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Review

The phytochemistry of the honeybee

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ABSTRACT

Honeybees rely on plants for everything they need to keep the colony running; plant nectar and pollen are their only carbohydrate and protein food sources. By foraging to satisfy their basic nutritional demand, honeybees inevitably gather specialized plant metabolites as part of the nectar and pollen. In general, these compounds possess biological activity which may become relevant in fighting pests and pathogens in the hive. The third plant derived bee product, besides honey and bee pollen, is propolis (bee glue), which comes from plant resins. It is not a food; it is used as a building material and a defensive substance. Thus, the beehive is rich in specialized plant metabolites, produced by many different plant species and the expression “Phytochemistry of honeybees” is not inappropriate. However, it is virtually impossible to perform a detailed overview of the phytochemical features of honey and pollen in a review article of this nature, for reasons of space. The present review deals with propolis, because it is the bee product with highest concentration of specialized plant metabolites and has valuable pharmacological activities. The most recent developments concerning plant sources of propolis, bees' preferences to particular plants, the application of metabolomic approaches and chemometrics to propolis research and the problems concerning standardization of propolis are summarized. The overview covers the literature published in the last decade, after 2007.

1. Introduction

Honeybees *Apis mellifera* L. have been living on Earth for over 125 million years, probably for as long as flowering plants. Nowadays bees are at home in almost all habitats in the world: from the equatorial rainforests and tropical deserts to the Subarctic regions of Eurasia and North America. This remarkable evolutionary success is as much due to the sophisticated social organization of honeybees as it is due to the products which honeybees manufacture, products which are essential for the life of the colony, serving as food, building materials and defensive weapons. Three of these products: beeswax, venom, and royal jelly, are chemically synthesized by the bees themselves. The other three - honey, pollen and propolis - are derived from plants and are modified by the bees for their own use.

Actually, honeybees rely on plants for everything they need to keep the colony running. The long co-evolution of bees and flowering plants has resulted in a complicated and mutually beneficial bee-plant relationship, combining activities of two very different types of genetic inheritance. For the honeybees, plant nectar and pollen are the main carbohydrate and protein food sources (Erler and Moritz, 2016). On the other hand, plants need pollinators and thus they have to attract bees, and in order to do so they offer different kinds of rewards. Floral nectar represents the main plant reward for many pollinators, including honeybees. Nectar is basically a sugar solution; the three most common nectar sugars are the disaccharide sucrose and the two hexoses glucose and fructose (of course, there are some differences between different plant species) (Chalcoff et al., 2005). Bees make honey mainly from the nectar by evaporating water from it, and while doing so secrete into it enzymes: invertase and glucose oxidase. The sucrose in the nectar is inverted to glucose and fructose in the bee's stomach through the action of the enzyme invertase. Preservation of honey is achieved through evaporation of water and hydrogen peroxide formation by the action of glucose oxidase on glucose. Honey is the bees' carbohydrate food source, while the most important source of proteins for bee survival is bee pollen. Collected by worker bees flower pollen is accumulated as pellets (corbicular pollen) in pouches on the rear legs of the bee and it is the mixture of these pellets that comprises bee pollen, which supplies the colony with proteins and amino acids (De-Melo and de Almeida-Muradian, 2017). Pollen also contains fat, vitamins, microelements, etc.

However, plants need to provide pathogen-free food for the bees if co-evolution is going to be a success (Erler and Moritz, 2016). Hence, the nectar offered by a flower should not be fermented nor should pollen be contaminated with fungal pathogens. It is therefore not surprising to see plants adding antibiotic specialized metabolites to the

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nectar to prevent bacterial fermentation (Stevenson et al., 2017), and that pollen also contains antimicrobial specialized metabolites, mainly phenolics (Ares et al., 2018). It is well known that bee pollen shows characteristic flavonoid patterns according to the plant origin. While foraging to satisfy their basic nutritional demand, honeybees inevitably gather specialized plant metabolites as part of the nectar and pollen. In general, these compounds possess biological activity which may be relevant for fighting pests and pathogens in the hive.

Unlike honey and pollen, the third plant derived bee product, propolis (bee glue), is not a food. Bees use it as both building material and defensive substance. They make use of the mechanical properties of this resinous material by applying it for blocking holes and cracks, repairing combs, strengthening the thin borders of the comb, etc. On the other hand, they make use also of its biological action: bee glue contains the putrefaction of "embalmed" intruders, killed in the hive and too large to be carried out, and it is responsible for the lower incidence of bacteria and moulds within the hive than in the atmosphere outside (Simone et al., 2009). To produce propolis, bees collect vegetable material and mix it with wax. It is now generally accepted that bees collect resinous plant materials, produced by a variety of botanical processes in different parts of plants. These are substances actively secreted by plants as well as substances exuded from wounds in plants: lipophilic materials on leaves and leaf buds, mucilages, gums, resins, latices, etc. (Crane, 1990). In some cases, bees may also cut fragments of vegetative tissues to release the resin used in propolis production (Salatino et al., 2005). Numerous plant species secrete highly antimicrobial resins to protect vegetative apices, young leaves and wounded tissues, and such secretions provide propolis raw material for bees. But why do bees need a special antimicrobial material? Honeybees battle an extensive assemblage of pathogens using both individual and "social" defenses. The collection of resins - complex plant secretions with diverse antimicrobial properties, acts as a colony-level immune defense for honey bees. Foraging for resins is energy demanding and provides no clear direct reward for the individual foraging bee, so resin collection and use functions as an aspect of the so-called "social immunity" (Simone et al., 2009).

The beehive is rich in specialized plant metabolites, produced by many different plant species, so the expression "Phytochemistry of honeybees" is not inappropriate. However, it is virtually impossible to perform a detailed overview of the phytochemical features of honey and pollen in a review article of this nature, for reasons of space. In addition, specialized metabolites are minor components of pollen and honey. The present review deals with propolis, because it is the bee product containing the largest amount of specialized plant metabolites (at least 50% of its weight). Moreover, it is not surprising that propolis, originating from protective plant secretions and serving as a defensive substance in the beehive, is also active against human pathogens. This has been proved by numerous publications, reporting the results of well-designed and reliable experiments (Burdock, 1998; Seidel et al., 2008). It has been demonstrated that propolis also possesses many other beneficial pharmacological actions (recent reviews: Sforcin, 2016; Pasupuleti et al., 2017; Zabaiou et al., 2017).

2. Propolis production by honey bees

Only a small number of worker bees collect resin for propolis production. According to Simone-Finstrom and Spivak (2012) "overall resin foragers consistently represent a minority of the foraging force (1% or less of total number of foragers)". The process has been described in detail in the works of Meyer (1956) and Morse (1975). They found out that only a few of the workers, not older than 15 days old, are specialized in propolis foraging. The resin forager bee cuts off a particle of resin (or resin containing plant tissue) with the mandibles, then transfers it to the corbicula on the hind legs (where bees carry pollen loads). After that the resin is brought back to the nest where it is mixed with varying amounts of wax and used as propolis. Foraging for resins is typically observed in the afternoon on sunny days, likely due to the increased pliability of resins at higher temperatures. The resin collection activity is most frequent in late summer through autumn when the honey flow is significantly reduced (Alfonsus, 1933; Meyer, 1956; Crane, 1990).

Bees have their ways of finding plant resins rich in biologically active phytochemicals (Salatino et al., 2011). The secretion of antimicrobial material is widespread in the plant kingdom; various plant species secrete highly antimicrobial resins to protect vegetative apices and young leaves, wounded tissues, etc. (Shuaib et al., 2013). However, there is a strong asymmetry between the wide available diversity of plants producing resin with high bioactivity, and the narrow spectrum of plant sources of propolis established in all parts of the world. Indeed, in a particular geographic region, bees usually demonstrate obvious preference for one or two source plants (Bankova et al., 2006). The cues used by resin foragers for finding a resin source are virtually unknown, although it is clear that they select specific sources. One leading hypothesis is that volatile compounds released from the resin play an important role in bees locating resin sources, signaling the availability of a material rich in bioactive compounds (Simone-Finstrom and Spivak, 2010). It is well known that worker honeybees respond to odors (chemical signals) in several behavioral contexts (Winston, 1987; Salatino et al., 2005). Workers learn the association of floral odors with the nectar and/or pollen rewards offered by the flowers, thus allowing for future identification of the plants from which food can be harvested (Smith and Cobey, 1994). So far no data has been gathered about the role of odors in foraging for propolis. It is possible that the same learning process occurs in the case of resin collection by honeybees (Bankova et al., 2014), taking into consideration the finding of Leonhardt et al. (2010), that stingless bees in Borneo use volatile terpenes as olfactory cues to find appropriate resin sources.

According to another recent hypothesis, formulated by Salatino and Salatino (2017) there seems to be a significant "constraint that honey bees have to face, a barrier that probably limits the number of plant species providing propolis resin". Some materials with good antimicrobial properties, such as latices, resins, and gums, are too sticky and hard to be collected by resin foragers. Bees normally gather exudates which form smooth solid films, which can be scraped by the bees from the plant surface and attached to their hind legs. Most of the materials containing antimicrobial specialized metabolites are too hard to be cut with the mandibles of honeybees, because their mandibles are delicate, as compared with mouth parts of typical chewing insects. A finding that probably confirms the importance of the mechanical properties of the source resins for propolis production is in the article of Simone-Finstrom et al. (2010), who proved that resin foragers were better able to learn tactile stimuli and discriminate between a rough and a smooth surface as compared to pollen foragers. The plant materials conventionally called "resins" in propolis research, are sufficiently soft and smooth to be either cut (apical buds and leaf primordia) or scraped from the plant surface (exudates) by the honeybees with their delicate mandibles (Salatino and Salatino, 2017).

3. Propolis plant sources

The identification of propolis plant source(s) is important, since propolis is one of the commercial bee products and contributes to the income of beekeepers. The knowledge of the plant sources can help to increase propolis production and to attain higher degree of standardization. Also, if appropriate sources are insufficient in the vicinity of the hive, bees have been observed to collect resinous matter from asphalt, fresh paint, etc., and such propolis is polluted (Alqarni et al., 2015).

Knowledge of the vegetable sources is also essential with respect to the health of the bee colony: in the last decade propolis collection and application by honeybees was identified as an important element of the "social immunity" of the colony. The term "social immunity" summarizes all antiparasitic and anti-infectious colony-level mechanisms