A Interface do Conhecimento sobre Abelhas 2

José Max Barbosa Oliveira-Junior
Lenize Batista Calvão
(Organizadores)

Atena Editora
Ano 2020

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APRESENTAÇÃO

A coleção “**A Interface do Conhecimento sobre Abelhas 2**” é uma obra que tem como foco principal apresentar um arcabouço de conhecimento científico sobre as abelhas. As abelhas desenvolvem papel fundamental para equilíbrio dos ecossistemas terrestres através dos seus serviços ecológicos. Também são considerados pela sua importância econômica e nessa perspectiva podem ser fontes de renda para agricultura familiar, por exemplo. Mas os produtores devem conhecer a composição base dos diversos vegetais em seu entorno para aumentar o valor agregado de seus produtos. Contudo, o cenário mundial atual de destruição dos sistemas naturais, uso indiscriminado de agroquímicos, pesticidas contribuem substancialmente isoladamente ou em conjunto para o declínio de suas populações. Essas atividades antrópicas promovem perda de hábitat e de recursos essenciais as abelhas. Assim precisamos compreender de forma integrada como promover a conservação desses organismos. Nesse contexto, o objetivo central foi apresentar de forma categorizada e clara estudos desenvolvidos que avaliam de forma sistemática a importância desse grupo para o planeta.

Em todos esses trabalhos a linha condutora foi o aspecto relacionado à taxonomia, diversidade, bioindicadores, distribuição geográfica através de lista de espécies, métodos de captura, propriedades enérgicas de sua produção, saúde humana e áreas correlatas. O abastecimento de conhecimento de forma concisa, esclarecedora e também heterogênea em sua essência permite o leitor adquirir conhecimento sobre o grupo biológico e também avaliar o seu papel na natureza, uma vez que, o avanço das atividades antrópicas tem sido um fator preocupante e muito acelerado nos últimos anos. Este aumento se dá por diversos fatores que devem ser discutidos e caracterizados pelas políticas ambientais. Outro fator relevante é a coleta, armazenamento e manutenção desses organismos em coleções, que é fundamental para aumentar os estudos do grupo, bem como a descrição de novas espécies para ciência.

Temas diversos e interessantes são, deste modo, discutidos aqui com a proposta de fundamentar o conhecimento de acadêmicos, mestres e todos aqueles que de alguma forma se interessam pelo assunto. Deste modo a seleção do tema voltado para as abelhas, para publicação da Atena Editora, valoriza o esforço de discentes e docentes que desenvolvem seus trabalhos acadêmicos divulgando seus resultados e traz uma heterogeneidade de assuntos de um táxon que nos permite mergulhar em uma profunda avaliação sobre o tema de forma contínua e atualizada.

José Max Barbosa de Oliveira-Junior
Lenize Batista Calvão

SUMÁRIO

CAPÍTULO 9

POLLEN GRAINS AND THEIR BENEFITS IN APITHERAPY

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ABSTRACT: Bee pollen can be used in medicine to improve human health. It will show how pollen grains contribute to the therapeutic treatments that use bee products (Apitherapy) due to their various nutritional functions and medicinal properties, as their biochemical components act beneficially on human health. The chapter will also show studies that corroborate that the biochemical components of pollen grains depend essentially on their botanical origin, specifically the plants that are attractive to bees in which they were produced. The pollen grains will be described briefly with respect to their morphological types and bee pollination processes. It also describes the collection methods of bee pollen in the apiaries and the laboratorial method of preparation. It will further describe the methods for identification of the botanical origin through pollen grains. At last medical evidence will be presented to corroborate the health benefits of the use of bee pollen through a synthesis of several results from bibliographic references in the scientific literature.
KEYWORDS: bee pollen, therapeutic use of pollen, pollen and medicine, pollen components, natural product

OS GRÃOS DE PÓLEN E SEUS BENEFÍCIOS NA APITERAPIA

RESUMO: O pólen apícola pode ser usado na medicina para melhorar a saúde humana. Será mostrado como os grãos de pólen contribuem para os tratamentos terapêuticos que utilizam produtos apícolas (apiterapia) devido a suas várias funções nutricionais e propriedades medicinais, pois seus componentes bioquímicos atuam beneficamente na saúde humana. O capítulo também mostrará estudos que corroboram que os componentes bioquímicos dos grãos de pólen dependem essencialmente de sua origem botânica, especificamente das plantas nas quais foram produzidos e que são atrativas para as abelhas. Os grãos de pólen serão descritos brevemente em relação aos seus tipos morfológicos e o processo de polinização pelas abelhas. Também descreverá os métodos de coleta do **pólen** apícola nos apiários e o método laboratorial de preparação. Descreverá também os métodos para identificação da origem botânica pelos grãos de pólen. Por fim, serão apresentadas evidências médicas para corroborar os benefícios à saúde do uso do pólen apícola através da síntese de vários resultados a partir de referências bibliográficas da literatura científica.
PALAVRAS-CHAVE: pólen de abelha, uso terapêutico de pólen, pólen e medicina, componentes de pólen, produto natural

1 | INTRODUCTION

Pollen grains contribute to therapeutic treatments that use bee products (Apitherapy) due to their nutritional and medicinal properties, as their biochemical components are beneficial to human health. They are notable for containing substances such as carbohydrates, sugars, proteins, lipids, amino acids, micronutrients, minerals and vitamins.

Bee pollen has pharmacological properties as it contains bioactive compounds with antioxidant activity, which helps protect the human biological system. Among the bioactive compounds found in pollen, carotenoids and phenolic compounds (such as flavonoids, phenolic acids and phenolic diterpenes) have various nutritional functions and have been described as biological response modifiers, acting as antioxidants.

The biochemical components of pollen depend essentially on their botanical origin, i.e., from attractive plants to bees in which they were produced. In areas with uneven vegetation, rarely are there any similarities between the bee pollen produced in two different apiaries. Differences occur throughout the year when various flowers blossom at each place. Bee pollen samples produced in each bee colony will present a different pollen composition and consequently unique therapeutic properties.

Knowledge about bee flora is still scarce, and therefore a great deal of scientific research is needed to learn more about plants supplying pollen to bees in different parts of the world. Considering this, it is important to understand how pollen grains are formed in flowers, their role in fertilizing plants and why they are collected by bees.

In industry, bee pollen is beneficial as a food supplement, medicine and for use in cosmetics. In order to produce bee pollen, proper quality control and thorough inspection of products sold on the market are essential. However, many countries adopt illegal practices with regards to registering with regulatory agencies and inspecting bee pollen production chains.

Changes in legislation are essential or even implementing laws and regulations in various countries concerning laboratory testing to certify the botanical origin and biochemical components, as well as for the various stages of production aiming at marketing and medicinal use.

Pollen is one more piece of evidence that shows what is good for bees is also excellent for humanity.

1.1 Pollen grains

Pollen grains are very important for plants that produce seeds (spermatophytes). In this category, approximately 270,000 plant species are included, most of them existing land flora. Pollen is important for plants because it contains the male gametes that fertilize the egg and, thus, the embryo is formed in the seeds. The seeds germinate to give rise to new individuals that colonize the environment, expanding their population. In nature, cross-sexual reproduction ensures the genetic variability of the species, between and among populations, enabling evolution.

Until the 17th century, nothing was known about pollen and its role as a fertilizing source. It was only in the 18th century that the first observations were made, and experiments were developed showing that without pollen, fruits and seeds did not develop [1].

Produced in large quantities in the anthers of male flowers of Monocotyledons (currently called Liliopsida, which include, for example, lilies, corn and palm trees) and Eudicots (Magnoliopsida, such as guavas, papayas and oranges) as well as, in the anthers of the male cones of Gymnosperms (pine trees, *Araucaria*, conifer trees, cypresses, etc.) together comprise the pollen load of the pollen sac (i.e., each locule of the anther) (Figure 1). Seen with the naked eye, it looks like a very fine powder of various colors from whitish beige to dark brown, going through various shades of yellow.

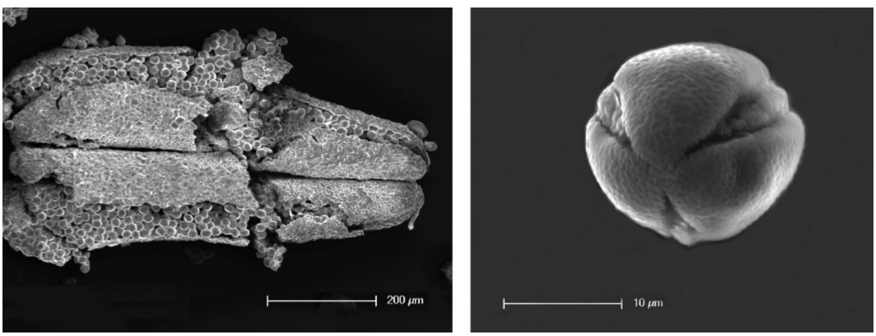

Figure 1. Scanning electron microscope images of anthers and pollen grains. **Left**: Anther with two locules of *Zornia diphylla* (L.) Pers, full of pollen grains. **Right**: A single pollen grain of *Zornia diphylla* (Source: Cynthia F. P. da Luz).

Transporting pollen to the female flower is called pollination and it takes place by pollinators such as wind, water (this type takes place in a small number of plants) or animals such as bees (Figure 2).

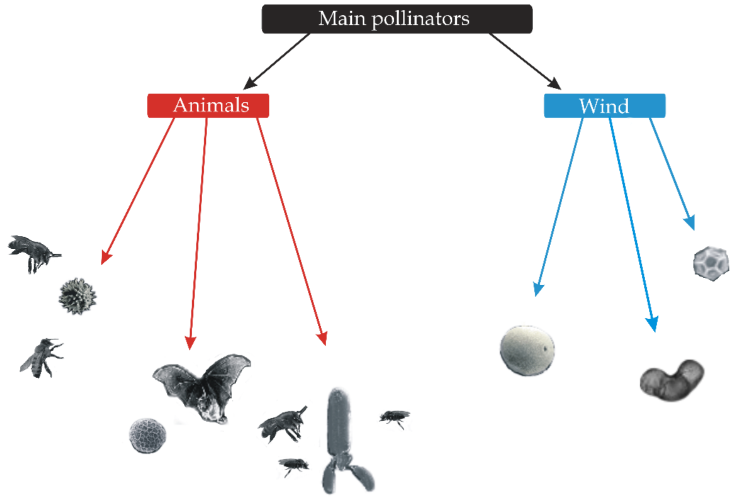

Figure 2. Pollen grains and main pollinators – the wind and animals (bees, bats, wasps, butterflies, flies). Zoophilous pollen grains generally show complex ornamentation structures and use oils to stick to the body of the animal and may be a single grain or grouped in more than one. The typical pollen grain pollinated by the wind has an aerodynamic shape with simple structures of ornamentation and sometimes with hollow spaces (Source: Cynthia F.P. da Luz).

For fertilization to take place, pollen grains have to attain the pistil of the stigma of the female flower of the same species and, afterwards germinate the pollen tube that takes the male gametes to the ovule inside the ovary (Figure 3). The pollen germ cells secrete enzymes that dissolve tissue and enables pollen tube cell growth.

In most plants, the proteins required for germinating pollen tubes are already found in mature pollen grains [2]. Germination of the pollen tube usually takes place very quickly and its growth rate is extremely high. In corn, for example, tube germination takes place within the first 5 mins of pollen deposition on the stigma, and this increases toward the ovary with rates close to one centimeter per hour, about 50 cm in 36 hours [3]. To conclude, pollen, which is a microscopic cellular structure, therefore has a strong vital potential.

Figure 3. Flower morphology of *Bombax* L. (Malvaceae). **a**. anthers, **b**. ovary with ovules; **c**. stamens; **d**. stigma; **e**. pollen grain (scale bar = 10 μm). Note that in this case, the flower has both male and female organs, which does not always occur in plant species. Drawing done by Cynthia F.P. da Luz.

The science of Palynology studies pollen grains in relation to their structure and formation. The history of Palynology is closely linked to the history of the microscope due to the small size of pollen grains ranging from 5 to 200 micrometers (a micrometer is equal to one millionth of a meter). Furthermore, all progress made in microscope technology reflected in developments concerning knowledge about pollen grain morphology as magnifying lenses and optical instruments had to be invented so that they could be studied.

A pollen grain imagined as a sphere has two poles and an equatorial area. The dimensions of the polar and equatorial axes vary among the pollen of plant species, which causes various shapes (circular, triangular, square, etc.) and surface ornamentation (Figure 4). Morphological analysis of pollen includes a number of descriptions about its size, symmetry, shape, type of apertures and aspects of its surface [4-6].

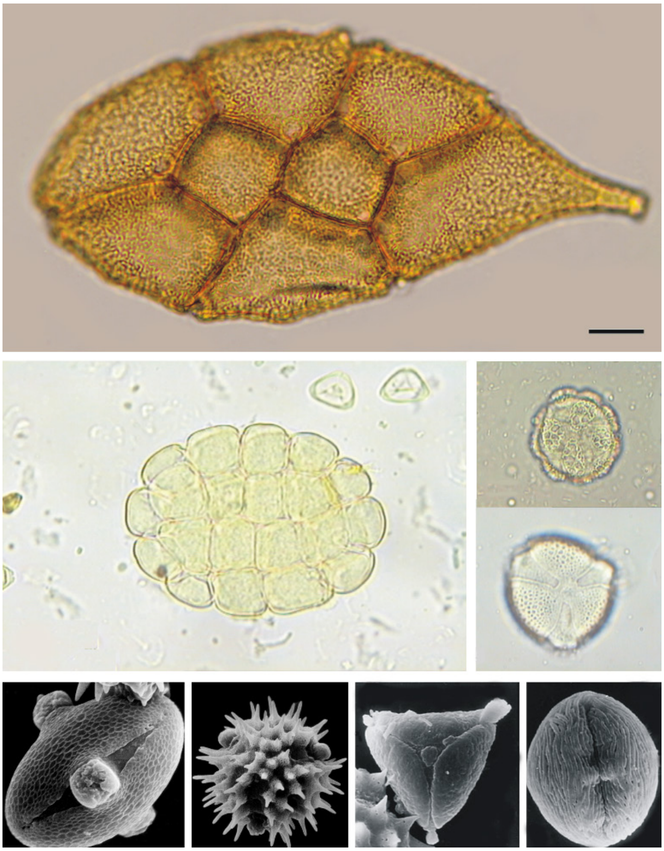

Figure 4. Light photomicrographs (LM) and scanning electron microscope (SEM) images of polyads and pollen grains. **Top**: general view of the polyad of *Calliandra* Benth. (Fabaceae) (LM). **Middle**: general view of the polyad of *Inga* Mill. (Fabaceae) (LM). *Mansoa* DC. (Bignoniaceae) pollen grain (LM). Caesalpiniaceae pollen grain (LM). **Bottom**: *Triumfetta* L. (Malvaceae) pollen grain (SEM). *Vernonia* Cass. (Asteraceae) pollen grain (SEM). *Eucalyptus* L'Hér. (Myrtaceae) pollen grain (SEM). *Schinus* L. (Anacardiaceae) pollen grain (SEM) (Scale bar of the LM photomicrographs = 20 μm).

The palynological bibliography is extensive as there are pollen catalogs, palynology identification keys and articles of pollen morphology that help identify pollen grains contained in bee products [7-14], among others.

Having this detailed knowledge, it was observed that unrelated plants may have similar pollen while some plant families have more than one type of pollen morphology among their species. Nevertheless, each species generally has pollen with a typical morphology and characterizing it helps palynologists to identify plants in which pollen derives. Thus, their botanical origin can be determined.

1.2 Honeybees and pollen: an ancient relationship

Flowering plants were the last group of plants that evolved on Earth. There is some controversy about which period they appeared. The dominant idea nowadays is that similar structures with floral apparatus first appeared during the Cretaceous period (from 130 to 140 million years before present), whose plants coexisted with dinosaurs. However, in recent geological surveys, six types of fossil pollen grains were found dating back about 250 million years, showing high diversification among flowering plants before the Cretaceous period, during the Triassic period, suggesting that flowering plants originated 100 million years earlier than scientists had previously thought [15-16].

On the other hand, the oldest traces of bees are preserved in small pieces of transparent amber. One of them is a bee which is believed to be more than 100 million years old [17]. Probably bees have an African origin and, as they are very old, they help explain the evolutionary diversification of flowering plants. As scientists suppose that bees essentially always pollinated flowers, it is assumed that they affected the evolution of plants, spreading pollen from certain types of flowers that they preferred to pollinate and, thus helped to “create” new species. Due to the close interaction with bees and other pollinators that are responsible for transporting and spreading pollen, flowering plants have developed various mechanisms to attract them and ensure fertilization, adjusting color, shape and floral scent to each specific pollinator, offering resources such as nectar and pollen [17-18]. Bees and plants mutually benefit from this relationship - the bees need nectar and pollen to sustain themselves and, in turn, the flowers need pollinators to initiate pollination and afterwards develop their seeds [19].

Hymenoptera is the third largest order in terms of number of species of the Insect class, which includes ants, wasps and bees. Most bee species (Apidae) are extremely dependent on floral sources for their survival [20]. Bees are considered more important for the conservation of plant species, for hosting the largest number of pollinators which are found in nectar and pollen, which is their main source of food and energy [21-22].

These sources are used for feeding the offspring (mainly pollen) and the adults (especially nectar) [20]. Worker bees collect pollen from flowers to perform their tasks in the hive due to its high nutritional value. When flying from flower to flower bees spread pollination and are responsible for the production of fruit and seeds of many plant species with seeds in the world and, thus, indirectly ensures the supply to countless other animals [23].

The pollen collected by certain species of bees (including the honeybees *Apis mellifera* L.) from the stamens of flowers is agglutinated into the tibia on its hind legs (called *corbiculae*, *scopas* or pollen basket), moistened with a small amount of nectar and salivary substances, which helps transport large quantities to the hive [24]. Therefore, it is called “pollen pellets”, “bee pollen” or “pollen load” (figure 5) to differentiate pollen from the anthers that were not moistened by saliva or nectar [25].

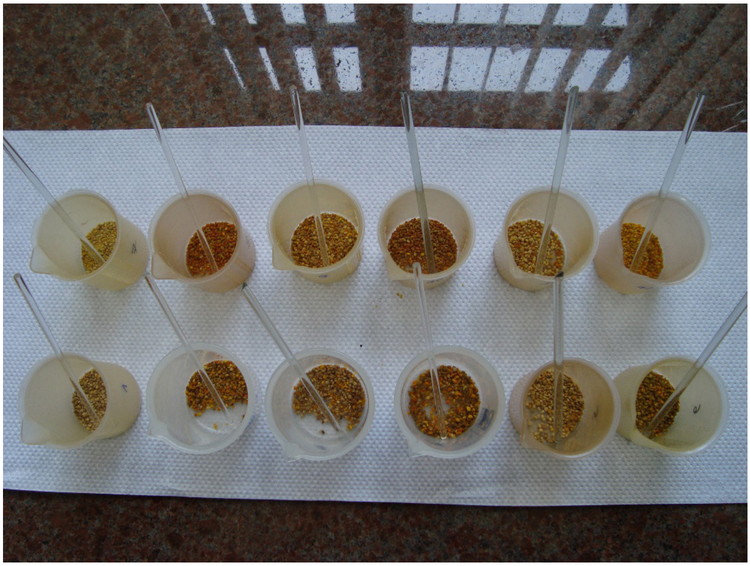

Figure 5. Bee pollen in preparation for laboratory analysis (Source: Cynthia F. P. da Luz).

Honeybees do not consume pollen directly at the harvest site. After flying from flower to flower to collect the pollen, they return to the hive loaded. Each pollen load can weigh from 7.60 to 10.7 mg in honeybees. For every flight that a honeybee makes, it returns to the hive with two pollen loads. Each honeybee can make 80 flights per day. This means that each individual can produce 160 pollen loads daily. As a mature beehive has on average 50,000 honeybees, the bee pollen production can be huge [26].

In a hive, the pollen pellet is pressed into the honeycomb cells that are suitable for storage, which are called “bee bread”. Phytocidal acid produced from the hypopharyngeal glands of these insects is added to the “bee bread” causing pre-digestion of pollen, which prevents germination and fermentation and helps to store it for a long period [24].

After honey, which is prepared from nectar and is the main source of carbohydrates for bees, pollen is the most consumed product in the hive and has the highest content of protein and contains minerals and lipids. Pollen is the main source of protein and lipids for larvae, perhaps for all species and genera of Apidae bees as the amount of protein and fat in the nectar is insignificant. Pollen contains most, if not all, of the essential nutrients to produce royal jelly. Adult worker bees obtain protein feeding directly from the pollen as the larvae of both sexes and the queen throughout her five-year life cycle receive royal jelly enriched with pollen produced by the worker bees [27]. It is estimated that every worker bee consumes around 120 mg of pollen during their short life of 28 to 48 days.

Therefore, for an average developed beehive, it is calculated that 40 to 60 kg of pollen is consumed per year [28]. Thus, pollen is essential for the growth and development of all individuals of a bee colony. Without it, the swarm does not develop and in a short amount of time (three to four days) it can die. However, pollen grains have different nutritional content for bees, as they differ in their chemical composition depending on the production plant [29-37]. Ultimately, their nutritional value depends on the physical and chemical availability of the soil and the environment in which the plant lives. It is known that bees fed with particular types of pollen develop faster than other types, because each pollen type has a different amount of vitamins, proteins, carbohydrates, minerals and sugars [38].

Therefore, it is important to emphasize that the medicinal properties of bee pollen depend on bee flora surrounding the apiary. In their daily search for pollen, honeybees tend to focus on only one flower species whose individuals are brought together in quite a dense population. However, if they do not find an enough quantity, they visit other flowers and can mix diverse pollen types in the same pollen load. The monofloral bee pollen (from the same species) has constant organoleptic and biochemical properties, while the heterofloral (from flowers of various species) have varying properties.

Knowing the bee flora, i.e. attractive vegetation for bees in an apiary, is essential for controlling a company´s productivity and the quality of bee pollen that is produced there. Calendars can be made regarding polliniferous flowering for each location by identifying the plants in an apiary, as well as analysing pollen grains in a laboratory. These calendars show the monthly flow of pollen supply to the bees. This information is essential to help beekeepers choose the best place to put their hives, as well as to check the waiting periods regarding pollen availability (inter-harvest or "non-flowering" period), and proving the botanical and geographical origin of the bee pollen aiming to certify samples sold on a commercial basis [39-43, 30, 44-48].

In countries as large as Brazil, there is very diverse vegetation. Certainly, many botanical species were not observed by beekeepers when the honeybees collected the pollen. Probably, these plants still cannot be found in the scientific literature concerning Neotropical bee flora. Therefore, much research needs to be carried out on the botanical and geographical origin of bee pollen.

2 | BEE POLLEN AND HUMAN HEALTH

For centuries, empirical medicine used pollen by attributing different virtues. In ancient Greece, for example, there was the "drink of immortality", which was a mixture of honey and pollen called "Ambrosia" and is considered by the Greeks as the inexhaustible source of power for the body [49-50].

Many benefits are attributed to pollen consumption such as being an extraordinary fortifier of the organism, stimulating and leading to well-being and physical force; a food supplement, which results in functional balance [51]. Various scientific studies have found that bee pollen benefits human health [52-57], among others. In others, their effectiveness could not be proved.

Various issues concerning their production, processing and storage need control to prevent marketing low quality products and ensure safety for human consumption [58].

2.3 Collection methods of bee pollen for consumption

Primitive people knew about honeybees and used their products and derivatives. In Europe, Asia and Africa there are reports and drawings that enable us to infer that bees have been exploited by man for almost 50,000 years. Prehistoric drawings in Valencia (Spain), dating back 10,000 years, show a man collecting honey from a hive [59]. However, without the development of beekeeping, i.e. rational beekeeping for commercial purposes, bee products could not have been used by people on a daily basis because removing them without any kind of handling is very complicated and always predatory. The earliest record of keeping bees in hives were found on the walls of the Sun Temple, erected near Cairo. The honeybee was the symbol of Lower Egypt and beehives made of clay and straw were unearthed in archaeological sites indicating that the Egyptians already knew how to handle them about 2,400 years BC. Papyri dated back to 256 BC show a beekeeper who owned 5,000 beehives. From Egypt, beekeeping spread among the Greeks and Romans. Excavations in the Gulf of Salerno found clay amphorae that were still full of honey from apiaries with beehives made of “straw” (“colmo”, “culmo” in Portuguese and Spanish languages) braided in a bell-shape, as the Romans also used to do. Using “straw” (“colmo”, “culmo”) for this purpose gives the name “colmeia, colmena” (honeycomb).

Honeybees have always been very important, so much so that various peoples valued the trade and literature, engraving and stamping bees onto coins, medals and clothing. During the Middle Ages, in some regions of Europe, swarms were recorded in the notary and left inheritance [60-61].

Aristotle, born in Athens (384 to 322 BC), was responsible for the earliest formal studies about bees. However, for centuries beekeeping was rudimentary and primitive. Only in the 17th century, with the help of the microscope, were important discoveries made about the biological aspects of bees and better equipment was invented for their rational culture and economic exploitation.

Nowadays, beekeeping is practised using highly technical and scientific methods. Beekeeping uses equipment and materials especially designed to help collect bee products from apiaries conveniently located aiming to improve the work of bees. Hives are placed in apiaries in strategic areas where there is bee flora, water resources and easy access to agricultural transport [62].

To collect bee pollen from the *corbiculae* of honeybees returning from fields and forests, pollen traps are set at the hive entrance of the strongest colonies, as they store the most. The pollen trap is a kind of screen with narrow openings. When the bees go through the screen, they dislodge the pollen from the *corbiculae*, which is stored on a tray outside. There are many types of pollen traps (Auger-hole, galvanized steel sheet, bottom, frontal, intermediate, among others). Not all the pollen is collected by the beekeeper so as not to weaken the hive that needs it for their food. As the screen also has larger holes, two thirds become "bee bread", which is consumed by individuals in the beehive.

Bee pollen has a range of colours due to the fact that it is collected from different floral sources (figure 6). It is not possible to evaluate its botanical origin only by the colouring, as various types of pollen are the same colour [63, 31]. It can be observed from scientific research already carried out that there is inaccurate product labelling of bee pollen sold on the market. Common names presented on labels show plants whose type of pollen was absent or present only at a low percentage, while the dominant pollen in the samples was not mentioned by the bee producer or was not correct. These results show the need to investigate pollen analysis (palynology) in laboratories to determine the floral origin and to certify the quality of the product, even where the biochemical composition meets the official specifications of each country [64]. The physicochemical characteristics and the botanical origin of bee pollen are still not well known, particularly in tropical regions where there is great diversity of bee flora associated with high temperatures and humidity [63, 31].

Figure 6. Apiary in the Atlantic forest at Tapiraí municipality, São Paulo, Brazil (24° 02' S, 47° 32' W) and light photomicrographs (LM) images of polyads and pollen grains from bee pollen samples produced in the local. **Top**: general aspect of the flora surrounding the apiary. **Middle:** polyad of *Anadenanthera* Speg. (Fabaceae). *Serjania* Mill. pollen grain (Sapindaceae). *Hyptis* Jacq. pollen grain (Lamiaceae). *Vernonia* Cass. pollen grain (Asteraceae). **Bottom:** general view of the mountains near the apiary and bee pollen collect at the apiary (Scale bar of the LM photomicrographs = 10 μm) (Source: Cynthia F. P. da Luz).

In Apitherapy, fresh pollen has a better reputation than dehydrated pollen, however, post-harvest handling is more difficult because it requires refrigerated storage [65]. This is because the composition of bee pollen with a high nutritional value encourages the growth of a wide range of microorganisms. Frozen pollen can have a higher content of phenolic compounds and a higher blocking effect, while dehydrated pollen has a higher content of total flavonoids and antioxidant activity [66].

Food safety is an extremely important aspect to be considered in bee pollen. Some beekeepers are not properly trained to control handling techniques of production and processing bee pollen, thus ensuring the quality of the product. Technical advice is required and questions arise concerning personal value changes, improving handling techniques, knowledge of good practices and, finally adopting permanent and routine control practices that maintain product quality [50]. As pollen has a level of protein, it can quickly lose its nutritional value if handled or stored improperly [67].

Cleaning equipment and materials used at different stages of producing and processing bee pollen is very important. Various studies have characterized the steps required for collecting, processing and marketing bee pollen [68-71, 58], etc. Quality is guaranteed using simple measures such as: 1) locating the apiary far from any agricultural area that uses pesticides; 2) not medicating bees; 3) maintaining a conduct of sanitation and hygiene at all stages of production and processing; 4) cleaning tools and machinery using hot water before use; 5) containers that hold the product must be new and approved for food use; 6) care must be taken when fumigating because it may produce nasty smells and an unpleasant taste of the bee pollen; 7) manipulation and mixing bee products should be avoided; 8) bee pollen should be stored in a freezer after harvesting it and, 9) never store other products, whatever they are, with bee products [72, 50].

The bee pollen collected from the pollen traps is placed into buckets that are left in freezers for at least 48 hours, before dehydrating the product. The cold destroys mites, eggs and moth larvae that might be in the pollen. The cold also works as a controller and stabilizer for the development and proliferation of microorganisms that came with the product from the fields. After freezing, thin layers of pollen are placed on trays, which are then dehydrated in an oven. The circulation of hot air at 40-42°C takes 8 to 12 hours (or longer, depending on the initial moisture as it arrives at the apiary with about 20-30% of its weight in water), until it reaches the maximum of 4% moisture. Dehydration preserves pollen from deterioration caused by fungi and bacteria [50].

The time of year, it was produced and the permanence of pollen in the apiaries influences contamination by fungi and yeasts, which can cause an accumulation of mycotoxins. These natural contaminants are a result of secondary metabolites of fungi and the most toxic are aflatoxins and ochratoxin A (OTA) [73]. Fungi producers of bee pollen mycotoxins belong to the genera *Aspergillus, Penicillium, Fusarium* and *Alternaria* [74]. The most commonly found yeast is *Candida magnoliae* [75]. It was observed that most species of bacteria found in pollen come from the pollen traps, suggesting that bees are the main source of contamination. The bacteria that predominated in bee pollen and “bee bread” were species belonging to the genus *Bacillus* (*B. subtilis, B. megaterium, B. licheniformis, B. pumilis* and *B. circulans*) [75].

Therefore, drying procedures should be optimised to obtain products free from microbial contaminants. According to [70], the drying temperature by hot air up to 42°C is too low and can still allow for microbial growth, and therefore its microbiological quality needs to be constantly evaluated. Microbiological evaluation is what informs us about the shelf life and potential risks to the health of the population, i.e., the potential to transmit food-related diseases [76].

In the scientific literature, there are even reports about bees collecting fungi (*Cladosporium* sp) to meet the protein needs of their colony due to the low supply of pollen near the apiary [31]. The nutritional composition of fungal loads presented higher levels of protein than the pollen collected in the same period and similar values of ether extract, organic matter, mineral matter and total carbohydrates.

Despite the high protein value of fungi, they are saprophytic (they absorb substances normally from decaying organic matter) and can induce the occurrence of diseases in hives, negatively affecting their balance [31, 77-79]. Nothing is known about the action of these fungal loads collected by bees. Fungal loads are black and have low moisture, which makes them very rigid, but for the layman consumer, there is no way to distinguish them in the commercialized batch as they are mixed with pollen. Only when an expert analyses them in a laboratory will their origin be clear, which is important in terms of quality control for human consumption.

Another relevant issue is buying counterfeit goods. Consumers who buy bee pollen should be aware of the labels, checking if they comply with the standard required by the government health authorities of each country. Analyses should be carried out jointly by various specialists, chemists, microbiologists and palynologists to ensure quality control of bee pollen and the real value of what is being consumed.

2.4 Using bee pollen as a food supplement

Conventional wisdom has always attributed healing properties to bee products. However, people do not usually consume pollen. This is probably because their extremely beneficial nutritional properties are unknown. Pollen is consumed daily as a food supplement by natural food lovers, who try to learn about the benefits of food, which make up a balanced diet.

What was considered conventional wisdom is confirmed by science because pollen has almost all the elements that humans need for feeding themselves [80]. Research compared the average protein, fat, minerals and vitamin content of bee pollen with other staple foods. It is richer in ingredients than beef, fried chicken, beans, whole wheat bread, apples, raw cabbage and tomatoes when compared on the basis of weight or calorie content. Although comparable in proteins and mineral content with meat and beans, the average thiamine and riboflavin of bee pollen are ten times higher than these foods or several times in relation to the content of niacin. However, it is usually consumed in such small quantities that the daily requirement of vitamins, minerals and proteins are not only met by using it. Yet, when consumed daily, it can be an important source of essential nutrients in a diet, especially in cases of chronic malnutrition [81].

It should be mentioned that 10 to 33% of bee pollen weight consists of proteins. Half of the proteins found in pollen is in the form of free amino acids: glutamic acid, arginine, cystine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Therefore, it can be observed how rich this food is as it is one of the few that has all the essential amino acids in its composition that the human body alone cannot synthesize and must be replenished daily by meals [50]. It is also rich in carbohydrates (20 to 40%) or/and lipids (1 to 14%) [82-86, 25, 27].

Bee pollen is called “a superfood” in academic circles because it contains protein similar to a beefsteak (around 20%). The lipid that the pollen may presents is a lipid with antioxidant properties. Bee pollen has 30% to 40% of three types of sugars: glucose, fructose and dextrins. As they are simple sugars, digestion is quick and the body absorbs them immediately. Therefore, they are excellent for those who need energy immediately. Pollen also provides cellulose fibre with the chemical structure of carbohydrates, which stimulates bowel movements. It also has organic acids, flavonoids, carotenoids, terpenes, free amino acids, nucleic acids, enzymes and growth regulators [82-85, 25, 27].

Bee pollen comprises between 2.5 to 3.5% of micronutrients and minerals (calcium, sulphur, phosphorus, magnesium, potassium, sodium, traces of aluminium, boron, chlorine, copper, chromium, tin, strontium, iron, fluorine, iodine, manganese, molybdenum, nickel, selenium, silicon, vanadium and zinc) [82-86, 25, 27]. Some minerals such as calcium, sulphur, phosphorus, magnesium, potassium, sodium and chlorine are required by our body in relatively high amounts (more than 100mg per day) and, therefore, are called mineral macronutrients or macroelements. Yet, other minerals, such as iron and zinc are called mineral micronutrients or microelements because they are needed by the body in relatively low amounts.

Bee pollen does not only have antioxidant vitamins (vitamins C and E, β-carotene, such as pro-vitamin A), but also those of the B complex (B_1, B_2, B_3, B_5, B_6, B_7, B_8, B_9) and vitamin D. The percentages of each vitamin vary according to the botanical origin of bee pollen; some have more than others [82-86, 25, 27]. The fat-soluble vitamins (A, D, E, K), which can be stored in body fat, can become toxic when consumed in excess. Whereas water-soluble vitamins (B_1, B_2, B_3, B_5, B_6, B_8, B_9, B_{12}, C) are normally not stored in significant quantities in the body, which leads to the need for daily consumption [87-89]. As pollen is rich in B complex vitamins, it is an excellent vitamin supplement [89].

Therefore, bee pollen may be regarded as a dietary supplement and a nutrient and energy source for humans. It is recommended that five grams of bee pollen is consumed per day or the equivalent of a tablespoon. Intake should not necessarily be in a pure state. There are already many flavoursome ways to ensure a daily intake of this food such as in salad dressings, fruit salad, pates, cakes, pollen biscuits and sweets.

It is worth mentioning, however, that a problem of standardising nutrients that may be present in each batch of commercialised bee pollen is, in most cases, due to the lack of identifying the floral species of the product. It is recognized that each plant species influences the type and amount of components in pollen. In other words, the physical and chemical composition of commercialised batches of bee pollen depends on its botanical and geographical origin, i.e., climate conditions and the type of soil where the plants grew, as well as the species, age and nutritional status of the plant that produced them. All the various types of pollen found in each sample also influence its biochemical components.

Preserving its biochemical components depends on the production conditions. Due to the nutritional and functional importance of the components found in bee pollen, the various development processes should be controlled and monitored so as to ensure consumers are supplied with all the nutrients to process it, as well as to meet the sanitary requirements and preservation of its organoleptic properties [71].

According to legislation from the Ministry of Agriculture, Livestock and Supply in Brazil [25], the quality of bee pollen must comply with certain physical and chemical standards and present maximum and minimum requirements: maximum moisture of 4% (dehydrated pollen) or 30% (fresh pollen); maximum ash of 4%; minimum lipids of 1.8%; minimum proteins of 8%; total sugar from 14.5% to 55%; minimum crude fiber of 2%; maximum free acidity of 300 mEq.Kg-1 and pH from 4 to 6. The standards are similar in other countries that already have them such as Argentina and Spain, among others.

Because of its high protein content, storing pollen incorrectly causes a rapid loss of its nutritional value due to Maillard reactions. The Maillard reaction is a phenomenon that affects foods which undergo heating processes, making them browner. On the one hand, the heat ensures microbiological safety, inactivation of certain enzymes, degradation of toxic substances and the development of substances responsible for the aroma, color and flavor, improving its palatability. On the other hand, it can interfere with important nutritional processes such as decreasing the bioavailability of minerals and the biological value of proteins [29, 90]. After consumers buy it, they should avoid storing pollen in places with direct sunlight or excessive heat.

2.5 Using bee pollen in cosmetics

In addition to using it as a food supplement, pollen is used in other sectors such as pharmacology, utilized as an ingredient in phyto-aromatic products and cosmetics in sunscreens, creams, masks, lipsticks, soaps, shampoos, etc. [91]. Bee pollen has been included in some cosmetics mainly for rejuvenation and skin nutrition. However, its effectiveness has still not been proved, on the contrary, there is a great risk of part of the population developing skin allergies [81]. Including alcoholic or aqueous extracts in cosmetic formulations does not appear to cause allergic reactions, or rarely causes them [81]. The cause of such allergies is attributed to the type of pollen used in cosmetic products. Some plant families include many plants that have allergenic pollen, such as Poaceae, among others. Therefore, more research is needed concerning this use of bee pollen.

2.6 Medicinal properties of bee pollen

Bee pollen stands out in terms of maintaining human health due to its richness in compounds and bioactive properties. Bee pollen can be considered a functional food supplement. "Functional food (containing bioactive compounds) is all food or ingredients that, apart from basic nutritional functions, when consumed in a normal diet, produce metabolic effects and/or is physiologically beneficial to health and should be safe for consumption without medical supervision" (Decree nº 398, 30/04/99 of the Sanitary Surveillance Administration from the Ministry of Health, Brazil). In other words, functional foods are those that contain physiologically active substances (bioactive compounds), providing health benefits, beyond nutrition.

Most functional nutrients have a common property: they are antioxidants. It is well known that free radicals are involved in almost all cell degradation processes such as cardiovascular disease, arthritis, cancer, diabetes, and may also be responsible for Parkinson's and Alzheimer's disease [92]. Epidemiological studies indicate that the main antioxidant nutrients are beneficial in preventing various chronic diseases caused by free radicals.

A large number of articles have been published in an attempt to find a relationship between the antioxidant activity and pollen components [93]. It was not possible to establish an absolute link between them, but it was observed that the main role can be attributed to the presence of phenolic compounds in bee pollen [94-98]. It was also observed that antioxidant activity is specific to the species of the pollen producer plant and is independent from its geographical origin and harvest season, and that it decreases when the pollen aged, especially if storage conditions are not ideal [99-100, 96]. In other words, the concentration of bioactive compounds and antioxidant activity of bee pollen may vary due to its botanical origin as observed in various studies [101-103, 86, 93].

Therefore, the antioxidant activity of pollen may be used as a marker for its bioactivity as it is possibly connected to others, for example, anti-inflammatory activity, antiatherosclerotic or anti-cancer [93].

In an interview on Brazilian TV [104], the researcher Dr. Ligia Bicudo de Almeida Muradian from the School of Pharmaceutical Sciences, the University of São Paulo (Brazil) said that research on bee pollen shows that it can help fight ageing diseases because "pollen has three antioxidant vitamins: beta carotene as pro-vitamin A, vitamin C and vitamin E". Pollen is also rich in vitamin B complex, which helps, for example, the central nervous system to function. It also helps to prevent and treat cataracts. The antioxidant vitamins of pollen are able to stabilise free radicals, which are involved in many degenerative diseases characteristics of ageing, which either come from the outside environment or are natural results of the natural metabolism. Concerning antioxidant activities, the bioactive properties of pollen enhance human health acting as a tonic, antibacterial, antifungal, anti-inflammatory, immunomodulatory and anti-cancer, exerting antioxidant functions and inhibiting the damaging action of free radicals, helping to delay ageing [104].

Research carried out on hydrosoluble and liposoluble extracts of bee pollen solution showed that pollen has no teratological effect (congenital malformation of the foetus). In addition, they increase protein synthesis, accelerate the wound healing process and increase hepatic triglyceride content. They have remarkable powers on anorexia, weight loss and weakness. In general, pollen shows a positive action on the digestive tract, in the increase in blood hemoglobin, it protects the vascular system; it has a beneficial effect on eyestrain and prostatism and attenuates brain ageing [27, 50, 70, 105-106].

Research carried out on elderly people showed that there may be a positive relationship between the daily consumption of bee pollen and oriented physical activity, enhancing dynamic balance, improving walking time and increasing the strength of the lower limbs [107].

Its effects on arteriosclerosis and tumours proved to be beneficial [108-109].

In traditional Chinese medicine, a mixture of bee pollen from *Radix polygoni multiflore, Semen ziziphi spinosae, Radix salviae multiorhizae, Fructus schisandrae* and *Fructus ligustris lucidae*, known as “NaO Li Su” is used as a “remedy” to treat memory loss [93]. But, [110] evaluated the effect of this preparation in 100 elderly volunteers verifying that after the treatment there was no improvement in memory. However, it generated a significant increase in the number of red blood cells and serum creatinine levels. Based on this study by [110], [93] suggested that the remarkable antioxidant activity of bee pollen may have been the key factor in achieving this result.

Research and clinical tests developed over several decades have shown that bee pollen is effective in the treatment of prostate problems ranging from infection and swelling to cancer [111-112].

Certain bacteriostatic effects have been demonstrated in bee pollen [113], but they were attributed to the addition of glucose oxidase (the same enzyme responsible for antibacterial action in honey) by bees when they mix regurgitated honey or nectar with the pollen in the corbiculae [114]. Therefore, this activity may vary between different pollen loads and is much higher in “beebread” [81].

There is evidence that ingested pollen can protect animals and humans against the adverse effects of x-ray radiation treatments [115].

Despite all the benefits of bee pollen, some care must be taken concerning contamination by toxic components that they may have. Normally, bee pollen is tolerated well by the human organism, although it is known that there are allergenic substances in some kinds of pollen, which can cause anaphylactic reactions in some allergic people [116-117].

Another problem that requires attention in particular, types of pollen is related to possible adverse effects due to pyrrolizidine alkaloids (PA) and their derivatives. The occurrence of PAs is restricted to Angiosperms and limited to four families: Asteraceae (Senecioneae and Eupatorieae tribe), Boraginaceae, Apocynaceae and the *Crotalaria* genus of Fabaceae [118-119]. The PA content in bee pollen is significantly less than the levels detected in flowers [119]. In 2008 [120] described these alkaloids in the bee pollen of *Echium vulgare, Echium plantagineum, Eupatorium cannabinum, Senecio jacobaea* and *Senecio ovatus*, which are known to have hepatotoxic, pneumotoxic, genotoxic and carcinogenic activity [121]. Any adverse symptoms after ingestion and the use of bee pollen should be investigated by clinicians. Considering the adverse effects of these alkaloids, the botanical origin of pollen for human consumption should be monitored, at least regarding the processing conditions and/or knowledge of the producing regions and should be clearly specified on the label of the commercialised product, which can significantly reduce bee pollen commerce potentially hepatotoxic, carcinogenic and genotoxic.

According to [119], a maximum allowed limit of PAs should be established for bee pollen commerce and bee pollen with high levels of PAs should be taken off the market. However, the author points out that it seems unrealistic, due to the common practice of mixing different batches of bee pollen in sales outlets. Thus, it is suggested that in terms of preventive consumer protection, it would be more effective to train farmers/beekeepers directly to avoid PA accumulating in bee pollen, avoiding bee pollen harvests in blooming seasons which present PAs or moving the hives to another place, minimizing its entry into the food chain.

One of the biggest problems that the world faces nowadays is the use of pesticides and other products in agriculture, which have contaminated bee products. Out of the total number of pesticides used in Brazil, about 30% are insecticides, and from these about 40% are considered toxic to bees [122-123]. Many of the insecticides currently used are systemic and can be found both in plants used by bees in bee products from them, such as pollen and nectar, as demonstrated by [124].

Studies have shown that there are pesticide residues in pollen and nectar samples in flowers and in colony stocks [125-128]. Thus, prior to commercialising bee products from areas affected by pesticide use, their levels in bee pollen produced there should be identified to find out the real risks to human health.

Furthermore, bee pollen analyses from urban areas have confirmed further contamination by pollutants. The scientific literature has shown that bee pollen could be a bio-indicator of environmental pollution and effective control is needed so that the quality is ensured for human consumption. In Brazil, the aluminum, barium and nickel content were the most common inorganic contaminants in bee pollen, followed by chromium, cobalt, cadmium, lead, arsenic, antimony and mercury. If bee pollen from polluted areas is regularly consumed by adults (daily intake of 25g), this can be a vehicle for aluminium and arsenic with average contents of 27% and 12%, respectively, of the tolerable weekly intake [129]. Studies on the botanical origin of samples which are based on Palynology are essential to identify the bee pollen commerce contaminated by toxic components.

3 | CONCLUSIONS

Bee pollen is considered a complete natural food due to the fact that it contains all the essential amino acids needed by human beings. Because of its bioactive properties, bee pollen has a preventive effect against some human diseases. Bee pollen is therefore considered a functional food of high nutritional value.

Given the growing Apitherapy interest in its use, the storage process of bee pollen needs to be controlled and monitored so as to ensure that the product provides consumers with all the nutrients available, maintaining its organoleptic properties. This care ranges from harvesting by beekeepers in the pollen traps to its final storage on the shelves of sales outlets.

Quality control of bee pollen must be strict, evaluating the accuracy of the information on the products´ labels. Ideally, each batch sold on the market would show its botanical origin, characterized by the Palynology, as well as its biochemical and nutritional composition according to systematic methods of characterisation concerning their constituents. The allergenic content for each batch of bee pollen commercialised, as well as its microbiological quality, presence of pyrrolizidine alkaloids, pesticides and pollutants should also be subject to ongoing assessment to ensure the safety of consumer products.

However, existing legislation in various places around the world requires none of this and what is worse is that many producers still adopt illegal practices, even selling counterfeit goods. In these places, there is still no official intervention from governments to address these issues of production and commercialising bee pollen. In the tropics, where the flora is diverse and uneven, the inexistence of these analyses leads to errors in labelling and uncertainties in the quality and bioactive properties of the product.

When the quality of the product is ensured, the numerous benefits of bee pollen for human health are guaranteed and its use in Apitherapy is extremely relevant.

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ÍNDICE REMISSIVO

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