *Wallacetrigona incisa* lives in the mountainous highlands of Sulawesi. It has many variations of nest architecture and places of nesting from natural habitats to residential environments that provide food plant sources of a variety of wild to cultivated plants. Stingless bees have a small body size, small honey pots, and limited foraging range and forage for more in short plants. In general, the forage distance and flight distances are correlated with the body size. The role of bees in nature is very important as pollinators and pray for protected wildlife and other insect eaters. Stingless bees also produce the products honey and propolis that are useful for humans. Indonesian meliponiculture provides benefits to the community but requires caution for the preservation of bees in their habitat. The transfer of colonies of stingless bees between islands and altitudes have caused many colony deaths and could not produce well. Conservation regulation is the solution.

Keywords: diversity, distribution, nesting, foraging, meliponiculture

1. Introduction

Besides honeybees (*Apis* spp.), stingless bees are a group of eusocial bees that live in colonies with defined castes that are morphologically fixed, including a reproductive female (queens), numerous sterile females (workers), and males (drones) [1].

Globally, stingless bees (Hymenoptera: Apidae: Meliponini), with approximately 500 species worldwide, are more diverse than honeybees (Apini: Apis: *Apis* spp.).

At least fifty species are known to belong to the Southeast Asia region [2, 3], out of 46 species of stingless bees that have been recorded in Indonesia that were recently grouped into 10 genera, namely Austroplebeia (1 sp.), Geniotrigona (2 spp.), Heterotrigona (10 spp.), Homotrigona (8 spp.), Lepidotrigona (6 spp.), Lisotrigona (1 sp.), Papuatrigona (1 sp.), Pariotrigona (1 sp.), Tetragonula (15 spp.), and Wallacetrigona (1 sp.) [4–7]. The main of those species are only found in Indonesia or neighboring countries in the same geographic region [4, 8, 9]. Of the species recorded, most of them were collected on the islands of Sumatera and Kalimantan [7], although sampling bias toward the western rather than the eastern islands may obscure the true pattern of distribution and diversity. Nonetheless, we have found an inline between the distribution of stingless bees' species and the known bioregional complexes of plants and animals.

Indonesia has a lot of naturally complex environmental conditions with macro and microclimates, topographical and other physical environments under complex geological history [10]. At least under the three biogeographic regions of the Indo-Malayan, Wallacean, and Australasia regions on the terrestrial ecological map [11], Indonesia has various species richness and high endemicity of plants and animals [12–15], also stingless bees are in accordance with these facts [7]. There is a pride fact in the distribution of the genus which is only restricted discovered on a certain island or regions, for example, genus *Austroplebeia* in Irian Jaya (Indonesian Papua); *Geniotrigona* in Kalimantan; *Heterotrigona* in Kalimantan; *Homotrigona* in Sumatera, Java, and Kalimantan; *Lepidotrigona* in Sumatera, Java, and Kalimantan; *Lisotrigona* in Sumatera and Kalimantan; *Papuatrigona* in Irian Jaya; *Tetragonula* in Sumatera, Java, Kalimantan, Sulawesi, Ambon, Maluku, and Irian Jaya; and *Wallacetrigona* in Sulawesi [7]. In a recent development, the species identification, apart from using morphological characters of stingless bees, has also been using distinctive characters of entrances, broods, and food storage and genetic markers [16–19], which gives rise to a complex species of stingless bees in the country.

2. Distribution and endemic species in Indonesia

Stingless bees' distribution globally has been categorized into four tropical regional groups, namely Neotropical, Afrotropical, Australasian, and Indo-Malayan [1, 9, 20–22]. In the tropical country of Indonesian archipelago, from the west to the east of the country are stretched tens of thousands of islands. The distribution of the stingless bee species is categorized as [10] explaining the complex geological history of Indonesia; the country is grouped into at least three major regions namely Indo-Malayan, Wallacean, and Australian tropical regions with different species endemics [7, 9, 23, 24]. The high species richness and uniqueness of stingless bee distribution in Indonesia may be related to the country's vast geographic expanse, diverse topological and environmental landscape, and complex geological history. There are at least 46 species of 10 genera of stingless bees recorded in Indonesia, spread across the archipelago. Starting from the west to the east of the islands, species of stingless bees have high endemicity, the westernmost being the islands of Sumatera (23 species), Java (7 species), Borneo (29 species), Timor (1 species), Sulawesi (3 species), and Ambon (2 species), and to the east is Irian Jaya (Indonesian Papua) (9 species) [7]. Species diversity, although dominated on the islands of Sumatera and Kalimantan, both of which are in the Indo-Malaysia region, most of which are not found in other regions [7, 21, 24], the same thing with the species of stingless bees found in the Indo-Australian region [4, 6, 7, 25, 26]. The species richness of stingless bees between the western and eastern regions gives an interpretation as if the diversity of stingless bees in eastern Indonesia is low, but if exploration is carried out more intensively, it is not impossible to find more species in eastern Indonesia.

The occurrence of the species *Heterotrigona itama*, *G. thoracica*, and *H. apicalis* and several species of the genus Tetragonula in the western part of the country are also distributed in mainland Asia as a unified distribution region of Indo-Malaysia. The Wallacean region of Sulawesi and the surrounding small islands have several species of stingless bees that are the same as those in the Indo-Australian region, although each island still has its species' endemic peculiarities. Currently, the Papuasia stingless bee includes 11 species that have been recorded from New Guinea (Papua, West Papua and Papua New Guinea Provinces) and the Solomon Islands (excluding the Santa Cruz Islands) (Engel and Rasmussen 2017). In Papua Province, 10 species have been recorded, and 7 of them are endemic species [27].

Tetragonula biroi was well known and first recorded in Papua islands, including the Indonesia Papuan [4], the recent distribution of the species was also recorded on Sulawesi Island by Indonesian researchers [16, 18, 19], and the first discovery of its presence in Halmahera island [28].

Eight species of stingless bees have been recently recorded in Sulawesi Island and surrounding small islands. *Wallacetrigona incisa* was collected from Sulawesi [29], and it was found in South Sulawesi [19], and it was reported by [6] from Central Sulawesi and North Sulawesi. Rasmussen et al. [9, 30] informed that *Lepidotrigona terminata* and *Tetragonula pagdeni* were found in Sulawesi, and [19] reported their founding of the

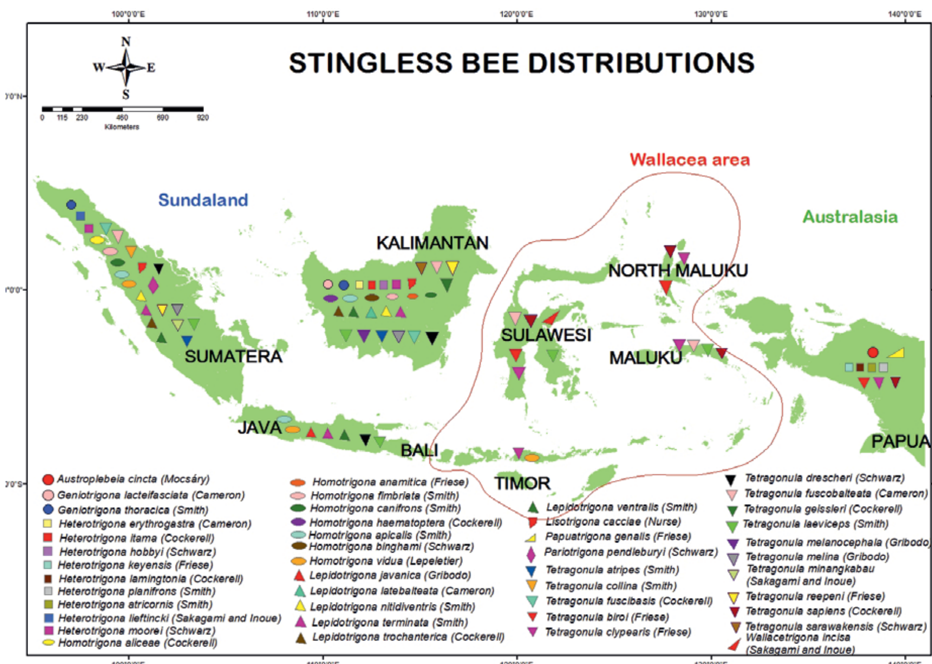

Figure 1.
Stingless bee species distribution map in three tropical regions of Indonesia [7, 19, 24, 28, 32].

species in South Sulawesi. Suriawanto et al. [18] reported their finding of *T. laeviceps*, *T. sapiens*, and *T. biroi* from Central Sulawesi, and [19] and [18] found *T. clypearis* and *T. fuscobalteata* from South and West Sulawesi, and [31] from Central Sulawesi. Sulawesi Island is the most interesting island for the Wallace endemic species of stingless bees and their microhabitat, namely *Wallacetrigona incisa*, who live only in the highlands of this island. The distribution of the species of stingless bees in Indonesia is shown in **Figure 1**.

3. Nest architecture and nesting behavior

The nest architecture of stingless bees has a basic structure that differs in shape, size, and appearance, which is also determined by microclimatic factors, availability of resin sources, colony age, and natural antagonists. Nest architecture could be utilized as a potential species identification tool [16, 18, 19, 33, 34]. Stingless bees build nests with several materials including resin, gums, plant exudates, and the stingless bee worker waxy secretions. All of these materials are mixed by worker bees known as cerumen [1, 34]. The composition of cerumen on the nest of *Tetragonula* sp. From Indonesia contains phenolic compounds, which act as an antioxidant in every part of the nest [35].

Stingless bees do not have an ability for digging solid objects, so they always use cavities of dead and living trees, soils, man-made structures like walls or cracks of buildings, drainage pipes, and poles, among others, or other materials with cavities [34, 36]. In Malaysia, stingless bee nests can mostly be found in rock crevices, underground cavities, and tree trunks or branches [37]. In Halmahera Island, it can be found in bamboo segments, logs, part of houses (part of roof, wall, and keyhole of the door), stone cavity, tree trunk, root of tree, foundation, wooden materials, furniture, electricity, iron cavity, and also over the land [28]. Stingless bees have distinct geographical distribution and nesting sites, inhabiting living and dead tree cavities, rock crevices, land, anthills, and termite nests [16]. The nests can be found under the surface of soil cavities, tree holes, wood cavities, hollow bamboo trees, and in gaps in the walls around the house [4, 38–40].

The nest of stingless bees consists of entrance, storage pots, and brood cells, which can be distinguished into outer and inner entrance, pollen and honey pots, and young and mature brood cells. Other important parts of stingless bee nests are completed with cerumen, batumen, and involucrum. Cerumen is a mixture of propolis (resin) and wax to construct storage pots and brood cells. Batumen consists of resin or wax as a layer of cavity nest [41]. Various shapes of batumen act as supporting parts of the nest to protect from sunrise, predators, and intruders [39, 42, 43]. The shape, color, hardness, and adhesiveness of the parts of the nest depend on the availability of the surrounding material.

Additional structures and shapes of the entrance are affected by environmental conditions, for example, its natural enemies, temperature, humidity, and wind speed. Growing colonies usually have rudimentary forms as part of the nests, for example, semi-spiral or semi-cluster brood arrangements before becoming spiral or cluster forms. Although many forms of the entrance are short because they are not yet developed, there are species of stingless bees that do not appear to have an entrance protrusion because it is only a hole whose surface is covered with resin. On the other hand, the nest entrance of *W. incisa* is hard and thick in texture and dominated by the black color. The brood cell was found only in the spiral form, with thick and stiff involucrum.

General brood comb constructions on stingless bees are spiral, stacked, layered, and clustered [44]. *Tetragonula biroi* and *Wallacetrigona incisa* has similar variable brood comb construction forms of either spiral, semi spiral, and irregular shape, surrounded by soft and slight involucrum [16]. *Tetragonula sapiens* in South and West Sulawesi have semi-cluster and semi-comb brood cells. *T. fuscobalteata* in South and West Sulawesi has cluster brood cells, which is different from *T. fuscobalteata* in

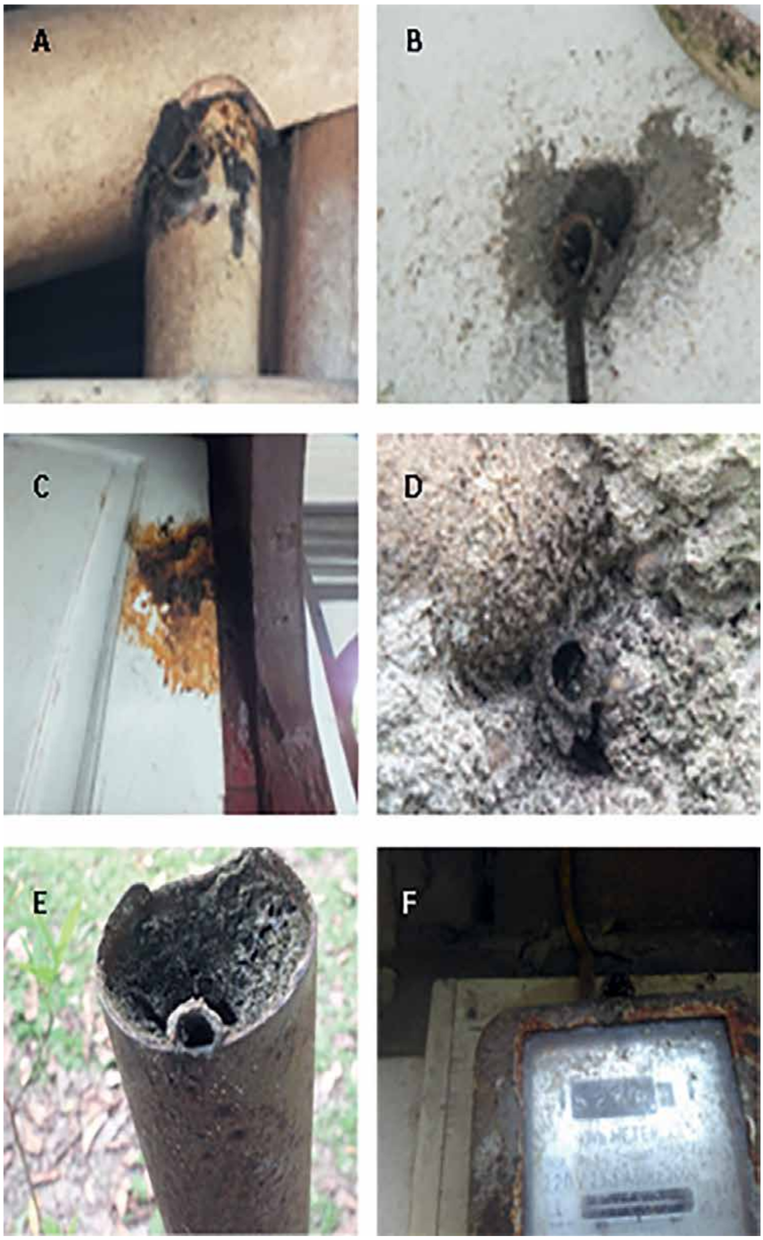

Figure 2.
Nesting preference of Stingless Bees in West Halmahera. A. Cavities in house parts; B. Cavities in walls of buildings; C. Cavities in oorways; D. Cavities in the foundation of a house; E. Cavities in an iron; F. Cavities in electric meter.

South Kalimantan which has cluster brood cells. However, It is similar to the brood cells of *T. drecscheri*, *T. melanocephala*. *Lepidotrigona terminata* in South Sulawesi has regular layered comb brood cells, similar to the colony found in South Kalimantan. *Heterotrigona itama* was found in South Kalimantan and has layered comb brood cells. The other species found in South Kalimantan has semi-comb brood cell (*Geniotrigona thoracica*), semi-cluster brood cells (*Homotrigona canifrons*), and cluster brood cells (*Homotrigona apicalis*) [19, 45].

Honey pots are generally oval, vertical, or rounded in shape and vary in size. In honey pots that are large, the honey can be harvested by sucking it, and conversely, the small ones are squeezed out. Within their nests, the bees deposit honey, pollen, and propolis, and all of these are exploited by humans [7]. Honey pots are usually located adjoining the nest entrance. Pollen pots are situated close to the broods, so feeding the chicks can be done more easily, and they also store food as honey, which can be exploited sustainably by humans.

Stingless bees, which are small, have adaptations to various small cavities in houses and buildings, including narrow doorways, foundations of house walls, and other cavities around the house, and those small cavities are likely as a trigger to the colonies to carry out the colony splitting (**Figure 2**).

4. Foraging behavior

Stingless bee workers are responsible for collecting resources, such as pollen and nectar, along with materials to build and protect the nest [1, 38]. Stingless bees actively fly from morning to evening on sunny days [46]; however, stingless bees first out of the nest later and last enter the hive quicker than honeybees [47]. Unlike honeybees, stingless bees forage randomly searching for food and resin sources without being guided by their senior bees. Every species of stingless bee has preferences for food resources, so they have different behaviors in foraging. Stingless bee workers, responsible for colony nursing, feed the larvae by putting food in incubation cells before the eggs are laid by the queen [48]. In general, the forage distance and flight distance of bees correlate with the body size; the bigger the body size, the longer the foraging and flying distance of bees.

The main species of stingless bees in Indonesia are small, which affects the limited flight range of bees in finding food. Unlike honeybees, in search for food, who are primarily guided by the senior workers, stingless bees individually and freely choose to take the material of the nearest distance from the colonies, as shown in the species *Tetragonula biroi* and *Heterotrigona itama* [49].

Like other groups of bees, stingless bees have a mutually beneficial interaction with flowers. Flowers provide food for bees in the form of nectar and pollen, and bees help attach pollen to the stigma so that both the survival of the bees and plant reproduction are guaranteed. Physical environmental features such as the temperature and humidity affect foraging behavior [50]. Stingless bees diligently perch on flowers until they are finished picking the ingredients they want [51, 52].

Stingless bees are generalist pollinators, and their role is evident in cross pollination. Stingless bees are small, so they can forage on small flowers without causing damage and can also take food from hidden flower parts that are not visible by other larger bees. Stingless bees are often seen foraging on large flowers, which are visited by larger bees, birds, and bats [53]. Pollen analysis shows that stingless bees collect pollen of various shapes, sizes, colors, and odors. Stingless bees frequently also visit flowers that are dull and musty in smell, typical of beetle and fly flowers; however, in a heterogeneous environment, stingless bees tend to choose flowers more typical of bees [53]. The high preference of stingless bees to collect nectar from short wild plants, which are commonly used as traditional medicinal plants, cause stingless bee honey to be used more as "jamu" or herbal medicine.

Stingless bees have the behavior of using various resins to protect the nest and make it difficult for predators to enter the nest. The sticky resin and slippery sand around the entrance are used to repel and trouble insect intruders. This type of protective resin varies depending on the environment in which the stingless bees' colony originates. *Wallacetrigona incisa* in the highlands of Sulawesi has an entrance covered with a hard and thick resin that protects the entrance of the nest from the forest rat [16]. Inside the stingless bees' nest is usually stored sticky and elastic resin, which is used to pile up the bodies of predatory intruder beetles that enter the nest.

Stingless bees exhibit defensive behavior when their colony is disturbed by animals and humans by ganging up and biting. Species that have relatively high aggressiveness compared to the other are *T. biroi* and *Homotrigona conifrons*. Behavior against ants by sticking sticky resin around the nest, against changes in light and wind by widening or narrowing the holes or making double entrances, for example *W. incisa*, *H. itama*, and *H. thoracica*. The zigzag flying of stingless bees while searching for food or flying around the nest is thought to be a way to make them less easily caught by predators. In multi-species stingless bee farming, *Lepidotrigona terminata* is known to steal resin from other stingless bees.

Stingless bees have a small body size, small honey pots, and limited foraging range and forage more for short plants. This range enables the stingless bee to visit fewer and different types of flowers. However, stingless bees have another advantage in that they do not rely solely on mass flowering plants. All these reasons cause stingless bees to produce less honey than honeybees. Stingless bees can visit small flowers that larger bees cannot; however, they are able to visit large flowers.

The mature queen is not able to change the nest by flying due to her body being disproportionately fat. Unlike honeybees, who conduct long and short migrations to find abundant food resources [54–56], stingless bees do not so but only coduct a short movement during a new queen swarming.

Stingless bees have the capability to protect the colony from natural predators and disturbances by using sticky resin around the entrance and weak parts of the nest targeted by intruders [57]. Stingless bees also construct additional configurations to protect the nest from intruders and natural enemies [33]. The highland endemic species of Sulawesi *Wallacetrigona incisa* builds a strong entrance to protect from the wild forest rat [16].

5. The benefit of Stingless bee

The archipelago country of Indonesia has very diverse natural ecosystems [58] that grow a huge number of wild and agricultural flowering plants, whose biodiversity has been well recorded at least in the islands of Java, Kalimantan, Sumatra, Papua, Sulawesi, Moluccas, and Lesser Sunda Islands [59]. As an agricultural country, a lot of flowering plants grow in various natural and agricultural systems [60] that exhibit diverse flower morphology, biology, and phenology [12, 61, 62], of those require various pollination by the flowers themselves, intermediary by wind, water, and animals [53, 63–65]. As a part of eusocial bees, stingless bees are potential and efficient pollinators among insects and other animal pollinators, as they visit diverse flowers, and therefore, as potential and efficient pollinators [1, 63, 65–67], they play an important role in ecosystem services in the tropics.

The ecosystem would not be able to maintain its stability without the contribution of bees. Bees serve an important function as pollinating insects [67, 68]. Without bees, the delicate balance of the ecosystem will be thrown off, which will have repercussions for many aspects of life [69]. Stingless bees have a significant value because they are the primary pollinators of both wild flowering and cultivated plants. As a result, they play an essential role in the preservation of biodiversity and the safety of food supplies. Another fantastic application for stingless bees is crop pollination. It is estimated that almost two-thirds of all cultivated plant species rely on bee pollination to bear fruits and seeds. Currently, honey production is the primary goal of meliponiculture, but crop pollination will require a significant number of colonies in the future and will most likely become the primary goal of the activity. Stingless bees can be readily managed to boost pollination and productivity of a variety of crops, including coconut, strawberry, tomato, and coffee. Bees pollinate many wild and cultivated flowers in addition to producing honey. According to [70], animal pollination accounts for around 35% of global food production volume, with [71] adding that this figure can rise to 78% in temperate zones and 94% in tropical areas. Many plants, including Arecaceae, Cucurbitaceae, and Solanaceae, have been reported to benefit from stingless bee pollination. Furthermore, stingless bees have been shown to be major pollinators of non-crop species in their natural environments [67]. According to reports, pollination activities are reducing as bee populations dwindle. The significance of bees as the primary pollinators of a wide range of wild flowering plants as well as almost 70 percent of all cultivated plants is acknowledged on a global scale [72].

In many areas of the world, stingless bees have been cultured in colonies and product management of meliponiculture [68, 73, 74], and it has been developed rapidly and spawned major industries as well as profited indigenous people who often have historical and important cultural practices associated with stingless bees [75].

Stingless bees collect nectar, pollen, and resin mainly from plants, of which the material in their nest cavities is stored as honey, bee bread, and propolis. Deposits of honey and bee bread in the nest are stored food, which will be consumed in the next lean season. Stingless bees mainly produce honey and propolis; the honey has a sour taste and is sometimes bitter [76]. This taste is the main reason why stingless bee honey is not the main choice for the public or inland people who live in the forest, who instead choose honey from honeybees (Kahono et al. unpublished data). Since ancient times, honey from stingless bees has been well known as a traditional medicine; however, they never carried out stingless beekeeping practices. Almost all species of stingless bees are targets for honey hunting. People gather honey from the wild and near their house. Initially, stingless bee honey was not found for sale in shops, only being sold by limited stingless bee breeder communities. Meliponiculture is the practice of raising stingless bees. These bees are raised primarily for their honey, which they store in cerumen pots, making it quite simple to extract [77], as well as for other valuable hive products such cerumen, propolis, and pollen. Additionally, these bees create strange items that humans can use. Their honey is their best-known item. The water content of stingless bee honey is larger (about 30%) than that of ordinary *Apis mellifera* honey, which is just 20% water [78]. Because of this, the honey undergoes natural fermentation processes after being kept by the bees, resulting in distinctive flavors and intriguing acidity. In addition, each type of bee produces a honey that is highly distinctive; some are more acidic, while others are sweeter.

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Stingless bee honey has a sour taste and sometimes bitter taste [76]. This taste is the main reason why stingless bee honey is not the main choice for the public. Initially, stingless bee honey was not found for sale in shops, only being sold by limited stingless bee breeder communities. Since the discovery of many bioactive medicinal compounds from honey and extracted propolis from various nest parts such as entrance, pots, involucrum, and others, that are good for human health, people have wanted to consume them. Stingless bee honey has anti-inflammatory, antimicrobial, anti-diabetic, skin aging delaying, antioxidant, hypolipidemic, anti-diabetic, and control and prevent infection. Numerous religious, nutritional, and commercial uses were also extracted from their hives [35, 79] which all have been imported as the high prices have increased public interest in stingless bees. In some cases abroad, stingless bees have a significant cultural and historical value because most of the products that are harvested from the hive are put to a diverse range of uses. Many indigenous and rural cultures place high importance on stingless bees culturally [80–83].

All of the promoted wax, honey, and resin are still being researched and haven't been used well in the country. Discovering many bioactive medicinal compounds from both honey and extracted propolis from various nest parts such as entrance, pots, involucrum, and others, is good for human health. Stingless bee honey has anti-inflammatory, antimicrobial, anti-diabetic, skin aging delaying, antioxidant, hypolipidemic, anti-diabetic, and control and prevent infection [35, 79, 84]. In some cases abroad, stingless bees have a significant cultural and historical value because most of the products that are harvested from the hive are put to a diverse range of uses. We do not discover in case of indigenous and rural cultures place a high importance on stingless bees culturally [80–83].

The most sought-after stingless bee is the most commonly distributed species that is well adapted to human environments, *Tetragonula laeviceps*. Honey hunting continues to dominate because it is easier and quicker than maintaining domestic stocks. Sustainable harvest systems are currently applied with attention to the safety of the bees, taking only a portion of the hive and using clean harvesting and packaging equipment.

Stingless bees play an important role in the food chain as the food of wildlife. The flying and the flower-visiting bees serve as the feed of predatory animals, that is, spiders, centipedes, predatory insects (beetles, bugs, grasshoppers, flies, and ants), reptiles, amphibians, birds, and mammals [85].

Stingless bee nests attract a variety of predators due to their abundance of food resources and abundance of breeding sites [86]. Females of the assassin bug *Apiomerus pillipes* (Reduviidae, Harpactorinae) are predators of workers of the genus melipona in Brazil, according to [87]. In Indonesia, stingless bees play an important role in the natural food chain as a prey for birds, honey bears, and other insectivorous. The predators can distinguish the bee comb to contain young stages (larvae and pupae), honey, bee pollen, and wax. The comb is a nutritious foraging target of animal predators including protected endemic animals of honey bear (*Heliarctos malayanus*), orangutan (*Pongo pygmaeus*), and other animals [56].

6. Meliponiculture in Indonesia

Traditional people utilize bee colonies around houses, taking honey without throwing away the hives and retaining them in the natural tree cavities. The basis of being able to breed stingless bees is the bee's life in a cavity that can be replicated with artificial boxes. Compared to honey beekeeping, maintaining stingless bee colonies and harvesting their productions is quite simple. Another advantage of stingless bees is that the bees have no sting so that maintenance is safe and harmless, making them friendly. Stingless bees have a high adaptation tolerance to environmental stress so that they usually survive a period of environmental stress so that they will develop again to enter the next good period.

Initial meliponiculture activity was raised by moving natural nests from forests and keeping them near residential, plantation, and agricultural lands. Furthermore, meliponiculture continues to develop semi-natural systems by adding wooden boxes for honey in the top of a colony in the natural tree trunk. Many of them move entire bee colonies from natural hives to new wooden or other artificial boxes [7, 88]. To imitate the artificial hives of honeybees, some local stingless beekeepers have made artificial honey pots so that the bees are efficient in time and energy to collect nectar and pollen.

In general, meliponiculture is usually carried out as a side activity by farmers, animal breeders, private workers, and government employees with a limited number of species and colonies. The first of the easiest to cultivate due to their wide distribution and high adaptation to various environments is *Tetragonula laeviceps*. This is the reason for the species to breed everywhere in the country [7]. Since Mapatoba Silla from Hasanudin University introduced the new model of meliponiculture with vertically stacked three nests for endemic species of Sulawesi, meliponiculture in the country even slow but show gradually increasing.

Although the size of the bees is related to the measure of honey production, however, a small species with a large amount of colony members of *Tetragonula biroi* shows very high production of honey and propolis. Some small species were used to be cultivated are *T. sapiens*, *T. fuscobalteata*, *T. clypearis*, and *T. dresscheri*; the medium is the species belonging to the genus *Lepidotrigona*, and the large species is *Heterotrigona itama*, *H. erythrogastra*, *Geniotrigona thoracica*, *Lophotrigona canifrons*, and *Wallacetrigona incisa*. There is no national production data for honey stingless bees, but there is a positive trend that the production of honey and raw propolis material to be extracted continues to increase. So, stingless bees have a crucial role in the small-scale economy, especially for villagers, by providing honey and raw propolis for industry [49].

Recently, the management of meliponiculture has been developed successfully in successive recent years. Many farmers have been doing it professionally with a greater number of species and bee colonies, as well as conducting bee ecotourism and selling bee colonies and beekeeping products.

Pest of natural colonies of stingless bees is not exposed properly due to the lack of research on it in the country. Flying bees in search for food resources become targets of insectivorous birds, and those that perch to collect the necessary material for the colony become targets of spiders, lizards, and chameleons. Large predators such as lizards, jumping spiders, and frogs stay near the nest entrance, watching in and out bees and catching them [33, 89].

Stingless bees are found in different countries and are adapted to the local ecosystem. Stingless bees can adapt to environmental changes (temperature, humidity, and rainfall) and changes in food sources within a few weeks to 3 months to form perfect colonies [90]. The transfer of colonies of stingless bees between islands in Indonesia and from the highlands to the lowlands on the island of Sulawesi has caused many colony deaths, and the colonies did not produce well [91].

Local peoples in the various regions of Indonesia have developed different methods of hives. Among the stingless bee species commonly cultivated in Indonesia are *Tetragonula laeviceps*, *T. biroi*, *Heterotrigona itama*, and *Wallacetrigona incisa*. These species are most commonly cultivated due to their active foraging colony behavior and large and saleable production. Traditional honeybee farming development opens new opportunities for people in rural areas, particularly women, and has the potential to improve the economic conditions of many households. Many people who avoid beekeeping due to honeybees' highly defensive behavior can be persuaded to try it, especially if flora resources are abundant. Beekeeping has long been practiced in Indonesia and is regarded as an important part of Indonesian culture, particularly among people living in villages or on the outskirts of forests. Apiculture and meliponiculture are also known as honey beekeeping and stingless beekeeping, though local beekeepers are unfamiliar with these distinctions. Beekeeping is popular in many parts of Indonesia, if not the entire country. As public awareness of stingless bees grows, so does interest in beekeeping in Indonesia. These bees are friendlier and easier to handle because they do not sting. Furthermore, the higher market price of stingless bee products makes them a viable cultivation option. However, the amount of honey produced is less than that of the Apis group, but beekeepers can profit from many colonies.

As stingless bee honey production from culture is not documented and the intensity of taking wild colonies is very high recently, scattered evidence suggests that stingless bee honey from nature has played a significant role in the national honey production.

In the national production of honeybees, there are well-known honey producing regions [7, 92]. Some areas have been started to be stingless bee honey producers, for example, North Luwu district (South Sulawesi) that provides lots of honey and raw materials for advanced products, including propolis.

In contrast to the stingless bee, the development of its cultivation occurs faster due to its easy-to-handle nature and body morphology, which is suitable for a wide range of flowers. The advancement of meliponiculture is not limited to pollination and honey production for commercial purposes. In the concept of stingless beekeeping, beekeepers have moved further into the realm of health by pollinating medicinal plants. As a result, stingless bees have been producing more medicinal honey, propolis, and derivative products over the last decade.

Indonesia needs to develop beekeeping by enhancing the existing natural ecosystems and artificial green environments as a source of feed, as well as promoting stingless native bees through formal education and bee ecotourism.

Managed meliponiculture in Indonesia is definitely the ideal choice because it can be done in all types of green environments, and meliponiculture can be conducted by ordinary people safely, cheaply, and easily and with less maintenance [93]. Recently, beekeeping of local species of stingless bees has been growing significantly in every region across their distribution, progressively increasing the national production of medicinal honey, raw propolis, and derivative products.

Consumption of honey from apiculture and wild honeybee hunting is more common as a daily drink and supplement drink in Indonesia than stingless bee honey is, mainly for traditional medicine. As a result, stingless bee honey is mostly collected in the wild or raised in small quantities traditionally.

7. Conservation

Despite the importance of bees and many other pollinators, they are threatened by a wide variety of factors. These factors include the loss of habitat because of deforestation, wildfires, changes in land use, excessive use of pesticides, and climate change.

These factors are causing alarming population declines, which will have severe consequences for ecosystems as well as the diet, health, and economies of people all over the world [81, 83]. Because of these factors, developing effective and permanent conservation strategies for stingless bees (and other pollinators) is an urgent matter [94, 95]. Beekeeping methods, land use conversion, pesticides, and changes in natural habitat are all variables that could impact bee populations [96].

Despite the significance of stingless bees, there are several dangers that are leading to alarmingly low population numbers. At the moment, the commerce of stingless bee colony was glow by move the endemic species to another endemic area, so it disturbed the natural distribution and guess of the bad side effect. Maybe the bees can survive at the area, but the bad side effect to local stingless bees was not researched. The bad effects that happen are the death of colonies on the trip, killed by natural enemies, the nest captured by other species [16], and instability of the native habitat because of the decreased ecological function caused by the takeover of the stingless bee colony.

The supervision of quarantine department needs to be increased to control the transportation of endemic species, especially those who get transported to other islands. The government needs to make an explicit regulation for the quarantine department about the conservation of endemic stingless bees.

8. Conclusion

Indonesia has various species of stingless bees that are spread throughout the Indonesian archipelago and are also endemic to at least three different regions. Wallacean island of Sulawesi has an endemic species of stingless bees, namely *Wallacetrigona incisa*, which lives in the highland mountains. In addition to morphological characteristics, stingless bees have various shapes, sizes, and nest architectural characteristics that can also be used as an identification tool. Nest architecture varies depending on climatic factors, availability of nest-building resin, colony age, and natural antagonists. The distribution of nesting sites ranges from natural habitats to residential environments.

Stingless bees are small bees that adapt to various sized cavities. In principle, in a good environment, all types of bees can be bred, although there is a tendency for breeders to choose superior types of bees. Stingless bees are important pollinators of the fruit crops and wild plants that are their food source. The most obvious advantage of stingless bees compared to honeybees is the production of raw propolis as the main ingredient in expensive liquid propolis,s which is important for human health.

Meliponiculture has begun to develop in Indonesia with a large number of colonies, although not evenly distributed. People conduct meliponiculture to improve their income. Moving colonies between islands and different environments is not recommended because it causes colonies to die and not develop. Likewise, the movement of endemic species to other endemic areas will disrupt their natural distribution and cause negative impacts. It is necessary to regulate the use of bees by considering their sustainability.

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Chapter 5

Diseases of Honeybee (*Apis mellifera*)

Muhammad Asif Aziz and Shah Alam

Abstract

Honeybees, important pollinators crucial for ecosystem health, are susceptible to a range of bacterial, fungal, and viral diseases that pose significant threats to their colonies. Bacterial diseases include American Foulbrood (AFB) caused by *Paenibacillus larvae* and European Foulbrood (EFB) caused by *Melissococcus plutonius*. AFB results in the death of honeybee larvae and the production of spores that contaminate the hive, while EFB primarily affects young larvae. Fungal diseases like chalkbrood are caused by *Ascosphaera apis*, Chalkbrood transforms larvae into chalk-like mummies Nosemosis is caused by two pathogenic spores *Nosema apis*, and *Nosema ceranae*, which infects the midgut of adult honeybees and viral diseases such as Deformed Wing Virus (DWV), Israeli Acute Paralysis Virus (IAPV), and Chronic Bee Paralysis Virus (CBPV) further weaken honeybee colonies, DWV and IAPV lead to deformed wings and premature death, and CBPV causes shivering hair loss, and paralysis. To manage these diseases, beekeepers employ various strategies including Integrated Pest Management (IPM) techniques, genetic selection for resistance, antibiotic treatments, and maintaining healthy hive conditions. Continued research, monitoring, and education are crucial for effective disease prevention and control, as well as the preservation of honeybee populations and the essential ecosystem services they provide.

Keywords: honeybee, bacterial, fungal, viral, diseases

1. Introduction

1.1 Bacterial diseases in honeybee

1.1.1 European foul brood

European foulbrood is a disease of bee larvae caused by the Gram-positive bacterium *Melissococcus plutonius* and worldwide distributed [1], which mainly affects the capped brood, and causes death [2]. The transmission of the pathogen in the hive depends on the survival, the bacteria remain viable in these deposits for long periods [3], and although the cells are cleaned, some bacteria may remain infecting new individuals [4]. Adult bees and honey can act as vectors and transport bacteria between hives and apiaries [5].

The infection process initiates when bees ingest food that is contaminated with the bacterium. Upon reaching the bee's midgut, the bacterium multiplies and induces physiological damage to the epithelial cells and the peritrophic matrix [6]. Additionally, malnutrition in larvae has been associated with this infection as the bacteria compete for nutrients [6].

Infected larvae within the bee colonies exhibit abnormal distribution and undergo a progressive color change, transitioning from white to yellow and eventually turning brown as they enter the final decomposition phase [7]. The mortality of larvae caused by *M. plutonius* infection holds significant importance, yet there remains limited knowledge regarding the pathophysiological mechanisms and virulence factors of this pathogen [8]. It has been suggested that variations in the genotypic structure of the bacteria contribute to the differences in virulence factors among different strains, which play a critical role in the development and severity of the disease. Therefore, understanding the molecular epidemiology of the bacterium and its association with the severity of pathophysiological processes is crucial for timely disease diagnosis and effective control in apiaries [9].

1.1.2 Symptoms

This disease can be identified through various characteristic symptoms. Infected larvae exhibit abnormal color changes as the disease progresses, initially appearing pearly white but later turning yellow, brown, or even dark brown [10]. Additionally, affected larvae become sunken and eventually disintegrate within the cells, leading to their decomposition. Twisted or contorted larvae are also observed, which serves as a distinctive symptom of EFB [10, 11]. Scale-like remains of disintegrated larvae can be found adhering to cell walls or the bottom of cells [9]. Infected colonies emit a foul odor resembling rotten or decaying broods [12], further indicating the presence of EFB. The disease also causes a spotty brood pattern within the colony, disrupting regular brood development and resulting in mortality [5]. Chalk-like scales left behind by disintegrated larvae may be visible on cell walls or debris within the hive [12]. EFB weakens honeybee colonies, leading to reduced population size, decreased honey production, and an overall decline in colony strength [11]. Severe EFB infections can increase adult bee mortality, further contributing to colony weakness [10]. Unlike American foulbrood (AFB), EFB does not exhibit rope-like structures when the brood is stretched with a toothpick or matchstick [5]. Early and accurate diagnosis of EFB is vital for effective disease management. Beekeepers and inspectors should closely observe these symptoms and, if necessary, confirm the presence of the bacterium *M. plutonius* through laboratory analysis (**Figure 1**) [10–12].

1.1.3 Diagnosis

Accurate diagnosis of European foulbrood (EFB) relies on a combination of clinical examination and molecular identification methods.

Clinical examination involves carefully observing the characteristic symptoms exhibited by infected larvae, such as larval discoloration, sunken and disintegrated larvae, twisted larvae, foul odor, and reduced brood pattern [5, 10, 12], and microscopic analysis, including Gram staining and observation of bacterial cells, can provide preliminary insights into the presence of the causative agent, *Melissococcus plutonius* [12–14].

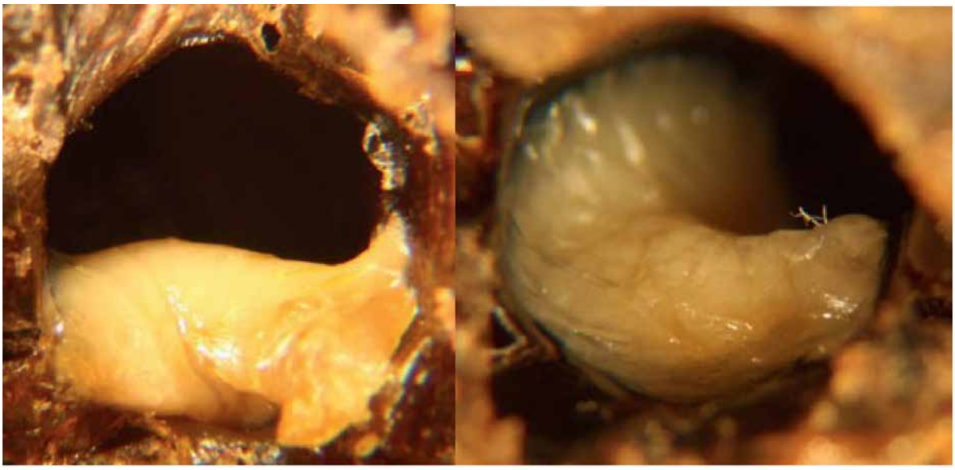

Figure 1.
A melted larva in its cell from a hive infected with European foulbrood and larvae curled upwards, flaccid, and brown or yellowish dead larva in its cell, [9–11].

To confirm the presence of *M. plutonius* and identify specific strains, various molecular techniques are used. Bacterial culture on selective media, such as MYPGP agar, enables the isolation and cultivation of *M. plutonius* from infected larvae [15]. Polymerase Chain Reaction (PCR) assays target specific regions of the bacterial genome, such as the 16S rRNA gene or the pMA plasmid, providing sensitive and specific detection of *M. plutonius* [12, 13]. Real-time PCR, or quantitative PCR, offers a rapid and quantitative assessment of *M. plutonius* presence in bee samples, contributing to accurate diagnosis [14, 15].

Molecular techniques like DNA sequencing allow for precise identification and strain characterization of *M. plutonius* by comparing obtained sequences with known references [13]. Multiplex PCR assays have been developed to simultaneously detect and differentiate between various honeybee pathogens, including *M. plutonius* and other brood diseases [16]. Fluorescent *In Situ* Hybridization (FISH) utilizes fluorescently labeled probes to directly visualize and identify pathogen spores in bee tissue samples [17]. Next-Generation Sequencing (NGS) technologies provide a comprehensive analysis of the microbial community, facilitating the identification of multiple pathogens, including *M. plutonius* [18]. Additionally, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has shown promise for the rapid identification of *M. plutonius* through the analysis of protein profiles [19].

Accurate diagnosis and molecular identification of *M. plutonius* using these diagnostic techniques are crucial for implementing appropriate control measures and preventing the spread of EFB in honeybee colonies. These methods serve as valuable tools for beekeepers and researchers in monitoring and managing EFB outbreaks [10, 12, 15, 17, 20, 21].

1.1.4 Control

Controlling European foulbrood (EFB) disease in honeybee colonies is crucial for maintaining colony health and productivity. Various strategies have been developed to manage and mitigate the impact of this disease. One approach is to encourage hygienic behavior in honeybees, as bees with high levels of hygienic behavior can detect and remove infected larvae, reducing the spread of the disease [22]. Timely removal and destruction of infected brood frames also play a significant role in preventing the further spread of EFB [22].

Another important strategy is to provide honeybees with a balanced and nutritious diet. This helps boost their immune system and makes them more resistant to EFB infection. Supplementing their diet with pollen supplements and ensuring adequate forage can contribute to improved colony health and resilience [23]. Additionally, genetic selection through breeding programs can be a long-term approach to combat EFB, selecting and propagating honeybee colonies that display resistance to the disease can significantly reduce its impact within the population [24].

Proper hive ventilation is crucial as it creates an environment that is less favorable for EFB development, good airflow helps reduce moisture levels and limits the growth of bacterial pathogens [22]. Regularly cleaning and disinfecting equipment and hive components, known as apiary sanitation, is another essential practice to prevent the buildup and transmission of EFB spores. Proper sanitation practices significantly reduce the risk of reinfection [25].

Educating beekeepers about EFB, its identification, and management strategies is crucial for effective control. Recognizing the signs of infection and implementing appropriate control measures contribute to disease prevention and control [26]. Antibiotics such as oxytetracycline may be used in severe EFB outbreaks but should be used cautiously and in accordance with local regulations [12].

Implementing an integrated pest management (IPM) approach that combines multiple control strategies can effectively manage EFB. By integrating various methods, beekeepers can achieve a comprehensive and sustainable disease management plan [22]. Regular inspection of colonies for EFB infection allows for early detection and intervention. Isolating infected colonies and implementing quarantine measures prevent the spread of the disease to healthy colonies [26]. Continued research, monitoring, collaboration among beekeepers, researchers, and regulatory authorities, and adherence to local regulations are also crucial for effective EFB control [12, 26].

1.2 American foul brood

American foulbrood (AFB) is one of the serious bacterial larval diseases of honeybees *Apis mellifera* [27, 28], caused by spore-forming, Gram-positive bacteria (*Paenibacillus larvae*) [29, 30]. The bacterium exhibits highly aggressive pathogenic behavior, resulting in the devastating collapse of bee colonies [30]. The spores of bacteria are ingested through food, specifically honey, pollen, and royal jelly [31] food exchange or feeding between larvae by nurse bees [32].

1.2.1 Pathogenesis

The infective stage pathogen is larval while clinical signs appear in the pupal stage, [27], and collapse the entire colony [28], after the hatching, the larvae are highly susceptible to pathogen infection [33]. Spores only infect the honeybee larvae midgut and multiply rapidly [34], vegetative cells infect the haemolymph and penetrate across the protective membrane [35], and the vegetative cells sporulate and transform into dry flakes which contain millions of spores [36].

1.2.2 Symptom

Capped brood that is sunken, perforated, or discolored, with a dark, coffee-like appearance" [13], and "Dead larvae that exhibit a rope-like consistency when probed with a matchstick or toothpick" [20]. The presence of foul-smelling, decaying larvae that may emit a sour or putrid odor" [28, 32]. Presence of spore-contaminated debris at the bottom of cells and on the hive floor" [20].

Following the initial diagnosis, a bacteriological examination is necessary to isolate and identify the bacteria responsible for the infection [37]. Molecular biology techniques like real-time PCR have become commonly used for identifying the specific bacterial strains causing the infection (**Figure 2**) [37, 38].

1.2.3 Diagnosis

In the field, as a method of confirming the disease, the beekeeper can drill several alveoli, swallowing the larvae and observing the liquid that is formed in this procedure. If the liquid obtained has a brown color and a pasty consistency, forming a filament is a sign of foulbrood [2].

The bacteriological diagnosis must be made, which includes the isolation and identification of the bacteria in the material from the infected colonies. Nowadays, molecular biology techniques such as real-time PCR are used to identify the infecting bacterial strains [37, 38].

The first PCR assay for the identification of *P. larvae* was developed in 1999 and was based on the 16S rRNA gene, of bacteria and is used for phylogenetic studies and detection of bacteria [38]. Nowadays, real-time PCR techniques are used for direct analysis [38].

1.2.4 Control

Rapid detection and destruction of infected colonies and equipment are essential to prevent the spread of AFB [20]. Strengthening honeybee colonies through proper nutrition and regular requeening can help minimize the impact of AFB [39].

One effective approach is selecting and breeding honeybee colonies with increased hygienic behavior, which involves the removal of infected broods to limit AFB spread [40]. Antibiotics, such as oxytetracycline or tylosin, can be used to control AFB at

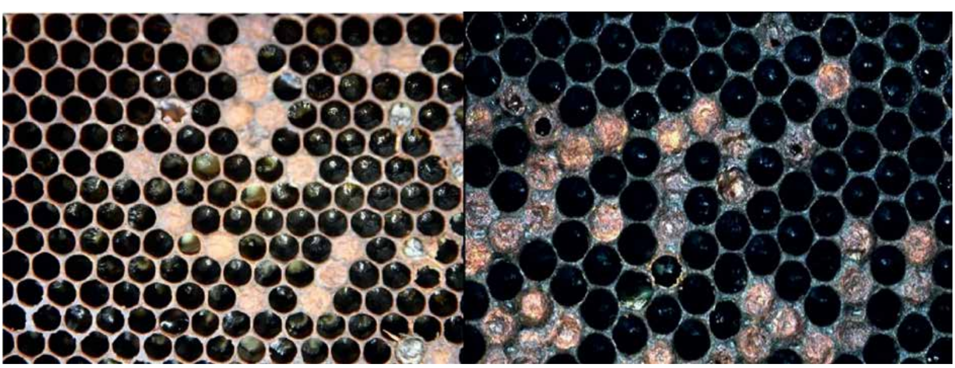

Figure 2.
The earlier stage infection and later stage infection of American foulbrood [20, 28].

the individual hive level, suppressing bacterial infection [21]. Other trisulfas (sulfacetamide, sulfadiazine, sulfathiazole), sodium sulfathiazole, and potentiated sulfonamides such as sulfamethoxazole + trimethoprim. Sulfathiazole sodium effectively suppresses AFB and further, antibiotics can be alternated using sulfonamides 1 year and oxytetracycline the next [41].

Another control method involves shaking bees off frames, destroying infected broods, and requeening with healthy queens to reduce the prevalence [40]. Proper disposal of infected material, including burning infected combs and equipment, is essential to prevent spore dissemination [39]. Implementing good apiary management practices, such as maintaining proper hive spacing, promoting ventilation, and reducing stressors, can help minimize the incidence of pathogens [39]. Beekeepers should also conduct regular surveillance for AFB symptoms and promptly report suspected cases to authorities to prevent further disease spread [21]. Adhering to biosecurity measures, including hive hygiene and equipment sterilization, is crucial for preventing AFB transmission [42]. Education and training programs for beekeepers on AFB prevention and control play a significant role in disease management [40]. Furthermore, the establishment of legislation and regulations pertaining to AFB control can support coordinated efforts at a national level [21].

2. Fungal diseases in honeybees

2.1 Chalk brood

Among the main pathogenic agents that affect bee colonies is the *Ascosphaera apis* fungus, which has spread in several countries worldwide causing ascospherosis, also known as chalk disease [43]. *A. apis* infection is an invasive mycosis that affects developing larvae [44]. It is generated when the larvae ingest the spores of the fungus with their food. The hyphae grow in the midgut, reaching the surface after sealing the cells, generating a microaerophilic condition, which allows the growth of fungi, later producing mummified corpses with the appearance of chalk [44, 45].

The fungus *A. apis* exclusively infect brood larvae and not adult bees [46]. Unlike other entomopathogenic fungi that can enter insects through their cuticles, the spores of *A. apis* cannot germinate on the larval cuticle and instead rely on the larvae ingesting the spores along with their food [44, 47]. Once the spores of *A. apis* are ingested, they require high concentrations of CO_2 to initiate germination, which occurs in the anaerobic environment of the larval intestine where CO_2 is produced by the larval tissues [48]. The optimal temperature for germination is 35°C [49]. Upon activation, the spores swell and develop germ tubes that grow into dichotomous hyphae, these hyphae penetrate the peritrophic membrane of the larvae and extend into the body cavity, eventually reaching the posterior end and breaching the barrier. As a result, spore cysts are formed. The infectious units that cause chalk disease are the ascospores, which form within spore balls localized in resistant cysts. The spores consist of two nuclei, with the larger one located at the center and the smaller one near the tip of the spore [49]. The spore wall is composed of three layers and primarily contains chitin, which contributes to the long-term viability of ascospores [46, 49].

2.1.1 Symptoms

One of the initial clinical signs of chalk disease is the presence of dead larvae that are covered in white fungal growth and usually appear swollen [49]. Over time, these larvae shrink and change color to black, gray, or white, depending on the presence of reproductive structures [50]. As the fungal development cycle progresses, the infected larvae become mummified [50]. Chalk disease can be easily identified by observing mummified bee brood, commonly referred to as plaster brood or plaster mummies, on the bottom board of beehives and in exposed cells [49]. However, it is difficult to detect low-level infestations of this disease (less than 12% infection) because worker bees eliminate the brood of infected bees (**Figures 3** and **4**) [51].

2.1.2 Diagnosis

Various methods have been developed to detect and confirm chalkbrood infection, including both traditional and molecular approaches.

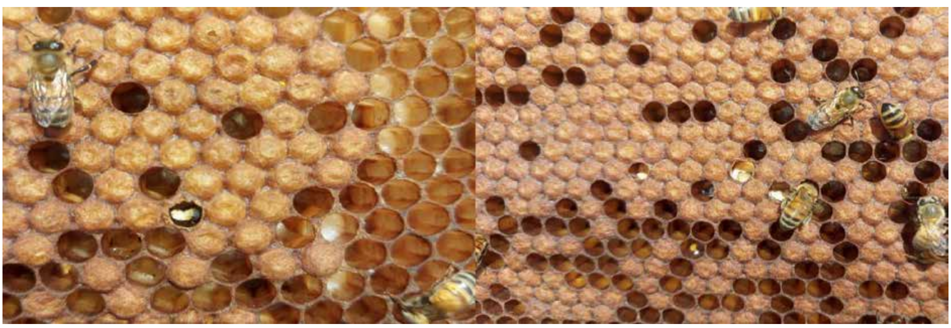

Figure 3.
White fungal growth and usually appear swollen [49]. Over time, these larvae shrink and change color to black, gray, or white, [50].

Figure 4.
Mummified bee brood, commonly referred to as plaster brood or plaster mummies, on the bottom board of beehives [49].

Traditional diagnosis involves visual inspection of infected broods, which appear as mummified larvae covered with a white down of mold [49]. However, visual examination alone may not provide definitive results, necessitating the use of molecular techniques for accurate identification of pathogens.

Polymerase chain reaction (PCR) and DNA sequencing detect and identify the presence of the fungal pathogen *A. apis*, which causes chalkbrood disease. PCR-based techniques target specific regions of the pathogen's DNA, such as the internal transcribed spacer (ITS) region, to amplify and detect its presence [52]. Additionally, real-time quantitative PCR (qPCR) has been employed for rapid and quantitative detection of *A. apis* in honeybee samples [53]. This method allows for the quantification of fungal load in infected colonies [54]. Furthermore, molecular techniques have facilitated the development of specific primers and probes to differentiate *A. apis* from other related fungal species, ensuring accurate identification [55].

In recent years, next-generation sequencing (NGS) technologies have been utilized for comprehensive analysis of the honeybee microbiome, including the identification of pathogens like *A. apis* [54].

2.1.3 Control methods

Controlling chalkbrood disease in honeybees is crucial for maintaining colony health and productivity. Various strategies have been employed to manage and mitigate the impact of this disease. These strategies include promoting hygienic behavior among bees to detect and remove infected brood, timely removal and destruction of infected brood frames, providing a balanced [22], and nutritious diet to boost the bees' immune system, ensuring proper hive ventilation and moisture control [23], breeding honeybee colonies for chalkbrood resistance, cautious use of fungicides such as oxytetracycline [56]. Maintaining optimal hive temperatures around 35°C can help suppress the disease [49], and practicing apiary sanitation to prevent the buildup and spore transmission [25].

Strengthening weak colonies [23], and educating beekeepers about chalkbrood disease [25]. Exploring biological control agents [57], implementing integrated pest management approaches, regular quarantine and hive inspection, and supporting ongoing research and monitoring efforts to enhance disease management strategies [22, 25]. Stimulating the feeding of colonies, with energetic liquid food and protein food, is also an important action, increasing the number of healthy bees and reducing colony stress [43, 58].

2.2 Nosemosis

2.2.1 Microsporidian of honey bee

Nosemosis is an adult honeybee disorder, that infects the epithelial cells of the intestines of adult bees [59], causing the integrity of the intestine to be destroyed and the digestive system to be disturbed [60], this caused a series of abnormal remarkable in worker bees [61]. Accelerate the development of bee behavior polymorphisms, that is, the early appearance of adult bee behavior [62], decreased ability to feed larvae [63], shortening life [64], and the increase in overwintering mortality [65]. Resulting in reduced bee colony productivity, fecundity, and viability [66]. The geographic environment will have an impact on the evolution of the pathogen [67].

2.2.1.1 Nosema apis

Nosemosis A is a disease caused by *N. apis* [68]. The high incidence of *N. apis* is from the autumn months, being during the winter as a result of increased colony mortality or collapse [69, 70]. It frequently shows apparent signs of the disease, caused by *N. apis* such as brown fecal stains with a peculiar acid odor observed on the frames and at the entrance of the hive [71]. As a result of high mortally and weak bees at the entrance of hives or swallow's abdomens [72].

2.2.1.2 Nosema ceranae

Nosemosis C is a disease caused by *N. ceranae* [73], it is present throughout the year and in the absence of clinical signs related to infection by *N. apis* such as fecal deposits or a dilated abdomen [74]. *N. ceranae* high virulent pathogen as compared to *N. apis* [75].

N. ceranae effect cellular defense mechanisms, and nutritional metabolism [76]. Expression of the hormone vitellogenin decreases [61], which participates in the synthesis of royal jelly [77], promotes immunity, acts on the response to stress and longevity of the bee [78].

2.2.2 Effects on colonies

Both type A and type C nosemosis have been shown to be extremely damaging to honey bee colonies [79, 80]. The most evident appearances of type A nosemosis are the unusual presence of feces and dead bees around infected hives, the increase in the size of the abdomen of the affected bees, and its seasonality, with infection peaks in spring and autumn [81]. Type C nosemosis is considered asymptomatic, at least during a long initial incubation period of the infection which, together with the imbalance of polytheism that it causes in bees, accelerates the onset of the hive. At early ages, have favored its relationship with the decline of bee hives [79, 80, 82, 83] and in particular with Colony Collapse Disorder (CCD), in different regions of the world [83–85].

The different pathogenicity of nosemosis has been related to the host response to the spore load administered to bees in experimental infections. Normally, the higher the dose of infection, the greater the effects on fecundity and host survival [83]. This phenomenon has been confirmed in experimental infections by *N. apis* and *N. ceranae*, where bees infected with higher doses presented higher mortality and higher sugar consumption [86].

There is clear evidence that *N. ceranae* affects various aspects of individual physiology (metabolism and immune response), morphology, and behavior of infected bees [61, 87–89]. For all these reasons, the infection unfavorably affects the viability and survival of the bees, and compromises the health of the hives, decreasing their productivity (**Figure 5**) [65, 90].

2.2.3 Diagnosis

For diagnosis, the Adult foraging bees are collected from the entrance of the hive and avoid the internal bee which is probably less infected by spores [71].

Figure 5.
Dysentery at the entrance of Nosema-infected bee hive.

2.2.3.1 Microscopic examination

Perform a microscopic examination of the midgut of the bee, generally infected bees have lighter and whitish color midgut [91], while uninfected bees are dark and dull midgut [71].

For the conformation of infected and uninfected colonies under microscopy, at least 30 bees are collected, and macerate of the midgut in 15 ml of water, three drops of the suspension are placed on a slide under a coverslip and examined at 400x magnification under a bright field or phase contrast microscope [71, 92]. Spores are dark outlines and oval-shaped spores and it is hard to differentiate the species under a light microscope [71, 93].

2.2.3.2 Molecular biology techniques

In Different molecular methods the Multiplex PCR as described by Martín-Hernández et al. [94] is used by small subunit 16S rRNA genes, with control of mitotic gene (COI) in a single reaction [95]. Further, quantitative PCR has been used for identification and also to quantify the degree of infected samples by Nosema [96], additionally, RT-PCR has been used for the number of infected cells quantification [71].

Furthermore, Aronstein et al. [97] developed a cost-effective ELISA technique for identifying *N. ceranae*, reducing the need for PCR. Characterization of monoclonal antibodies (mAc) against *N. ceranae* and *N. apis*, enabling the development of a rapid and sensitive indirect immunofluorescence (IFI) technique for the identification of both species in infected bees samples [98]. Ptaszynska et al. [99] introduced a loop-mediated isothermal amplification (LAMP) technique for distinguishing *N. apis* from *N. ceranae*. The method utilizes multiple pairs of primers, increasing sensitivity compared to conventional PCR by specifically targeting the DNA sequence of interest.

2.2.4 Control

Fumagillin was the most widely used active product to control nosemosis, supplied in a mixture of sugar and water [71, 100, 101]. In 1949, fumagillin; from (*Aspergillus* *fumigates*) has been used to treat *N. apis*, and was later used to treat *N. ceranae* [102]. Recent studies show that fumidil-b (antibiotic) is effective temporarily against *N. ceranae* [103]. Further, fumagillin is toxic to bee-caused alterations in the ultrastructure of the hypopharyngeal glands of bees [102].

As an effective method, the replacement of the queen each year achieves a high survival ability for hives and the production of honey [104]. The application of natural compounds (phenolics and organic acid) is one of the best chemotherapeutics against nosema, further, tannic acid, toltrazuril, and resveratrol reduce the infection of *N. ceranae* [105], Porphyrins (aromatic compound) used in treatment to control Nosemosis [106].

2.2.4.1 Natural product

Natural substances, such as oxalic acid and formic acids, are mainly used to control varroa mites [107], and also reduce the parasite load of bees infected by *N. ceranae* [108]. The use of caffeine, gallic acid, and kaempferol supplement diets increased the survival rate of *N. ceranae*-infected bees [109].

Feeding with thymol a decrease in the parasite load in bees infected with *N. ceranae* compared to controls [110]. Porrini et al. [111] observed a decrease in spore counts in bees infected with *N. ceranae* that were administered syrups supplemented with plant extracts of *Artemisia absinthium*, *Allium sativum*, and *Ilex paraguariensis*, obtaining significant results with *L. nobilis* where one of the major components is thymol. Damiani et al. [112], similarly observed similar results against *N. ceranae* in the application of extracts of *L. nobilis*.

2.2.4.2 RNA interference

RNA inferences (RNAi) are post-transcriptional gene silencing techniques that develop for the controlling of *N. ceranae* and significantly decreased the parasite load percentage of nosema spores [113]. RNAi from Nosema spp. genes that code for ATP/ADP transporter proteins, for the polar tube protein 3 (PTP3), and for a cuticle protein (nkd) of the insect [113, 114]. The microRNAs (miRNA) expressed by bees infected with the microsporidium *N. ceranae*, by ultra-sequencing, suggest that there is a differential expression of these miRNAs in response to infection [115]. These RNAi reduced the spore load in the host and increased the immune response of the bees [116]. Other works target genes from the bee itself that are essential for the development of the pathogen so that its silencing is capable of effectively reducing its proliferation without affecting the host cell itself [117].

3. Viral disease of honeybee

Single-stand RNA viruses are highly infected pathogens of honeybees [118] about 20 bee-identified viruses, that is, Sac-brood virus (SBV), Deformed-wing virus (DWV), Kashmir-bee virus (KBV), Israeli-acute-paralysis virus (IAPV), Chronic-bee-paralysis virus (CBPV), Acute-bee-paralysis virus (ABPV), and Black-queen cell virus (BQCV) [119–122], and KBV, IAPV, and ABPV complex viruses reported last year caused several colonies losses and bee mortality [123–125].

Due to the great diversity of virus species, aspects such as pathogenicity, virulence, and impacts (at a social and individual level) on honey bees are not constant traits, nor are they independent of host conditions. For example, the DWV and VDV-1 viruses are part of the same viral complex with DWV type A and DWV type B, respectively [126], both viruses cause abnormal conditions on the wings and abdomens of adult bees of any caste, leading to early mortality [127, 128]. On the other hand, the BQCV virus can infect any caste of honey bees, but they are only pathogenic for queen larvae, causing sterility [129]. Through these examples, we can visualize how the susceptibility of the different strains to viruses depends directly on the species of the virus.

3.1 Symptoms

IAPV infect bees having the following symptoms as paralyzed bee, decrease flying ability, crawling, changing in orientation, and shaking of wings [130].

ABPV caused loss of hair from the bee thorax, and paralysis [123].

KBV declined bee population [131].

CBPV can paralyze bees, No fling ability, loss of hair from the abdomen, trembling, and the bee to black in color [132].

DWV as the name indicates the deformed bee wings, shortened abdomens, and hypoplastic glands (**Figures 6** and 7) [133–135].

3.2 Transmission of viruses

Viruses in honeybees can be transmitted through various pathways, contributing to their spread within colonies. Vertical transmission occurs when infected queen bees pass on the virus to their offspring through infected eggs or sperm [136]. Horizontal transmission, on the other hand, involves the spread of viruses between individual bees within the colony through direct contacts, such as grooming or feeding activities [136]. Varroa mites (*Varroa destructor*) play a significant role as vectors in virus transmission. As these mites feed on honeybees' hemolymph, they can introduce and transmit viruses directly into the bee's body, facilitating their dissemination within the colony [137].

Contaminated food and water sources within the hive can also serve as vehicles for virus transmission. Bees may consume infected pollen, nectar, or honey, resulting in the ingestion and subsequent spread of viruses [138]. Robbing behavior, where bees

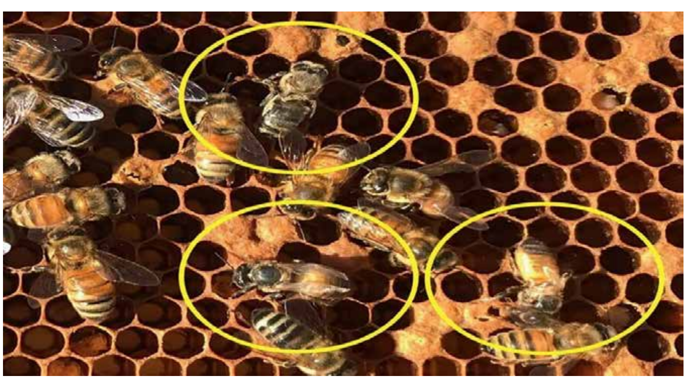

Figure 6.
The deformed bee wings, shortened abdomens, and zero fling ability [133–135].

Figure 7.
IAPV infect bees are paralyzed bee, decrease flying ability, crawling, changing in orientation, and shaking of wings, and CBPV can paralyze bees, loss of hair from the abdomen, trembling, and the bee to black in color [130, 132].

from different colonies steal resources from each other, can contribute to the transmission of viruses. Infected bees involved in robbing activities can introduce viruses into previously healthy colonies, thus spreading the infection [137]. Furthermore, certain beekeeping practices can inadvertently facilitate virus transmission. The use of contaminated equipment or the sharing of beekeeping tools between infected and healthy colonies can lead to the transmission of viruses [139].

Bee-to-bee transmission through direct contact is another important pathway for virus dissemination. Bees in close proximity can transmit viruses to each other, such as when Nosema-infected bees pass viruses to healthy bees through mutual grooming behaviors [140]. Pollination activities involving honeybees can contribute to virus transmission between different colonies. Bees may carry viruses on their bodies or in their honey stomachs, transferring them to flowers and subsequently infecting other colonies during pollination [139]. Fecal-oral transmission also plays a role, as viruses present in infected bees' feces can contaminate hive surfaces and resources. Healthy bees can then come into contact with these contaminated surfaces or ingest the infected feces, leading to virus transmission [137]. Additionally, environmental factors, including temperature and humidity, can influence the survival and transmission of viruses in honeybees. Higher temperatures and increased humidity can enhance viral replication and increase the likelihood of virus transmission [141].

3.3 Diagnosis

Various methods are used to identify and detect viral pathogens in honeybee samples. Molecular techniques such as (PCR), Reverse Transcription PCR (RT-PCR), and Quantitative PCR (qPCR) are widely used for their ability to detect and quantify specific viral genetic material [142, 143]. These methods enable the identification of RNA viruses and provide valuable information on virus levels within honeybee populations [144].

Immunological assays like Enzyme-Linked Immunosorbent Assay (ELISA) are used to detect the presence of viral antigens or antibodies, indicating the presence of viral infection in honeybee samples [145]. Dot blot assays involve the immobilization of viral genetic material or antigens on a membrane, allowing specific probes to detect the presence of viral infection [146]. Western blotting, on the other hand, is a technique that detects and identifies specific viral proteins in honeybee samples, providing valuable information on viral infections [147].

Advanced technologies such as Next-Generation Sequencing (NGS) and metagenomics offer comprehensive approaches to identifying known and novel viral pathogens in honeybee samples [148, 149]. These methods involve high-throughput sequencing of the entire DNA or RNA content, enabling the detection and characterization of viral pathogens present in the sample. Viral microarrays utilize DNA or RNA probes immobilized on a solid support to simultaneously detect multiple viruses, providing a comprehensive viral profile [147].

Microscopy techniques such as Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) allow for the direct visualization of viral particles, aiding in the identification of viral infections [150, 151]. In situ hybridization utilizes labeled probes to detect and localize viral genetic material within honeybee tissues, providing visual confirmation of viral infections [152]. These techniques enhance the accuracy of viral diagnosis and aid in understanding the spatial distribution of viruses within honeybee samples.

Other diagnostic approaches include Multiplex PCR, which enables the simultaneous detection of multiple viral pathogens, providing a cost-effective and efficient method [153]. High-Resolution Melting Analysis (HRM) differentiates between viral species or strains using PCR and melting curve analysis [144]. Loop-Mediated Isothermal Amplification (LAMP) is an isothermal amplification technique that allows for the rapid detection of viral RNA or DNA in honeybee samples, providing a simple and cost-effective diagnostic approach [153]. Additionally, serology methods detect specific antibodies produced by honeybees in response to viral infections, providing indirect evidence of viral exposure or current infection [154].

3.4 Control

Firstly, maintaining hive hygiene through regular cleaning, sterilization of equipment, and removal of infected brood and contaminated comb can limit the spread of viruses [140]. Secondly, effective varroa mite management, using integrated pest management strategies and mite-resistant honeybee stocks, is essential for reducing viral transmission [155]. Additionally, genetic selection for honeybees with traits associated with resistance to specific viruses has shown promise in disease control [156].

Biosecurity measures, including preventing the introduction of infected bees or equipment, and quarantine protocols for newly acquired colonies or queens, can minimize the risk of viral transmission [137, 156]. Regular monitoring of honeybee colonies for viral pathogens using techniques like PCR or ELISA allows for early detection and timely intervention [157]. Vaccination strategies targeting specific viruses are being researched as a potential means of disease prevention and control [141].

Reducing stress factors on honeybee colonies, such as providing adequate nutrition, minimizing pesticide exposure, and ensuring proper ventilation, can enhance their immune response and resilience to viral infections [137]. Selecting honeybee strains with enhanced hygienic behavior, which remove diseased brood, can reduce the viral load and prevent infection spread [22]. Maintaining high-quality queen bees by regularly replacing aging or diseased queens can improve colony resistance to viral infections [155]. Integrated pest management (IPM) strategies that combine various control methods, such as monitoring, cultural practices, biological controls, and targeted chemical treatments, can also help reduce the impact of viral infections [158].

Providing honeybees with a diverse and nutritious diet through pollen substitutes or access to diverse forage sources boosts their immune system and resistance to viral infections [159]. Educating beekeepers on best management practices, disease identification, and prevention strategies is crucial for effective viral infection control [158]. Selecting suitable apiary locations away from potential sources of viral contamination, ensuring strong and populous colonies, establishing treatment thresholds for varroa mite management, regularly sterilizing beekeeping equipment, replacing colonies with high viral loads, and promoting collaboration and research among beekeepers, researchers, and government agencies are additional measures that contribute to viral infection control [158, 159].

4. Conclusion

In conclusion, honeybees face significant threats from bacterial, fungal, and viral diseases. American Foulbrood (AFB) and European Foulbrood (EFB) are bacterial diseases that result in larval death and hive contamination. Chalkbrood, caused by a fungus, transforms larvae into chalk-like mummies. Nosemosis, caused by two spore-forming parasites, affects the midgut of adult honeybees. Viral diseases like Deformed Wing Virus (DWV), Israeli Acute Paralysis Virus (IAPV), and Chronic Bee Paralysis Virus (CBPV) weaken colonies, causing deformities, premature death, and paralysis. The transmission of these diseases is facilitated by external factors such as Varroa destructor mites, which also act as viral vectors. Beekeepers employ various strategies like Integrated Pest Management (IPM), genetic selection for resistance, antibiotic treatments, and hive maintenance to manage these diseases. However, continued research, monitoring, and education are essential for effective prevention and control, ensuring the preservation of honeybee populations and the critical ecosystem services they provide.

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Chapter 6

Biological Control of Honey Bee Diseases and Pests

Mehtap Usta

Abstract

Beekeeping makes significant contributions to both the agricultural economy and crop production through pollination. Protecting the health of honey bees is of critical importance. It is evaluated that in an environment without bees, crop production may decrease by 47%. Many factors in the beekeeping sector negatively affect honey production. Among these reasons, microorganism-induced diseases as well as organism-induced diseases and hazards are at the forefront. Various strategies are used to protect the health of honey bees. However, pests and diseases are still not prevented. The most important of these are chemicals due to their widespread use. These products jeopardize both bee health and bee product quality. Methods using biological materials, which are more environmentally friendly than chemical control, should be preferred. Among these methods, biological control method stands out. As a result, the use of biological products as an alternative is critical for both the health of the organisms and the elimination of residues. The use of microorganisms and their products as biological control agents in the protection of bee health will be an important step in this regard.

Keywords: biological control, honey bee, honey bee diseases, honey bee health, microbiology

1. Introduction

Honey bee growth stages can provide an ideal setting for a variety of disease causes and pests. For this reason, many pathogens and pests can cause disease in honey bees [1]. However, due to rapid global movement, commerce of bees, bee products, and beekeeping supplies between continents and nations, bee illnesses quickly spread to all countries [2]. Similarly, the rapid spread of diseases and pests within the country is an important factor in migratory beekeeping [3]. One of the most significant challenges delaying the growth of beekeeping and restricting production efficiency in Turkey is honey bee illnesses and pests [4]. Bee illnesses generate major losses in Turkish beekeeping, and it is impossible to establish if medications are used on purpose. Furthermore, the environmental damage caused by the usage of chemical pesticides is enormous. As a result, new ecologically friendly solutions for bee illnesses and pests should be employed and developed [5]. Bee illnesses are classed as adult or brood diseases based on the source of the disease (bacterial, fungal, viral, parasitic, or protozoan) or the host where the disease occurs (adult or larva).

2. Honey bee diseases

2.1 Bacterial diseases

American foulbrood is a deadly and widespread brood disease in honey bee larvae that causes them to die and stink. The disease is caused by a spore-forming bacteria called *Paenibacillus larvae subsp. larvae*. Dr. GF White was the first to identify it in 1906 [6]. *Paenibacillus larvae subsp. larvae* is a gram-positive bacteria that causes no illness in adult bees since its spores are harmful to the larvae. Bacterial spores delivered to larvae through food cause illness [7]. It is easily disseminated and can cause considerable losses in bees because of bacterial transmission. The infected honeycomb sample delivered from Pınarhisar area of Kırklareli in 1947 was the first official record and definite diagnosis of the illness in Turkey. A detailed investigation done in Turkey in 1991 discovered that this condition is seen in nearly every location [1]. The safest and most successful way to tackle this illness, which must be notified in Turkey, is to fully burn the sick colonies [3]. Aside from that, no biological control agent has been produced. However, research into this condition continues [8–10].

European foulbrood is another foulbrood disease that occurs across the world and also in Turkey except in New Zealand. This name was given because the first studies on the disease were carried out in Europe [11]. The causative agent of the disease is a gram (+) bacterium called *Melissococcus pluton*, which does not form spores [12, 13]. It is accompanied by certain secondary microorganisms. *Paenibacillus alvei*, *Bacillus laterosporus*, *Achromobacter euridice*, *Enterococcus faecalis*, and *Enterococcus faecium* are the strains [7, 13]. The harmful bacteria enter the digestive tract of bee larvae via the food delivered by the feeder bees. After the pup enters the pupal stage, bacteria that settle in the larva's digestive tract mature in the gut, and the disease agent is discharged into the honeycomb with excrement. While the worker bees clear these leftovers from the comb cells, they spread the sickness to the healthy larvae. This illness has no effect on adult bees that are carriers [14, 15]. The first line of defense against the illness is to keep the hives healthy. Because the illness is most damaging in weak hives [16]. Because the appearance of European foulbrood is directly related to colony stress, behaviors that may cause stress in the colonies should be avoided [11]. In the battle against this disease, the tactic of destruction is also applied. A product for use as a biological control approach has yet to be created.

Septicemia is a disease of adult honey bees caused by the bacterium *Pseudomonas aeruginosa* (=*Pseudomonas apiseptica*). *Pseudomonas apiseptica* is a gram (−) and non-spore forming bacterium [17]. In nature, this bacteria may be found in damp soils, plants, stagnant water, and marshes. *Pseudomonas apiseptica* causes disease by entering the bee's respiratory (tracheal) system and then spreading to the circulatory fluid in numerous ways. The disease is seen in hives with no air and high humidity [1, 14]. There are no known bee breeds or lines that are resistant to septicemia. There is currently no therapeutic approach for the condition. The illness is avoided by locating the apiary in a dry, clean, sunny location, providing the essential feedings, and reducing stressors for the bees [15].

2.2 Viral diseases

There are about 20 viruses that cause disease in adult bees. Some of these viruses are more dangerous than others, such as sacbrood virus, wingless bee virus (DWV), and chronic and acute bee paralysis virus (CBPV and ABPV) [18]. Some of these viruses cause harm because they are transmitted by mites. In the signs of viral disease, it can be shown that the bodies of the bees are hairless, shiny and oily. Furthermore, its legs and wings twitch regularly. Because the liquids in the honey stomach cannot be discharged, the honey stomach's abdomen swells. They are unable to fly since their wings have been shattered [4, 7, 19]. Precautions for viral diseases are often regarded as physical precautions. Precautions include maintaining the hives in wet areas, positioning supports 30–40 cm above the ground, and replacing the queen bee [20]. In Turkey, viral investigations are largely diagnostic; no biological preparation that may be used to treat illnesses has been created [21–24].

2.3 Fungal diseases

Chalkbrood disease is a puppy illness caused by the fungus *Ascosphaera apis* [25]. It was discovered in Turkey in 1988. According to 1989 study, the illness was found in all regions of Turkey, and according to research performed in the Southern Marmara Region, the sickness was found in 25% of the hives [26–28].

Aspergillus flavus is the most common cause of stone disease. The causative agents are sometimes *Aspergillus fumigatus* or other *Aspergillus* species. The color of *Aspergillus flavus* is yellow green, while *Aspergillus fumigatus* is gray green. These fungi are found in both soil and plants. It infects both young and mature bees. This fungus also infects other insects, animals, birds, and people [1, 29, 30]. By transporting sick combs to healthy colonies and feeding the bees tainted honey, fungal infections can be transferred to other bees. Fungal illnesses can emerge as a result of factors like as inadequate hive ventilation, excessive moisture content, and loss of bees' natural gut flora owing to antibiotic usage [17]. Because human ingestion of honey from infected hives causes carcinogenic consequences, these honeys and combs must be destroyed [4]. The best way to prevent fungal illnesses is to destroy sick bees, honeycombs, completely clean the hives, and replace the queen bee.

2.4 Protozoan diseases

Nosema disease is one of the adult bee diseases caused by the protozoan *Nosema apis* [17]. Zander originally identified *Nosema apis* spores in Germany in 1909. Except for Central Africa, it has spread practically everywhere [14]. The first report of *Nosema apis* infection in Turkey occurred in 1952, and the disease was first diagnosed in 1986 [29]. *Nosema* is a prevalent ailment in Turkey, particularly in the Marmara and Black Sea regions, and it should be addressed. The primary signs of the condition include wing separation, abdominal swelling, loss of sting reflexes, inability to fly, and crawling on the ground [31]. In the therapy, bees are given medications containing Fumagillin in addition to syrup. Preventive actions should be implemented to keep the illness under control [32].

A disease identical to *Nosema* was discovered in the eastern honey bee *Apis cerana* in 1996, giving rise to the term *Nosema ceranae*. Little is known about the impact of this disease and its progression in Asia today. Until recently, it was considered that this chemical was exclusively found in the eastern honey bee, *Apis cerana*. However, for the first time, Chinese researchers reported finding *Nosema ceranae* in the western honey bee *Apis mellifera* in Taiwan in 2005. *Nosema ceranae* was discovered in the western honey bee *Apis mellifera* in Spain. While *Nosema* losses in Spain were 10% in 2000, they quickly grew to 20%, 30%, and eventually 88% in 2004. *Nosema ceranae* was also discovered to be the cause of large-scale bee losses in Spain during the summer of 2005. Furthermore, incidents in the apiaries, such as very severe *Varroa* infestation and bees fleeing their hives totally, have been documented [33, 34].

3. Honey bee pests

Trache mite (*Acarapis woodi*) is an internal parasitic mite that lives in worker bees' respiratory tracts. It is sometimes found in queen bees and drones. Rennie initially discovered *Acarapis woodi* in England in 1921. The mite first discovered in England and Scotland moved to Europe, Australia, New Zealand, Asia, America, and South Africa. In Turkey, there is no information or research on the occurrence of tracheal mites [35]. Trache mite-infected bees exhibit symptoms similar to *Nosema*, chemical poisoning, and other diseases that induce paralysis in bees. As a result, the final diagnosis should be made after the infected bees have been checked in the laboratory [20]. Fumigant medications with the active components bromopropylate, menthol, and formic acid are used to treat the condition.

The bee mite (*Varroa destructor*) is a highly hazardous external parasite that feeds on the larvae, pupae, and adults of the honey bee (*Apis mellifera* L.), multiplying fast and causing mass bee mortality. *Apis cerana*, the Indian and Far Eastern bee, is the primary host of *Varroa*. *Apis cerana* has developed a natural defensive mechanism against *Varroa* as a result of living with the parasite for many years, and no pesticides are required to control the parasite [36]. It was discovered that *Varroa* was present in 35% of the hives in the Southern Marmara Region and 41% of the hives in Turkey [29, 30]. The optimal temperature for *Varroa* development is 34°C. The formation and propagation of *Varroa*; genetic variables, the appropriateness of colony circumstances, the amount of brood area, and the colony's *Varroa* infection rate. The sex and race of the larva on which it develops are also important in *Varroa* reproduction [37]. Because synthetic chemicals can harm human health by leaving residues in honey and beeswax, and mites develop resistance to these drugs, the use of natural licensed drugs such as formic acid, lactic acid, and oxalic acid, as well as essential oils such as thymol, has recently begun in the control of *Varroa*. The most extensively utilized biological control strategy is to feed the hive honeycombs containing drone cells. Because *Varroa* favors male brood eyes, it places its eggs in them. These frames are removed from the hive when the eyes have closed, lowering the *Varroa* population [37].

Honeycomb moths have two species, one giant *Galleria mellonella* and one tiny *Achroia grisella*, and they frequently do severe damage in weak colonies. The giant honeycomb moth is the more dangerous of the two. The adult honeycomb moth, which is only dangerous during the larval stage of its life, lives in the bush [3]. In the world and also in Turkey, various chemical substances (paradichlorobenzene, ethylene dibromide, sulfur dioxide, acetic acid, calcium cyanide, methylbromide, etc.), physical applications (heating, cooling), and biological applications (*Bacillus thuringiensis* bacteria) are used in pest control studies [26]. The addition of the bacterium *Bacillus thuringiensis*, which is applied as a biological control, to the basic honeycombs is applied in different countries and this application is not yet done in Turkey.

Bee diseases and pests are one of the most serious issues impeding the growth of beekeeping. As a result, beekeepers must be knowledgeable with the signs and features of the most prevalent parasites and illnesses in bees, as well as the ways for battling them. Unconscious and incorrect procedures will result in economic losses as well as the spread of the illness to healthy colonies. Care should be made to combat infections in a timely and effective way. It should not be forgotten that any chemically utilized substance will harm human health by leaving residues in honey and beeswax [1–3].

4. Biological control

Biological control is the use of other living creatures to lower pest numbers rather than chemicals to reduce pest populations. It is the labor done to maintain pest populations beneath the economic harm threshold by employing organisms that live in farmed plants and control pests and weeds [27]. Harry Scott Smith coined the phrase "biological control" during a 1919 conference of the Pacific Slope Branch of the American Association of Economic Entomologists in Scottford, California. Biological control methods as we know them now first appeared in the 1870s. During this decade, Missouri State Entomologist C. V. Riley and Illinois State Entomologist W. LeBaron pioneered the use of parasitoids for crop pest management in the United States. Charles V. Riley sent the first international shipment of an insect as a biological control agent to France in 1873 to aid in the fight against the predatory mite "*Tyroglyphus phylloxera*," the "vine phylloxera" (*Daktulosphaira vitifoliae*), which is damaging grapevines in France. Following the founding of the Department of Entomology in 1881, the United States Department of Agriculture (USDA) began research in the subject of classical biological management [28].

This section will discuss the use of microorganisms, in addition to the use of several biological control approaches.

Biological control bacteria infect insects through their digestive systems. *Bacillus thuringiensis* is a soil-borne bacteria that is employed against Lepidoptera (moths and butterflies), Coleoptera (insects), and Diptera (flies). Farmers get the bacteria in dry or packaged form, which is combined with water and sprayed onto sensitive plants such as fruit trees. Some *Bacillus thuringiensis* bacterial genes have been inserted into some GMOs, causing the plant to manufacture bacterium toxins, which are a form of protein. These repel insect pests, reducing the need for pesticides. If pests develop resistance to these crops, *Bacillus thuringiensis* will become obsolete in organic farming. The bacteria *Paenibacillus popilliae*, which causes white spot illness, has been discovered to be useful in the management of the Japanese beetle by destroying its larvae. It is a host-specific bacteria that causes no damage to vertebrates or other invertebrates [38, 39].

Baculoviridae viruses are species-specific and have been found to be beneficial in biological control. *Lymantria dispar* multicapsid nuclear polyhedrosis virus, for example, has been used to spray extensive woods in North America where *Lymantria dispar dispar* (gypsy moth) larvae cause significant defoliation. The viruses it consumes kill the moth, and its rotting stems shed viral particles on the leaves, infecting additional larvae [40, 41]. According to the literature, Baculoviruses used in biological control have not been used in terms of honey bee health [42].

In terms of bee health, fungus, bacteria and bacterial products are mostly used. The use of biological control agents; predators, parasitoids or pathogens to control pests can be considered as suitable options. Biocontrol agents are expected to manage the population of bee pests without causing harmful effects on honey bees and without contaminating valuable bee products.

Fungus applications have been used more especially in mite control. In this context, *Metarhizium anisopliae* and *Beauveria bassiana* are the fungal species used in the literature [43–45]. Applications with the fungus were tested both in the laboratory and in the field. While successful results were obtained in the laboratory (85–100%), the results in the field were not very favorable due to the negative effect of conidia and the effect of different external conditions. In the laboratory, the day of death varies between 5 and 10 days depending on the type of fungus [44, 46, 47].

In honey bee pests, wax moth (*Galleria mellonella*) is the biggest pest. There are not many studies using microorganisms or their products in the control of wax moth [48]. In one of the nine different strains used in studies with *Bacillus thuringiensis* in the literature, after 3 days 50–83.3% success was achieved. Studies on these bacteria and their products are still ongoing. In addition, other entomopathogenic microorganisms (fungi, nematodes, etc.) have been tried on the wax moth, but no results have been obtained as an effective agent [49–53].

As a result of the study with *Steinernema riobrave* and *Heterorhabditis indica* nematode species, 76–94% results were obtained in 19 weeks. Although the rate seems to be high, it is not very suitable for effective use because the application time is too long [54].

Although even 100% results are obtained in laboratory application, the same efficiency cannot be obtained in field application. However, for field application, both the use of nematodes and the use of fungi need to be further developed [42, 55, 56].

5. Harmful effects of pesticides on bees

Many research have been conducted on the impact of pesticides on bees. Some chemicals have been found to create aberrations in bee communication. For example, it was discovered that when a deadly amount of parathion was administered to bees, the bees made errors in determining the direction and distance to the location of the nutrients [57]. Gels et al. investigated the effects of lawn pesticides on the bumblebee *Bombus impatiens* Cresson. The study discovered that after applying Imidacloprid in the form of granules and sprays, it is innocuous to bees in the event of irrigation, but has a detrimental effect on colony viability in the absence of irrigation [58]. Many research have identified the harmful effects of neonicotinoid group medications on honey bees, although the effect of low dosages of these chemicals on bee behavior is not entirely known, according to El Hassani et al. [59]. Under controlled laboratory circumstances, researchers administered acetamiprid and thiamethoxam orally and topically to bees and discovered that acetamiprid was more effective on bee movements than thiamethoxam, although this impact was not different from the control group [59]. According to Johnson et al. [60], pesticides reduce honey bee products, particularly wax production. Researchers also discovered that a combination of chemicals, rather than a single pesticide, had an impact on honeybee health [60]. Doğaroğlu claimed that Turkey holds an important place in the world of beekeeping due to its diverse ecological circumstances and honey bee races and ecotypes, and that our local bee breeds should be safeguarded [61]. The effects of thiametoxam, a popular pesticide in Turkey, on wasps (*Vespa* sp.) were studied. According to the findings of the study, the prescribed amount and diluted concentrations of the pesticide diminish the life span of wasps and kill bees [61].

6. Biological control for harmful insects and diseases

Bacteria, fungi, viruses, protozoa, and nematodes are examples of entomopathogens utilized in biological insect control. Protozoa and nematodes are investigated in independent categories with their own names in certain publications. However, only a small number of them are utilized in pest control [62]. Entomopathogens are naturally occurring pathogens that attack, infect, and occasionally kill insects. Many entomopathogens are mass-produced and sold as "biological insecticides." *Bacillus thuringiensis* is one of the most well-known, and it has been employed successfully against a wide range of insect species. Entomopathogens are often administered by combining them with common spraying instruments or irrigation water. Because these commercially generated entomopathogens are usually species-specific, they may be utilized safely in biological management. Unfortunately, these preparations account for just around 2–5% of the global pharmaceutical industry [63]. Studies on this topic are ongoing, and it has been discovered that numerous entomopathogenic species provide promising results in biological control. On the other hand, it should be attempted to boost their efficiency by developing ideal microhabitats for naturally occurring entomopathogens.

7. Conclusion

Pollinators, especially honey bees, are in many respects almost keystone species for the ecosystem, but pesticides, whose use has increased significantly in recent years, have become a threat to honey bees and other pollinators. The toxicity of pesticides on honey bees has been scientifically proven by laboratory research. Under natural conditions, honey bees are exposed to the synergistic effect of multiple pesticides rather than a single pesticide as in laboratory studies. As a result, although it is not possible to compare the findings obtained under laboratory conditions with those obtained under natural conditions, it is sufficient to grasp the current situation. Predators, parasitoids and/or microbial biopesticides have the potential to be used against honey bee pests.

In general, these biocontrol agents are better applied within well-planned integrated pest management programmes to control pests than used alone.

The development of more specific and effective biocontrol agents is particularly will be even more needed in situations such as climate change, which can greatly affect honey bees.

In addition, the microorganisms and their products to be used for cost-effective honey bee pest control are of constant importance. Furthermore, the correct application methods of microbial products need to be further developed to ensure the sustainable release and thus long-term use of these microorganisms.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 7

Propolis in Dentistry

José Eduardo Orellana Centeno
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Abstract

Propolis is a product derived from bees and consists of a dark coloured sticky material, which is collected from various plants and mixed with wax. Propolis is found coating a thin layer of this substance on the inner walls of their hives in order to repair or protect the hive from invaders. In dentistry, consideration should be given to the large number of medicinal plants and other natural products, including those from the animal kingdom such as propolis, which can make an important contribution to the area of health and be willing to scientifically study their therapeutic properties, so that after verifying their value, they can be introduced more constantly in dental treatments and in some cases replaced definitively due to their properties, especially their biocompatibility, with the aim of massively increasing their application.

Keywords: propolis, dentistry, medicinal plants, public health, alternative medicine

1. Introduction

Propolis is a product derived from bees and consists of a dark coloured sticky material, which is collected from various plants and mixed with wax. Propolis is found coating a thin layer of this substance on the inner walls of their hives in order to repair or protect the hive from invaders.

2. Propolis in dentistry

Dentistry is a branch of health sciences that deals with the study, diagnosis, treatment and prevention of diseases affecting the stomatognathic apparatus composed of the teeth, oral cavity, jaws, muscles, covering tissues (skin and mucous membranes), vessels and nerves of this part of the body. The most common conditions of dental patients are dental caries and periodontal diseases. The pain, inflammation and infection that accompany these pathologies have been studied and treated by stomatologists throughout human history [1].

Propolis is a product derived from bees and consists of a dark coloured sticky material, which is collected from various plants and mixed with wax. Propolis is found coating a thin layer of this substance on the inner walls of their hives in order to repair or protect the hive from invaders; it is an embalming substance, which is responsible for the low incidence of bacteria inside the hive [2].

Propolis is a substance that has the advantage of being a natural and inexpensive product, which makes it available to everyone. Despite this, there is little evidence on the use of propolis in various oral conditions. Due to the scarcity of information on propolis in the different dental disciplines, the aim of this study was to conduct a literature review on the properties and uses of propolis in dentistry [3].

The mechanism of the antimicrobial activity of propolis is complex and can be attributed to synergism between some of its compounds, such as flavonoids, aromatic acids, fatty acids, esters, hydroxy acids, sesquiterpenes and other phenolic compounds present in its composition. In general, the antimicrobial activity of this compound is more active against Gram-positive bacteria than against Gram-negative bacteria; however, its inhibitory character has been demonstrated against Gram-negative oral microorganisms involved in cariogenic and periodontopathogenic processes such as *Streptococcus mutans*, *Prevotella intermedia/Prevotella nigrescens*, *Porphyromonas gingivalis*, and even yeasts such as *Candida albicans* [3]. Its anti-inflammatory potential has been attributed to its ability to stimulate cellular immunity by promoting phagocytic activity and inhibiting the synthesis of prostanglandins, which mediate this process [4].

The chemical composition of propolis is extremely complex and is not fully known because it depends on the flora of the region where it is collected; this influences the way it is used within the hive as it can serve as an embalming substance or as a coating for the hive. This means that different parts of the hive will have different propolis composition, so it will be very difficult to find two hives producing identical propolis even if they are located in the same geographical area, as they elaborate it according to their needs and available raw material sources [5].

A higher percentage of phenolic compounds have been found in the propolis that coats the combs than in the propolis intended to reduce the entry of foreign agents into the hive. More than 160 compounds have also been identified in propolis, 50% of them phenolics, to which pharmacological action has been attributed [6].

The differences in composition are mainly determined by the flora of the ecological area, the evolutionary cycles of the resin-supplying plants that condition changes in the concentrations of these, micro-organisms present in the geographical environment, climatological factors, as well as the macroscopic and organoleptic characteristics of the propolis and the technique used to obtain it. However, qualitatively speaking, there are numerous substances that are found in propolis in a constant and relatively stable way [7].

Propolis has some components that make it up as can be seen below, where resins and balsams predominate in the highest percentage and wax, these two components give the shape and structure of propolis, see **Table 1**.

The wax and some other components present in propolis are considered to have no proven therapeutic activity and normally constitute about 40–50% of the total mass

Resins and balsams	50–55%
Wax	30–40%
Aromatic volatile oils	05–10%
Pollen	05%
Organic and mineral substances	05%

Table 1.
Components of propolis.

in a propolis sample; the rest corresponds to the biologically active part. The fraction related to the polyphenols of aromatic acids constitutes 2/3 of this amount, to which the pharmacological action is attributed [8].

Among the organic substances contained in propolis, although it makes up only five percent, when in fact it shares some of the main characteristics for the therapeutic properties used, the following is a description of the organic substances contained in propolis:

- Organic acids: benzoic acid and derivatives (hydroxy-4 benzoic acid, methoxy-4 benzoic acid, protocatechic acid and gallic acid).
- Phenolic acids: caffeic acid, phenylic acid, isophenylic acid.
- Aromatic aldehydes: vanillin, isovainillin.
- Unsaturated aromatic acids: cinnamic acid and derivatives p. coumaric acid, ferulic acid (4-hydroxy-3-methoxybenzaldehyde) and isoferulic acid.
- Coumarins: esculethol, scopolethol.
- Flavonoids: ecacetin, yellow chrysin, pectolinarigenin, tectochrysin, galangin 3,5,7-trihydrixiflavone, isalpinin, rhamnocitrin, isorhamnetin, quercetin, quemferido, butelenol, ermanin, pinobanksin and apigenin, 5,7-dioxy-3,4-dimethoxyflavon; 3,5-dioxy-7,4-dimethoxyflavone and 5-oxi-7,4-dimethoxyflavone.
- Flavonones: pinostrobin, sakuranetin.
- Quercetin derivatives: alpha-acetoxybetulenol [9].

Up to now and based on propolis characterisation processes up to twelve different types have been described, but it is type six that has shown the highest inhibition against GTF-B and GTF-C enzyme activity. The possible active biological component that modulates the GTF inhibitory activity is unknown, but it is thought that it may be due to the action of non-polar chloroform, ethanolic extract of propolis, and especially the hexane fractions that compose it; it is also suggested that flavonoids present in propolis may be involved in the enzymatic inhibition [4].

3. Preventive dentistry

Propolis has anti-cariogenic properties, so its use in preventive dentistry is mainly to reduce the incidence of caries and prevent the accumulation of dental plaque in vitro and in vivo. The antimicrobial effectiveness of the extracts depends on the solvent used, although the solvent used is not definitive as it is the actual substances or elements contained in the propolis, the origin of the propolis and the microbial species evaluated, with ethanolic extracts of propolis being the most effective, contents such as apigenin (flavonoid) and t-farnesol (terpenoid) have been shown to have the strongest antimicrobial properties against Streptococcus mutans micro-organisms, based mainly on their ability to inhibit glycosyltransferases and their bactericidal effect [10].

The fatty acids in propolis have been shown to provide a cariostatic effect by decreasing acid production. It has been proven that one of the most widely used preventive products is the use of rinses, with propolis being used as the main substance in mouthwashes with an aqueous solvent or carrier, compared to a chlorhexidine (CHX) rinse or others containing an alcohol solvent or carrier, which are among the most commonly used types of rinses, those with a chlorhexidine-based substance being one of the most recommended by periodontists, due to its capacity to eliminate a large number of microorganisms, but it has some side effects such as altering the sense of taste, its prolonged use can damage the oral microbiota because the elimination of microorganisms is not specific but eliminates indiscriminately, something that would not be a problem with propolis which is a biocompatible substance and that its prolonged use has no side effects [11].

In the case of mouthwashes in which the vehicle is alcohol, they are contraindicated because they cause high oral sensitivity, as well as when there are lesions in the mucosa due to their caustic and astringent effect, although propolis can be used in this vehicle, due to these characteristics, it is recommended to use it in an aqueous vehicle. Propolis has proven to be more effective against the following bacteria: *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus acidophilus* and *Enterococcus faecalis*, which are the most common bacteria for oral diseases such as caries and gingivitis [12].

4. Endodontics

In the field of endodontics, propolis has been shown to have biocompatibility as well as antimicrobial properties compared to one of the most commonly used substances in endodontic treatments, calcium hydroxide as an intra-canal medication. In addition, studies have been conducted comparing and evaluating the antimicrobial activity of calcium hydroxide. Another substance or medication is the use of triantibiotic mixture (TAM), as opposed to ethanol extract of propolis as intracanal medication in root canals infected with *Enterococcus faecalis* (microorganism present in most endodontic complications), propolis was more effective than the triantibiotic mixture against *E. faecalis* and, at seven days, both were equally effective, reducing the healing time [13].

Propolis and calcium hydroxide show synergistic effects with other antibiotics such as ciprofloxacin against *E. faecalis*. Another use of propolis in endodontics is as a solution to irrigate and disinfect the canals, as is the case with sodium hypochlorite as a solution, being just as effective as the latter; with the advantage that propolis acts against periapical inflammation and protects periodontal cells due to its antibacterial properties, unlike sodium hypochlorite, which only cleans and eliminates organic tissue but does not have antibacterial properties [14].

5. Periodontics

In the treatment of periodontal diseases, propolis has demonstrated antibacterial, anti-inflammatory, anaesthetic and healing activity in certain lesions such as chronic ulcers or in various types of periodontitis. The action of propolis is directly against supragingival plaque due to its stimulating action on tissue recovery, which is due to the biocompatibility of propolis, acting against Gram-positive microorganisms and its anti-inflammatory property, which allows for a better local immune response.

As an anti-inflammatory, propolis inhibits the synthesis of prostaglandins and helps the immune system by promoting phagocytosis and stimulating cellular immunity. Another property of propolis is that it is an adjuvant in the healing process, which in the case of chronic gingivitis and recurrent and non-specific mouth ulcers, is very useful for promoting the process in the gum and accelerating the healing process [15].

Propolis for the treatment of periodontal diseases has been used in many different forms, such as patches that continuously release propolis in the gum area affected by periodontitis, favouring the recovery of the tissue due to the prolonged and constant release of propolis in the gingival area with the pathology [16]. Another of the most commonly used presentations is as an irrigant prior to periodontal treatment, with good results being observed. Propolis has also been used for the treatment of herpes simplex virus in a solution form, which slows down the progression of the virus and is reflected in the skin changes that occur at the onset of the disease [17].

Propolis tincture is another presentation that has been used, 0.12% being the most common, after the initial phase of periodontal treatment and also in periodontal surgery in cases of periodontal disease, gingival enlargement and high frenulum insertion. After the initial phase of periodontal treatment, cotton swabs soaked in propolis tincture were administered and the procedure was repeated 24 and 48 h after the intervention [18]. Propolis, which has a healing property, makes the gingival tissue have a faster and more successful postoperative period, reducing the time of evolution, improving haemostasis and adequate tissue healing. Propolis as a paste and mouthwash were shown to have the ability to inhibit the growth of supragingival biofilm, representing an approximate reduction of 80–88% [19].

Chronic periodontitis (CP) is one of the most common types of periodontal disease. It presents clinically as an infectious inflammation that affects the supporting tissues of the tooth, causing loss of attachment and alveolar bone. It is asymptomatic, which means that it progresses silently and for this reason it cannot be diagnosed early and it cannot be treated in the early stages of the disease, which is why the clinical manifestations are more severe [20]. It is initiated by bacteria present in the biofilm; however, specific immune response and inflammation play an important role in the development of the disease [21]. Assessment of the periodontal status should be made by observing the degrees of inflammation of the periodontal, supporting and surrounding tissues of the tooth. This is reflected by a reddish discolouration, bleeding, sometimes with a crepitant sensation and tissue that does not appear to be firmly attached to the bone [22].

6. Surgery

Regarding the participation of propolis in oral surgery, it has been used in post-operative treatment, due to the healing property that is used in the majority of dental surgical treatments, improving the process of alveolar closure, using the presentation of propolis in the form of 5% propolis tincture. This has led to a remission of symptoms and a shortening of the evolution period [23].

Propolis has been administered topically in combination with drugs such as dexamethasone directly to oral surgical wounds, with concentrations of 30% ethanolic extract of propolis (EEP) and 0.1% dexamethasone being the most recommended for topical treatment with orabase gel. From this, they found that ethanolic extract of propolis generated greater anti-inflammatory effect and reduced wound healing time compared to dexamethasone gel alone [24].

7. Prosthodontics

In prosthodontics, where patients using removable partial prostheses or total prostheses have soft tissue damage due to inflammation or poor hygiene that causes some opportunistic microorganisms to appear, including fungi such as *Candida albicans* [25]. Some propolis-based products such as ointments or rinses are used, as they have great antifungal properties, especially on *Candida albicans*, which is why, once again, propolis can be of great use in prosthodontics [26]. Oral candidiasis is an infection of the mucosa of the oral cavity caused by yeasts of the *Candida* genus in populations that use removable dentures [27].

One of the most common diseases for older adults is prosthetic stomatitis, which is a chronic form of infection by the fungus *C. albicans* that affects the mucosa of patients with removable total and partial prostheses. *Albicans* fungus that affects the mucosa of those patients who wear removable total and partial prosthesis, the reasons can be discomfort caused by the prosthesis, it is poorly adjusted, it means that in some part of the prosthesis there is an excess or surplus of acrylic and that hurts the patient's mucosa, another of the reasons may be null or poor hygiene, as well as the patient carries the prosthesis all day even at bedtime, when it must remain out of the mouth at night to clean it [28]. The treatment normally used is with antifungal agents such as nystatin suspension or miconazole gel, with favourable results for the pathology in question. However, despite this, there have been failures in antifungal therapy, which is why biocompatible options are needed, with propolis being one of the best natural products for treating prosthetic stomatitis [29].

8. Pulpal therapies

One of the concerns in paediatric dentistry is the preservation of the pulp of primary teeth, whose main objective is to maintain the space of the dental arches, but also to allow the permanent teeth to erupt when the primary tooth is exfoliated. One of the pulp therapies in which propolis has been successful is pulpotomy, which is a pulp therapy that is performed when the dental pulp is exposed and therefore the inflamed coronal pulp tissue has to be removed leaving only the root pulp [30].

For a long time there has been a search for the ideal medicine, treatment or substance to carry out pulp therapy for pulpotomy, which must be antimicrobial, biocompatible, have the capacity to heal the inflamed pulp tissue, and not affect the resorption process of the teeth. The most commonly used substance or medicine has been formocresol, which is a substance that has the capacity to mummify the pulp tissue, preventing the pulp tissue from becoming infected [31]. The problem with the use of this substance is that it has carcinogenic and mutagenic properties, which has led to its use being banned in some countries, and in others it continues to be used but with certain restrictions and vigilance, hence the importance of using a different substance or medicine [32].

One of the advantages demonstrated by the use of propolis as an alternative in the treatment of pulpotomy is that it is easy to handle, biocompatible and does not mummify the tissues, allowing a more natural recovery [33].

9. Conclusions

In dentistry, consideration should be given to the large number of medicinal plants and other natural products, including those from the animal kingdom such as propolis, which can make an important contribution to the area of health and be willing to scientifically study their therapeutic properties, so that after verifying their value, they can be introduced more constantly in dental treatments and in some cases replaced definitively due to their properties, especially their biocompatibility, with the aim of massively increasing their application.

Propolis is a medicine based on knowledge of herbal and holistic medicine with therapeutic properties, because it has components with analgesic and antibiotic characteristics, and due to these properties, its usefulness in dental materials is the subject of research. It is a natural resin with diverse biological properties developed by bees of the Apis millifera species that is responsible for protecting the hive and also has antifungal activity, so it has been used for the therapeutic management of fungal infections.

The results of this research must be corroborated by subsequent studies because the results presented cannot be considered conclusive. The more clinical evidence there is of these natural products, the more confidence there will be in their application in patients.

The great difficulty in the use of propolis in general medicine and dentistry lies in the difference of the components that this antimicrobial action yields depending on geographical location, harvesting season, and method of extraction.

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Chapter 8

Propolis from *Meliponinae*: A Highway from Ancient Wisdom to the Modern Medicines

Ariane Storch Portal and Caio Mauricio Mendes de Cordova

Abstract

Propolis has been extensively studied and several chemical constituents, mainly flavonoids, terpenes and phenolics, have been identified. With the emerging microbial resistance to antibiotics, the interest in the search for active compounds, mainly secondary metabolites of plants, has been increasing significantly. In this chapter, we describe the characteristics of the main species of native stingless bees found in South America, especially in Brazil, the ancestral use of propolis produced by them, its chemical composition and its potential for the development of new therapeutic compounds. Its chemical composition is very rich, and for many bee species it remains to be unfolded. Its biological properties evaluated so far include the antibacterial, antimycoplasmic, antifungal, antiviral, antioxidant and anti-inflammatory activities, but not for all known bee species. However, their existence is threatened by the introduction of exotic bees in the environment or using pesticides that annually kill millions of individuals. As if that were not enough, we face the destruction of original forests themselves, and the reforestation strategies with exotic plants.

Keywords: natural products, stingless bees, bioprospecting, terpenoids, phenolic compounds, antimicrobial, antibacterial, antiviral, antifungal

1. Introduction

Humanity has employed natural resources for healing, particularly plants, animals, and minerals, since the beginning of time. One of the earliest applications of natural products may have been the search for pain alleviation and disease cures through the consumption of herbs [1]. Plants produce a wide range of substances, many of which are connected to defensive mechanisms, such as antibacterial activity. This enormous variety of phytochemicals results in part from the requirement for evolution to contend with microbial, insect, nematode and other plants. Most plant pathogen infections are successfully avoided by this defense mechanism [2].

One of the natural items that has been used by humans for a variety of therapeutic purposes is propolis. It is made up of a combination of the wax and saliva of these insects with resin from plants that bees have collected [3]. This resinous substance is combined with the soil by some stingless bee species to create the substance known as geopropolis [4]. As it is used for building nests and sealing the hive, maintaining the internal temperature and preventing the entry of unwelcome visitors, it is crucial for the colony's health. Due to its antibacterial qualities, propolis also functions chemically as a protection against microbes [3].

About 50% of it is composed of vegetable resins, 30% of it is beeswax, 10% is essential oil, 5% is pollen and the remaining 5% is made up of wood and soil debris [5]. The yield of the volatile fraction, however, has typically been described as being in the order of 1% [6]. Propolis has a distinctive perfume that comes from its volatile components, which also have a lot of biological activity. The majority of the chemicals in their makeup were mono- and sesquiterpenoids [7]. Studies have shown how important these chemicals are in the fight against harmful pathogens, including Gram-positive and Gram-negative bacteria [6, 8].

2. Importance of the prospection for new medicines and the role of propolis

Newman and Cragg highlighted in an extensive review in 2020 [9] the importance of natural products and structures derived from or related to natural products as a source for new molecules. In the period between January 1981 and September 2019, 126 antibacterial molecules were approved (disregarding prophylactic agents). Of the 126, about 48% (78) are derived from natural products or unaltered natural products. However, since the advent of antibiotics in the 1950s, virtually no antimicrobials have been developed from plant sources. The main natural sources of these agents were bacteria and fungi. With the emergence of resistance to antimicrobials, interest in the search for other sources, mainly secondary metabolites from plants, has increased significantly [2, 10].

To produce propolis, bees collect the plant resins by selecting the best compounds capable of protecting the nest and ensuring its survival. Considering this natural preselection based on the knowledge of these insects, the composition of propolis and its biological activities have aroused the interest of several researchers [11]. Despite the great diversity of the bees native to Brazil, most of the studies investigating the composition and activities of propolis are carried out with samples produced by exotic bee species introduced in the country. Studies on its volatile fraction are even more scarce. Knowing that the chemical composition of propolis is quite variable and is related to the species of bee that produces it, seasonality and its geographical location, studies with propolis and geopropolis of native stingless bees provide potential unprecedented results and the discovery of compounds with antimicrobial activity [12].

3. Bees' diversity

Since ancient times, bees have aroused curiosity and human interest. In Greece, they were seen as priestly and chaste animals. The local currency had the image of a bee, symbolizing wealth. The Romans considered them as a representation of territorial defense [13]. In Christianity, they were related to various qualities, above all hope, due to their tireless work, and resurrection, for disappearing in winter and resurfacing in spring [14].

Bees have developed behavioral characteristics, such as the distinction of colors and aromas, which aid in the search for nectar and pollen: their main food sources.

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These characteristics also benefit the plants, because during foraging, incidentally, they transfer the pollen of the anther of one flower to the stigma of another, performing pollination [15].

The order *Hymenoptera* is one of the most diverse groups of species in the class *Insecta*. *Hymenoptera* presents a wide variety of complex habits and behaviors, culminating in the social organization of wasps, ants and the most important pollinating agents: bees [15]. The bees belong to the superfamily *Apoidea*, which has several families, among them the *Apidae*, which has more advanced social habits and is divided into four subfamilies: the *Apinae* (bees of the genus *Apis*, with about 11 species), the *Meliponinae* (stingless bees, with hundreds of species), the *Bombinae* (bumblebees, with about 250 species) and the *Euglossinae* (orchid bee, with about 175 species) [16].

4. *Meliponinae* subfamily

The first written records about the meliponines arrived in Europe in the sixteenth century and were made by Spanish and German explorers in Central and South America. These reports and later archeological studies, particularly in the Mesoamerican region, contributed to the discovery of important data on the traditional search for honey and on the management of meliponines, which was already well structured at least by 300 BC. More advanced research was conducted only in the nineteenth century, almost two centuries after the beginning of scientific research on bees [17].

The subfamily *Meliponinae* is believed to have been the oldest to branch off from less social ancestors and develop high social behavior. A fossil of a female stingless bee about 80 million years old has been found wrapped in amber. This bee is considered the oldest known species of social bee and has characteristics similar to those of living species [18].

Meliponines are also known as stingless bees (SBs) or indigenous bees because they are traditionally bred by indigenous populations [8]. More than 600 species have been described and are found in South America, Central America, Southern North America, Africa, Southeast Asia and Northern Oceania. In Brazil, more than 200 species can be found, distributed in 29 genera, being *Plebeia*, *Trigona*, *Melipona*, *Scaptotrigona* and *Trigonisca*, the ones with the largest number of known species [3].

Brazil is home to the largest diversity of SBs in the world. Native species are distributed throughout the Brazilian territory, mainly in the Amazon region. The warm climate and the abundant flora in species that can provide nectar, pollen and resin are favorable conditions for the existence of these bees [19]. They are especially sensitive to low temperatures and depend on the structure of their nests for the thermoregulation of the hive [20].

The nests of the meliponines are considered the most elaborate among bees. Some species nest in exposed places, such as on branches, but more often the nests are found in preexisting cavities in tree trunks or anthills and abandoned termite mounds. They are protected by one or several layers of a casing composed of wax and resin, which assists in maintaining the temperature. The colony is delimited by the bitumen that consists of a mixture of wax, resin and clay [16, 21]. The upper layer of bitumen is usually compact to avoid infiltration and the lower one is riddled, allowing the flow of water [22].

The entrance of the nest presents particular characteristics for each species and can be constituted of geopropolis, clay, cerumen or pure wax [23]. Some species build quite narrow entrances, which are guarded by a single bee, while others build larger entrances that allow the circulation of multiple guards [24]. Camouflage and occlusion of the entrance are strategies that can also be undertaken to prevent invaders [13].

Female bees have a well-developed ovipositor (egg-laying organ), which can be modified to form a stinger, which is used as a defense mechanism [15]. However, female meliponines have a stunted stinger and are therefore unable to use it, and so are known as stingless bees [13].

Female bees are considered more docile; however, they are not helpless bees. When they feel threatened by large invaders, such as men, they can curl up in their hair or fur, pinch with their sharp jaws, penetrate holes such as ears and nostrils or release unpleasant odors. Some species produce formic acid in their mandibular glands, which when released, can cause serious burns [13, 25]. The SBs with tamer behavior protect their nests by building them in places of difficult access, including inside anthills or near more defensive bee nests [26].

These bees have eusocial behavior, that is, they live in well-organized communities, where the queen is responsible for reproductive work and the workers take care of the offspring, the provision of food and the construction and defense of the nests [24]. Food storage is done in pots consisting of cerumen, where pollen and honey are stored separately [22].

Stingless bees differ from bees of the genus *Apis* in several aspects. While *Apis* exhibit uniform behaviors and morphology, meliponines are quite diverse. Some species are very small, smaller than even fruit flies and others have robust bodies. The size of the colony is also variable and can house tens to thousands of individuals. The nests may be arranged in clusters or combs. Foraging can be done by groups or solitary bees and is generally characterized by shorter flights, which imply the elaboration of propolis using the plant resources closest to the colony [27, 28].

The ecological relevance of these insects is undeniable. They act as pollinators of several native and cultivable plants, contributing to the conservation of different biomes and agricultural production, directly impacting the production of fruits and seeds and consequently the economy [29]. The honey produced by SBs is marketed in some regions of Brazil for its appreciated flavor and medicinal properties. These bees also produce propolis, which has been the subject of studies around the world due to its pharmacological properties [3].

5. Important *Meliponinae* species

5.1 *Melipona quadrifasciata*, Lepeletier, 1836

Melipona quadrifasciata is a robust bee, which measures between 9 and 10 mm in length, has blackish coloration on its thorax and head, rusty on its wings and yellow bands on its abdomen. It is considered a bee of tame behavior, however, it has quite strong colonies that can act defensively. It can be distinguished into the subspecies *Melipona quadrifasciata quadrifasciata* and *Melipona quadrifasciata anthidioides*. The subspecies are differentiated by the pattern of yellow bands present on the dorsum of their abdomen, which consists of three to five continuous bands in *M. q. quadrifasciata* and two to five discontinuous bands in *M. q. anthidioides* [30].

Melipona quadrifasciata anthidioides can be found in hot climate regions, often in the states of Rio de Janeiro and Minas Gerais (Southeast Brazil). The subspecies *M. q.*

quadrifasciata prefers regions with colder climates, such as the states of Paraná, Santa Catarina and Rio Grande do Sul (South Brazil). It can also be found in high-altitude regions in the states of São Paulo, Rio de Janeiro and Minas Gerais [31].

'Yra-maya' is the indigenous appellation (Tupi) for bee, where 'Yra' means honey and 'may', 'manha' or 'manda' means watchman. *M. q. quadrifasciata* is popularly known as 'mandaçaia', where 'çai' in the indigenous denomination refers to smart [32]. The cleverness of the nest watch is one of the most impressive features for traditional peoples. In fact, the mandaçaia is an attentive watchman: a worker bee always keeps guard at the entrance of the nest, to defend the hive.

The crops pollinated by this species are pumpkin (*Cucurbita moschata*), pepper (*Capsicum annuum*), chili pepper (*Capsicum frutescens*) and tomato (*Lycopersicon esculentum*). There are also plant species that are attractive to these bees, such as 'assa-peixe' (*Vernonia polyanthes*), 'estoraque' (*Styrax ferrugineum*), 'murici' (*Byrsonima intermedia*), 'picão' (*Bidens segetum*), 'picão-de-cipó' (*Bidens gardneri*) and witches' broom (*Ouratea hexasperma*) [33].

Melipona quadrifasciata quadrifasciata was classified as endangered in the List of Fauna Species Threatened with Extinction in Rio Grande do Sul, Brazil, in 2002 [34]. In 2014, the Zoobotanical Foundation (FZB) with support from the State Secretariat of the Environment (SEMA) updated these data, where the species remains with the same classification.

5.2 *Melipona compressipes manaosensis*, Schwarz, 1932

Melipona compressipes manaosensis is popularly known as 'jupará' or black 'jandaíra' of the Amazon. In the central Amazon, its products are used in the feeding and income provision of local populations, in addition to playing an important role in the pollination and dispersal of seeds that occur during the collection of plant resins [33, 35].

This species can be found in Amazonas, Amapá and Pará in Brazil and Guyana, French Guiana and Suriname [36]. The worker bees of this species measure about 13 mm and have little defensive behavior. The colonies are made up of about 5000 workers, 250 bumblebees and a queen. The entrance to their nests consists of a mixture of vegetable resins with clay, resulting in a whitish mixture [37].

A study identified the plant origin of pollen grains collected by SB species for 1 year [38]. Some plant species where pollen resources were collected by *M. compressipes manaosensis* were 'guava-de-tapir' (*Bellucia grossularioides*), 'cássia-rosa' (*Cassia grandis*), 'embaúba' (*Cecropia* sp.), 'jenipapeiro' (*Genipa americana*), 'araçá' (*Myrcia amazonica*), 'pau-rosa' (*Physocalymma scaberrimum*), 'cajazeira' (*Spondias mombin*) and 'baginha' (*Stryphnodendron guianense*).

5.3 *Melipona bicolor schencki*, Gribodo, 1893

This robust species measures about 9 to 10 mm. Its voluminous body is covered with hairs, the coloration of which varies throughout its life, being lighter when young and darker with time. It is considered a bee of tame behavior and easy management. Two subspecies of *M. bicolor* were distinguished: *M. b. bicolor* and *M. b. schencki*. They differ in the coloration and geographic area they are distributed. The first can be found in Brazil in the states of Bahia (Northeast), Espírito Santo, Minas Gerais, Rio de Janeiro and São Paulo (Southeast). And the second, which has darker hairiness, can be found in Paraná, Rio Grande do Sul, Santa Catarina (South) and in high and cold areas of Southeastern Brazil [36].

Melipona bicolor schencki is popularly known as 'guaraipo', 'guarupú' or 'pé-de-pau' [36]. In the Tupi nomenclature, 'guarabu', sometimes written 'guaripú' or 'guaraipo', comes from 'guará' which refers to the habit of drilling and 'ubu' or 'ibú' which means land. This species found in forest regions usually nests in hollow sticks, or tree cavities close to the ground, which justifies its popular name [32, 39].

While almost all colonies of social bee species are led by only one queen, *M. bicolor* presents a unique behavioral characteristic: facultative polygyny. It is the only species of the genus *Melipona* where cohabitation of queens can occur for a considerable period, not only transiently [39].

Melipona bicolor schencki is on the List of Endangered Fauna Species in Rio Grande do Sul, Brazil, in 2002 [34], which was updated in 2014 by the Zoobotanical Foundation (FZB) with support from the State Secretariat for the Environment (SEMA), remaining in the 'endangered' classification.

5.4 *Melipona marginata*, Lepeletier, 1836

These bees, popularly known as 'manduri', live in small colonies with about 300 individuals. The entrance to their nests is striated and narrow, allowing the passage of one bee at a time [40]. In Brazil, it is found in the states of Bahia, Ceará and Pernambuco (Northeast), Espírito Santo, Rio de Janeiro and São Paulo (Southeast), Goiás and Minas Gerais (Center), and Santa Catarina (South) [36].

This species is considered one of the smallest in the genus *Melipona*, measuring between 6 and 7 mm in length. Despite their size and the low number of individuals in their colonies, they are proportionally large honey producers. They have a very defensive behavior and can nibble strongly when they feel threatened. It is a polytypic species, which presents subspecies with different colors of hairiness, related to the altitude of its location. This characteristic was observed for the first time in 1875 by the naturalist Fritz Muller in Blumenau, Santa Catarina, Brazil, when describing the species now called *Messor obscurior* [41].

There are records to prove that this species can colonize termite mounds, limiting the area with the use of resins. They also use the same material to enlarge the delimited area, invading the space of the termite mound. Their nests can also occupy tree hollows, lined with bitumen consisting of resin and earth [42].

5.5 *Melipona seminigra merrilae*, Cockerell, 1919

Melipona seminigra merrilae, popularly known as 'uruçú boca-de-renda', is a medium-sized SB species, about 11 mm long. It has a robust body and dark brown integument. Its elongated abdomen has a bright reddish-brown color and predominantly yellowish-fawn-hollow hairiness [26].

This species has been reported only in the states of Amazonas and Pará. It inhabits floodplain and 'igapó' forests, nesting in trees 8 to 10 m from the ground. The entrance to their nests is tubular consisting of geopropolis. It is commonly ornamented with brightly colored seeds and resins [26, 36]. Its colonies are populous, housing about 2000 individuals. The queen is slightly smaller than the workers and has darker coloration. This species adapts well to different environments and collects nectar throughout the year, including in the rainy season [26].

A study was able to verify some plant species visited by *Melipona seminigra merrilae*, such as 'pau-de-facho' (*Aparisthmium cordatum*), 'guava-de-tapir' (*Bellucia grossularioides*), 'cássia-rosa' (*Cassia grandis*), 'embaúba' (*Cecropia* sp.), white wren

(*Matayba* sp. nov.), 'araçá' (*Myrcia amazonica*), 'pau-rosa' (*Physocalymma scaberrimum*) and 'cajazeira' (*Spondias mombin*) [38].

5.6 *Melipona fasciculata*, Smith, 1855

Melipona fasciculata, also known as 'tiúba' or 'uruçú' gray, is a species of SB about 12 mm long and predominantly grayish in color. It has abundant whitish hairiness on its body and near the wings the hairs are reddish. Along its abdomen, it has clear bands that can be continuous or interrupted [43]. In Brazil, this species can be found in the states of Maranhão, Pará and Piauí (North), Mato Grosso and Tocantins (Center) [36]. It is rare on dry land and usually nests in mangrove regions, where it shelters its colony in tree hollows. In the mangroves, they are found in the 'siriúba', a popular name for the species *Avicennia nítida*, family *Avicenniaceae* [44].

The indigenous peoples like Timbiras, Tupinambás, Guajajaras, Tremembés, Awha-Guajás, Vultures and Gavião domesticated this species, passing on the knowledge about its creation over generations [45]. This species pollinates the crops of 'açaí' (*Euterpe oleracea*), eggplant (*Solanum melongena*), cashew (*Anacardium occidentale*), tomato (*Lycopersicon esculentum*) and annatto (*Bixa orellana*). Other plants are also attractive to these bees, such as giant stink (*Senna alata*), 'lobeira' (*Solanum grandiflorum*), 'jurubeba' (*Solanum juripeba*), seal (*Vismia guianensis*), myrtle (*Myrcia eximia*), thrush (*Mimosa caesalpiniifolia*), crab (*Miconia minutiflora*), 'siriuba' (*Avicennia nítida*) and 'tapiririca' (*Tapirira guianensis*) [33].

6. Indigenous bees: sociocultural importance and ethnoknowledge

Indigenous peoples have a close relationship with the meliponines. Before the introduction of the species *Apis mellifera* and the culture of sugar cane in the Americas, the honey of the native bees was used by the indigenous peoples as an indispensable source of energy for the great journeys in the search for food. The popular names of many species, such as Jataí, Tiúba, Jandaíra, Guarapu and Manduri, are part of the sociolinguistic indigenous heritage [22].

Indigenous knowledge is closely related to nature. The legacy of these peoples has immeasurable value and has been passed down through the generations. In Mexico, the creation of SBs and the use of its products date back to the pre-Columbian period. For the Maya people, one of the most relevant ancient civilizations in history, the SBs played an important role in religious ceremonies, feeding and treating diseases [46].

In Brazil, the Kayapó people use elaborate techniques for the management of swarms, such as platforms that allow the reach of hives in tall trees. Among these people, bee specialists are shamans, who possess extensive knowledge about the anatomy and behavior of various SB species. The people use honey and pollen in food, cerumen and resins in the waterproofing of canoes, in addition to being inspired by the social organization of these insects [25]. For the Pankararé, indigenous peoples in northeastern Brazil, the creation of 'abelha mansa' (meek bee) is a recreational and medicinal activity. Honey is used in food as an energy source and is extracted in a nonpredatory way, ensuring the maintenance of the tree from where it was collected and the colony [24].

The knowledge about the SBs of the Guarani Mbyá people of the Morro da Saudade village, in the city of São Paulo, Brazil, is transmitted orally through the generations. The teachings are followed and improved by the younger ones, and some members of the community are seen as great connoisseurs of the subject. The products from bees are used in handicrafts, religious rituals and medicinal potions, as well as activities related to spiritual and contemplative life [47]. A study was conducted on the traditional knowledge about the SBs of the Enawene-Nawe people of the state of Mato Grosso. It was verified that the people were able to identify several species of SBs not only by morphology but also by the ecological and social characteristics of these insects [48].

Meliponiculture has also been practiced for generations by other traditional communities like 'quilombolas' (descendants of enslaved Africans), 'ribeirinhos' (inhabitants of riverbanks), 'sertanejos' (inhabitants of the hinterland), 'caipiras' (countrymen) and 'caiçaras' (traditional fishing communities). The products from the SBs are used for subsistence family consumption and are a source of complementary income. These people contribute to the conservation of bees, through the management of species that are practically no longer found in natural habitat, due to the devastation of the native forest. The rational beekeeping of SBs can therefore be a sustainable strategy to promote biodiversity conservation [23].

7. Introduction of exotic bee species in Brazil

The introduction of the European bee species *Apis mellifera* in Brazil occurred around 1839, brought by the Portuguese to produce wax candles. In 1845, German immigrants brought more bees and settled in the south of the country. Other colonizers have also introduced European bees into different regions [49]. In 1956, the biologist and geneticist Dr. Warwick Kerr, at the request of the Brazilian government, brought from Africa some queens of bees to perform crossing by artificial insemination, which would originate a species capable of producing more honey in the tropical climate [25, 49].

The following year, swarms and their respective queens escaped accidentally and ended up crossing with the European species already introduced in the Brazilian territory, emerging hybrid populations, today called Africanized bees. This incident had an extensive environmental impact since these bees spread to almost the entire American continent [25, 49].

8. Impact of anthropogenic actions on bees

The introduction of exotic species, accidentally or for economic purposes, can cause significant changes in natural environments, such as changes in habitats, hybridization and competition with native species. In addition, the intensive occupation of the environment by man impacts bee populations by eliminating food sources, by destroying substrates necessary for the construction of nests and by using pesticides [8].

Due to the intense bee die-off, several studies were conducted to evaluate the toxicity of agrochemicals, but most of them were carried out with the species *Apis mellifera*, endemic in several regions of the world. A study evaluated the effect of the insecticide dimethoate on the SB species *Melipona scutellaris*, where the average lethal concentration (LC50) was 320 times lower than for *Apis mellifera* larvae, demonstrating the sensitivity of this species to the pesticide and the need to conduct further studies using native bees [50].

The deforestation of forest areas for urban and agricultural use has also significantly reduced bee populations. The scarcity of resources, competition between species, predation by invaders and inbreeding due to population decrease, can make swarms captive to a small territorial space, or lead them to extinction [8].

Even if the melipones adapt, it was verified that the major volatile compounds in the propolis of native bees in the Itajaí Valley region, in Southern Brazil, are compounds found in Pinus and Eucalyptus, indicating that this is the most important plant source in the elaboration of propolis by those specimens [51]. This 'reforestation' with exotic trees certainly contributes to the difficulty in discovering new molecules with biological activity and potential for the development of new drugs.

As bees contribute to the maintenance of forests through pollination, several species of *Meliponinae* depend on forest environments and are not found in anthropogenic environments, except near forests. This issue is an aggravating factor in Brazil, considering that some species of the genus *Melipona* are translocated to regions distant from their natural occurrence [8].

9. Bee products beyond honey: propolis

Propolis is a mixture of substances used by bees in the defense of the hive. Worker bees collect resinous materials from shoots, exudates and other parts of plants and pack them in their corbicles (part of the posterior tibia used to transport pollen, clay and resin). These resins are biotransformed by bees with the addition of their salivary enzymes and wax [52, 53]. Physically, propolis has quite variable characteristics. It may have a hard and brittle consistency or be sticky and elastic. The coloration can also vary between cream, yellow, green, light brown or dark brown [54].

The name propolis is derived from the Greek words pro, in defense of, and polis, the city, e.g.: 'in defense of the city or the hive'. In fact, this material has great importance for the health of the colony. Bees use propolis in the sealing and repair of crevices to prevent the entry of invasive insects and maintain the internal temperature. In addition, it is used against microorganisms in the asepsis of the places where the laying of eggs is made. And if the bees cannot remove a dead invader from the hive, they use propolis to embalm it, which prevents decomposition and bacterial proliferation in the nest [54, 55].

The plant materials used in the composition of propolis are produced by various botanical processes in different parts of the plants. The collection of these materials is a difficult activity to observe and often occurs high in the trees. Among the substances collected, lipophilic compounds from the leaves, mucilage and resins can be highlighted [7].

10. Historical and popular use of propolis

Propolis has been used in traditional medicine since antiquity. The Egyptians used it to embalm the dead and thus prevent putrefaction. Its properties were also recognized in Greek and Roman medicine by Aristotle, Dioscorides, Pliny and Galen, who employed it in asepsis and wound treatment and as an oral disinfectant. Its use was perpetuated in the Middle Ages in Arab medicine and was recognized by New World civilizations [56].

In the seventeenth century, propolis was included in the London Pharmacopeia. Between the seventeenth and twentieth centuries, its use became popular in Europe due to its antibacterial action. It is currently used in various pharmaceutical forms, such as extracts, mouthwash, lozenges and formulations for topical use. Also, its employment in the food and cosmetic industries has been benefited from the propolis properties [56].

In 1908, the first scientific work on propolis, its chemical properties and composition was indexed in Chemical Abstracts. The first patent appeared decades later in the same index of scientific literature, in 1968, where Romanian propolis was employed in the production of bath lotions. Since then, several studies on propolis have been published, possibly due to its panacea characteristic and its added value [57].

Currently, propolis is used for various therapeutic purposes as an antibacterial, antifungal, antiviral, anti-inflammatory agent and for increasing the body's natural resistance to infections. Formulations for external use are employed in the treatment of dermatitis and wounds. It is also available in capsules of pure extracts or in combination with other natural products. Throat lozenges and spray, mouthwash and hydroalcoholic or glycolic extracts are widely used as a folk remedy [56].

11. Geopropolis

Some SB species add soil to propolis, giving rise to the so-called geopropolis (**Figure 1**). Although it is not its main constituent, the presence of earth is a differential in the composition of this product. Its coloration is variable and is related to the plant origin, the soil used in its constitution and the species of bee that produces it. The chemical composition and biological activities of this bee product are still little known [29].

Fritz Müller, a German naturalist who migrated to Blumenau, Santa Catarina (Brazil) in the mid-nineteenth century, in one of his letters to Charles Darwin, about the habit of various insects, mentioned his observations on the genus *Melipona* and the constitution of propolis. He observed that these bees not only used wax in the construction of the nest structures but added other materials such as resins and soil [58].

12. Chemical composition of geopropolis from meliponines

In 1998, more than 50 compounds were identified in the Brazilian geopropolis [59]. Among them, the geopropolis of *Melipona compressipes* and *Melipona quadrifasciata anthidioides* collected in the states of Piauí (Northeast) and Paraná (South), respectively. The extracts of these samples were submitted to chemical analysis through gas chromatography mass spectrometry (GC-MS) and a complex chemical composition was observed. The samples showed significant amounts of lactic, phosphoric and long-chain fatty acids, such as stearic and palmitic acids.

The chemical composition of *Melipona fasciculata* geopropolis, collected in Maranhão state (North), was also analyzed [60]. The main constituents found were carbohydrates and their derivatives (19.8%), triterpenes (15.9%), hexoses (11.9%), anacardic acid (8.3%), lupeol (7.3%) and alkylresorcinols (5.9%). Disaccharides,

Figure 1.
Geopropolis over a Melipona bicolor schencki hive (photograph by Ariane Storch portal).

glucuronic acid, salicylic acid and isomers, β-amyrin, inositol and xylitol, among others, were also found.

Samples of geopropolis of the species *Melipona interrupta* and *Melipona seminigra*, collected in municipalities of Amazonas (North Brazil), were evaluated for their composition, evidencing the significant presence of phenolic compounds [61].

The composition of the aqueous and hydroalcoholic extracts of *Melipona quadrifasciata* could be demonstrated. Rutin, gallic acid, gallocatechin, epicatechin gallate and syringic acid were identified in the aqueous extract. In the hydroalcoholic extract, the main constituents found were quercetin, epigallocatechin, p-hydroxybenzoic acid, epigallocatechin gallate and coumaric acid [62]. The flavonol sakuranetin and gallic acid were also identified [63].

Propolis contains volatile constituents to a lesser extent. This fraction, however, can provide relevant information about the antimicrobial activity and elucidate the classes of compounds present in its composition, contributing to the identification of its botanical origin [64]. Volatile compounds are among the main secondary metabolites present in plants. They perform important functions that ensure survival and adaptation to the environment. Among these functions can be mentioned the attraction of pollinators and seed dispersers, protection through repulsion or intoxication and antibacterial, fungicidal and insecticidal action [65].

Volatile oils (VOs), also called essential oils (EOs) when obtained directly from plants, are a complex mixture of lipophilic substances, consisting mainly of terpenes, fatty acid derivatives, amino acid derivatives and phenylpropanoid compounds. In propolis, terpenes constitute a large part of the volatile compounds [66].

Terpenes are classified according to the number of isoprene units (C_5H_8) present in their structure: monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), triterpenes ($C_{30}H_{40}$), tetraterpenes ($C_{40}H_{64}$) and polyterpenes [67]. Monoterpenes make up a large part of the EO and can contribute more than 90% of its total composition. Sesquiterpenes are another important group found in the EOs. Terpenes can be biosynthesized by the classical mevalonate pathway in the cytosol or by the alternative deoxyxylulose phosphate pathway that occurs in plastids [68].

Essential oils can be obtained by distillation or pressing of plants or parts thereof. At room temperature, they are liquid and volatile, with a characteristic odor. The density of EOs is generally lower than that of water, but sassafras, cinnamon and clove oils may have higher densities [68]. The composition of a VO can present a major constituent, facilitating chemical correlation with biological activity. However, small amounts of other substances present can act synergistically, contributing to a certain biological action [69]. However, the composition of these oils is variable and is directly related to seasonality and the place of collection [12].

13. Biological activities of geopropolis compounds

Some studies have highlighted the importance of investigating the biological potential of geopropolis. **Table 1** presents selected studies that investigated the antibacterial, antimycoplasmic, antifungal, antiviral, antioxidant and anti-inflammatory properties of *Melipona* species geopropolis.

It was soon evidenced as regards the antioxidant potential of the hydroalcoholic extract of geopropolis of *M. fasciculata* [71]. The action was correlated with the presence of phenolic compounds, such as phenolic acids, gallotannins and ellagitannins. It was also suggested that the high concentration of phenolic acids, such as gallic and ellagic acids, was responsible for the antioxidant action of the samples tested [72].

The antibacterial activity of the hydroalcoholic extract of *Melipona orbignyi* geopropolis was evaluated against the Gram-positive bacterium *Staphylococcus aureus* (sensitive and methicillin-resistant strains) and the Gram-negative *Escherichia coli* (sensitive and cephalosporin-resistant strains) and *Pseudomonas aeruginosa* (strains sensitive and resistant to amphotericin B). The antibacterial action was attributed to the presence of phenolic compounds, which have some known mechanisms of action, among them the permeabilization of the microbial cytoplasmic membrane [62].

Others investigated the antimicrobial potential of the volatile oil of *Melipona q. quadrifasciata* geopropolis against bacteria with and without cell walls. The antimicrobial action with the lowest minimal inhibitory concentration (MIC) was displayed against the bacterium without cell wall *Mycoplasma pneumoniae* 129 strain. The volatile oil was fractionated and its subfractions were tested, but these subfractions showed no improvement in antibacterial activity [75]. This characteristic had already been observed by other authors, who concluded that the isolated compounds from propolis did not present better results regarding the activities tested, suggesting that the synergy between the compounds favors biological actions [70].

In the literature, it is possible to find studies that confirmed the antimicrobial activity of the volatile constituents of propolis against several microorganisms. Against Gram-positive bacteria such as *S. aureus*, *Staphylococcus epidermidis*, *Micrococcus glutamicus*, *Bacillus subtilis*, *Bacillus cereus*, *Sarcina lutea*, *Streptococcus pyogenes*, *Streptococcus mutans*, *Streptococcus faecalis* and Gram-negative bacteria such as *E. coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *P. aeruginosa* [6].

Bee species	Biological activity	References
Melipona compressipes	Antibacterial	[70]
	Antifungal	[70]
	Antiviral	[70]
M. fasciculata	Antioxidant	[71, 72]
M. compressipes fasciculata	Antibacterial	[4, 73]
M. q. quadrifasciata	Anti-inflammatory	[74]
	Antioxidant	[27, 63]
	Anti-herpetic	[27]
	Antibacterial	[27, 75, 76]
	Antimycoplasmic	[51, 75, 77, 78]
	Antiadhesive	[51, 77]
	Antifungal	[28]
M. seminigra	Antioxidant	[61]
M. marginata	Antibacterial	[28]
M. q. anthidioides	Antibacterial	[70, 79]
	Antifungal	[70]
Melipona bicolor schencki *M. compressipes manaosensis* *M. fasciculata* *M. quadrifasciata* *M. marginata*	Antibacterial Antimycoplasmic	[51]

Table 1.
Biological activities of geopropolis of bees of the genus Melipona already studied.

A study conducted in Greece investigated the chemical composition of volatile propolis compounds from different geographic regions. The predominant constituents were terpenoids, especially α-pinene. Other components have also been identified as α-eudesmol, δ-cadinene, α-muurolene, guaiol and trans-β-terpineol. The *in vitro* antimicrobial activity was evaluated against six species of bacteria, including *S. aureus*, *P. aeruginosa* and *E. coli*, confirming the antimicrobial potential of the samples tested [80].

In another study, the volatile constituents of propolis samples collected in three municipalities of Rio Grande do Sul state (South Brazil), α-pinene and β-pinene, were presented as major constituents. The antimicrobial activity was evaluated by the agar diffusion method against *S. aureus*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *E. coli*-type strains. The antibacterial activity was classified as moderate and the presence of monoterpenes in the samples was attributed to this activity [81].

The antimicrobial potential of the volatile oil of *Apis mellifera* propolis collected in Mato Grosso do Sul (West Brazil) was also evaluated. The activity of the said volatile oil was evaluated by the broth microdilution method against strains of *P. aeruginosa*, *K. pneumoniae*, *Enterococcus faecalis* and *S. aureus*. The volatile oil and two of its isolated constituents—(E)-nerolidol and spathulenol—showed antimicrobial properties [82]. Others tested the antimicrobial activity of the volatile oil of *Apis mellifera* propolis collected in Rio de Janeiro. The microorganisms such as *S. aureus*, *S. epidermides*, *S. pyogenes* and *E. coli* were susceptible to the sample tested. The chemical composition presented as major constituents β-caryophyllene (12.7%), acetophenone (12.3%) and β-farnesene (9.2%) [12].

It was found that the *M. b. schencki* geopropolis VO with the best minimal inhibitory concentration (MIC) was 424 ± 0 μg mL^{-1} against some mycoplasma strains. Fractionation of the VO resulted in a reduction of 50% of the MIC. However, its compounds' synergism seems to be essential to this activity. Antibiofilm assays demonstrated 15.25% eradication activity and 13.20% inhibition of biofilm formation after 24 h for one subfraction at 2x its MIC as the best results found. This may be one of the essential mechanisms by which geopropolis VOs perform their antimicrobial activity [51].

The antiadhesive activity of *M. quadrifasciata* geopropolis was demonstrated against mollicutes (mycoplasmas), where a dichloromethane subfraction was highlighted in the antiadhesive assay with an inhibitory activity of 21.6%. A synergistic effect of the nonpolar compounds in *M. quadrifasciata* propolis was also suggested as responsible for its antibacterial activity. The hexane (MIC = 62.5 mg/L) and dichloromethane (MIC = 125 mg/L) fractions presented the most promising antibacterial results against *M. pneumoniae* [77].

14. Bacterial resistance to antibiotics and the need for new drugs

The increasing microbial drug resistance has been observed worldwide and with increasing mortality, prolonged hospital stays and rising costs, with other sectors of society being impacted beyond healthcare [83].

Between 2011 and 2014, the National Healthcare Safety Network (NHSN) in the USA reported high levels of resistance to various antibiotics in Gram-positive bacteria including methicillin-resistant *S. aureus* (MRSA), and Gram-negative bacteria such as third-generation cephalosporin-resistant *E. coli* and carbapenem-resistant *P. aeruginosa* [84].

Data from the European Centre for Disease Prevention and Control from 2015, when compared to US data, showed lower rates of resistance in Gram-positive bacteria and equally worrisome rates among Gram-negative bacteria [84].

The U.S. Centers for Disease Control and Prevention (CDC) estimates that at least 23,000 deaths from resistant infections occur each year in the USA. In Europe, in the year 2007 alone, 25,000 deaths were attributed to infections by resistant microorganisms [85].

If this current trend continues, it is estimated that by 2050 there will be 10 million additional deaths due to antimicrobial resistance, surpassing other significant diseases such as cancer and diabetes. In addition, in the same year, microbial resistance is estimated to cumulatively cost $100 trillion worldwide [85].

Natural products offer an obvious source for the research and development of new pharmacological treatments for infections. Moreover, it is more rational to use the millenary wisdom of bees, which learned to look for the best chemical compounds in nature to produce their propolis for the defense of their hives, in every environment, instead of testing individually each plant species for antimicrobial compounds. Unfortunately, we risk losing this wisdom while putting native bee species in danger of extinction.

15. Conclusion

Propolis from native stingless bees has a very rich chemical composition and an enormous potential to be unveiled, both in its use in the form of herbal medicines and in the discovery of new molecules with potential for drug development. Its properties evaluated so far include mainly the antibacterial, antimycoplasmic, antifungal, antiviral, antioxidant and anti-inflammatory activities, but not for all known bee species. Many species of bees have not even had their propolis chemically characterized. Furthermore, their existence is threatened being by the introduction of exotic bees in the environment or using pesticides that annually kill millions of individuals. As if not enough, we also have to deal with the destruction of the natural forests themselves and the use of foreign flora for reforestation. Hence, it is necessary that their environment be preserved, and that the bees themselves be preserved, so that humankind can benefit from this ancestral treasury.

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Conflict of interest

The authors declare no conflict of interest.

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Melittology - New Advances beckons curious minds to immerse themselves in the latest revelations within the realm of bee research. The journey starts with the exploration of the antimicrobial properties of natural honey, where the chemical composition, antibacterial activities, antifungal effects, and antiviral properties of honey are meticulously unraveled. It invites readers to transcend the scientific realm and delve into a narrative woven with historical, sacred, and holistic dimensions of honey that explores how honey evolves beyond its scientific attributes, transforming into a cultural and spiritual entity. Readers are then transported to the unique world of honey derived from Butia yatay palm savannas to learn about the distinctive characteristics of honey from this botanical and geographic origin. Moreover, the book sheds light on vital aspects of meliponiculture, emphasizing the imperative need for conservation efforts and explores the diversity, distribution, nesting, and foraging behavior of stingless bees, offering insights crucial for their preservation. It also provides an in-depth examination of symptoms, diagnosis, and management of various afflictions, underscoring the ongoing need for research, monitoring, and education for the preservation of honeybee populations. The book also delves into strategies to manage different microbial diseases, along with pests of honeybees, and illuminates the potential and prospects of biological control, offering sustainable solutions to combat challenges. It also uncovers the therapeutic potential of propolis in the dental realm, outlining its multifaceted applications in various dental contexts. Finally, the book explores diverse aspects, including the sociocultural importance of indigenous bees, historical uses of propolis, and the chemical composition of geopropolis, and provides deep insight into ancient wisdom as well as the modern medicinal applications of bee products. *Melittology - New Advances* invites readers to embark on an enlightening journey, fostering a profound appreciation for the integral role bees play in our world.

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