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Management Strategies of Honey Bee Diseases

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ABSTRACT

Most diseases that affect honey bees are just a nuisance, but some are serious not only to the individual bees but to the whole colony. To diminish the impact of diseases in honey bees is of interest not just because of the well-being of the insects and the value of the honey that they produce for the beekeepers, but for the value of pollination that many important crops are dependent on. However, the decline in the bee populations seen recently had worried the scientific community, the beekeepers and the general public. Raising awareness about this threat has led to an extensive development of strategies that target protecting beehives and restore or increase honey bees population. Breeding for bees hygienic behavior as well as the use of biocontrol agents seem to be the most promising. The present review summarizes the various constraints facing honey bees as well as some of the recent progresses in controlling them.

Key words: Honey bee diseases, biological control agents, antagonistic bacteria, natural products

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INTRODUCTION

Honey bees (*Apis mellifera*) are among the most important insects in the ecosystems. They are not only important for the honey they produce but also as pollinators of field, horticultural and vegetable crops. Beekeeping currently faces many challenges including the changes in agricultural practices (Kremen *et al.*, 2007; Steffan-Dewenter and Westphal, 2008), the extensive use of pesticides (Barnett *et al.*, 2007; Desneux *et al.*, 2007) and the development of pathogens.

Honey bee brood and adults are hosts for a number of microorganisms such as bacteria, fungi, protozoa, virus and parasitic mites (Floris *et al.*, 1996; Genersch *et al.*, 2006; Qin *et al.*, 2006; Aronstein and Murray, 2010; Forsgren, 2010; Genersch, 2010) that cause serious losses in the beehives production and a decline in the bee populations. To control these pathogens and parasites beekeepers often rely on using antibiotic and pesticide treatments. Several problems associated with this extended use of antibiotics and pesticides is leading to both short-term impacts on beekeepers and long-term effects on the ability of bees to evolve resistance toward their pathogens and favor the spread of more virulent pathogen strains (Cox, 2000; Miyagi *et al.*, 2000). This diminishes the lifetime expectancy of honeybees (Martel *et al.*, 2006) and causes disequilibria in the normal microbiota of the beehive (Charbonneau *et al.*, 1992), increasing the risks of contamination of hive equipments and products (Lauro *et al.*, 2003). For instance, chemical residues can persist in the honey affecting its quality for human consumption (Martel *et al.*, 2006). Chloramphenicol has been detected in honey and other apiary products in numerous countries (Bogdanov, 2006; Sheridan *et al.*, 2008).

Given the economic importance of the honey bees, it is undeniably necessary to develop effective, sustainable and eco-friendly strategies to control of bee diseases. These include changing management strategies, breeding bee lineages showing natural resistance to diseases and/or hygienic behavior (Spivak and Reuter, 2001) and the use of biological control agents (Bastos *et al.*, 2008; Lee *et al.*, 2009). The combination of all these strategies will provide a novel way of controlling honeybee diseases and reducing the use of antibiotics and pesticides in the beekeeping operations. Such a reduction will guarantee human health and safety by preventing the risk of contamination of honey and hive products and promoting the well-being of the bees.

ECONOMIC IMPORTANCE OF HONEYBEES

The value of honey bee pollination to worldwide agriculture has been estimated to be about 215 billion dollars (Gallai *et al.*, 2009). Besides their role as pollinators of many horticultural, vegetable and field crops as well as wild flowers, honey bees are the source of honey and other hives products such as propolis, royal jelly, venom and beeswax. The worldwide production of honey totals over a million tons, yielding an exchange market worth over US$ one billion (FAOSTAT, 2009).

Plant pollination: Pollinators strongly influence the ecological relationships, ecosystem conservation and stability of the genetic variation in plant communities. Over 35% of crops and 60 to 80% of wild plant species rely on the activity of pollinators (Klein *et al.*, 2007). Honey bees are among the major pollinating insects that play an important role in guaranteeing yield and quality for a number of horticultural, field and vegetable crops. They are also the most economically important pollinators of crop monocultures worldwide (Watanabe, 1994). Without the activity of these insects, yield of some fruit, seed and nut crops would decrease by over 90% (Southwick and Southwick, 1992). It is undoubtly clear that any decline in the pollinator populations will compromise agricultural production and consequently the economy.

Honey and other hive products: Since, humans first began keeping bees, their principal aim has been the harvest of honey. In 2005, the worldwide honey production exceeded the 1.4 Million tons mark (FAOSTAT, 2009) with about 64.5 Million beehives in 2008 (FAOSTAT, 2009). Other products of the hive include pollen, brood, propolis, royal jelly, venom and beeswax. The world production of beeswax exceeds 61.2 thousand tons (FAOSTAT, 2009). Less than a half kg of beeswax, containing about 450,000 wax scales, is enough to make 35,000 hexagonal cells, which may store up to 10 kg of honey. Beeswax also has many uses worldwide, including the production of candles, cosmetics, electronics, lubricants, leather and fabric preservatives, polishes, inks and paints, models for dentistry and beer. Cosmetics represents one of the largest beeswax user industry while an important portion of the by-product is still recycled within the bee industry to produce the foundation for new honeycomb and queen cell cups. Propolis is used in the attachment of combs to the top and sides of the hive, as well as for filling cracks, reducing the size of the hive entrance and embalming intruders.

The pollen, queen and worker bees jelly has always represented an appreciated nutrition source in human societies since ancient times. Both by-products are also used in various cosmetics, lotions and dietary supplements. An interest in collecting and commercializing bee venom for therapeutic uses has been emerging in recent years. In addition, commercial beekeeping increased the interest in other hive products, i.e., the queen and worker honey bees to establish new colonies or replace those lost to natural or catastrophic causes.

HONEY BEES CONSTRAINTS

Although, their importance in the natural ecosystem and for agriculture, their production of value-added by-products, for human consumption or commercial and therapeutic uses, honey bees populations have suffered a dramatic decline in recent years due to a number of abiotic and biotic constraints. Abiotically, honeybee health is negatively affected by the intensive use of pesticides and fungicides in agriculture (Fletcher and Barnett, 2003; Barnett *et al.*, 2007) and the chronic exposure to pesticides needed to combat the parasitic mite *Varroa destructor*. Destruction and fragmentation of natural and semi-natural habitats as well as the intensification of agriculture and change in landscapes and crops biodiversity had dramatically affected honeybees and other pollinators (Larsen *et al.*, 2005; Cane *et al.*, 2006). In terms of biotic stress, honey bees are the targeted host of many bacteria, mites, fungi, protozoa and viruses.

Beekeeping practices: Increased urbanization and suburban sprawl, combined with an increasingly intensification of agriculture worldwide have decreased available apiary sites. As a result, the total number of colonies has been decreasing although, difficult to ascertain. Facing this challenge, beekeepers seek alternative sources of income by leasing their colonies for the pollination of horticultural, field and vegetable crops that are entirely dependent on the activity of bees. This practice has tremendous negative impacts on the nutrition of bees and their habitat causing a variety of stresses related to nutrition, colony staging and transport (Van Engelsdrop *et al.*, 2008).

Pesticides: Most intensive agricultural systems have recourse to the use of a number of pesticides to control pathogens and pests. When used, insecticides cause major losses in the populations of honey bees (Laurent and Rathahao, 2003). In systems, where bees are required for pollination, a careful management is required to minimize these losses. During growing season, bees poisoning symptoms due to acute insecticides exposure such as an increase in bee death can be seen at the entrance of the colony (Faucon *et al.*, 2002). Other alterations in the bee's behavior and sense of orientation can also been detected (Decourtye *et al.*, 2004). For instance, a wide spread loss in bee colonies was reported in France in recent years and have been ascribed to the effect of nicotine-like insecticide i.e., Imidacloprid (Rortais *et al.*, 2005). Because of its low mammalian toxicity, high effectiveness and high mobility in plant and mammalian tissue, it is often used as systemic insecticide for the control of sap-sucking insects in crops, as well as blood-sucking insects in companion animals (Tomizawa and Casida, 2003). There is an ongoing debate about the chances of this happening to a degree that bees are being considerably endangered. Some studies report residues of Imidacloprid in the nectar and pollen at levels that are potentially dangerous to bees (Chauzat *et al.*, 2006). In the contrary other experimental assays, consisting of feeding Imidacloprid to bee colonies in syrup or pollen at amounts likely to be found in the field, have shown no significant differences in terms of development and survival of colonies between the Imidacloprid-treated and non-treated control. The authors reported also that the exposure of bees to pollen from corn plants treated with the Imidacloprid did not have any significant effect on their longevity (Bailey *et al.*, 2005; Faucon *et al.*, 2005).

Diseases: Honey bees are affected by a large number of parasites and pathogens. The American foulbrood (AFB), the most economically devastating disease and potentially lethal to infected colonies and European foulbrood (EFB) caused by *Paenibacillus larvae* and *Melissococcus plutonius*, respectively (Forsgren, 2010; Genersch, 2010), are widely distributed. There is also a fungal disease of the brood that is due to *Ascosphaera apis* (Aronstein and Murray, 2010). All these microorganisms have a certain preference for larvae and pupae, where they induce distinctive symptoms, in comparison with adult bees seemingly not-affected.

The parasitic mite *Varroa destructor* was also reported to infest brood cells and to phoretically live on adult bees (Rosenkranz *et al.*, 2010). Under heavy mite infestations, an accelerated rate of death becomes obvious among the colonies.

A protozoan, *Nosema apis*, is known to infest the guts of adult bees and to cause dysentery and early decline of adult workers, especially when the infestation is at its highest level. A new *Nosema* species, *N. cerana*, has been recently identified from the Asian hive bees *Apis cerana* (Chen *et al.*, 2009) and has now been found also on *A. mellifera* in Europe (Fries, 2010).

Most adult honey bees carry symptomless viral infections (Chen and Siede, 2007; Ribiere *et al.*, 2008; Ribiere, 2010). However, under conditions of stress caused by poor nutrition, inclement weather, or parasitism by *V. destructor* or *N. apis* (Yang and Cox-Foster, 2005; Yue and Genersch, 2005), viral infection can overpass the non-detectable threshold, causing symptoms in adult bees.

CONTROL STRATEGIES

A number of strategies have tentatively been applied in recent years to protect honey bees against pathogens and parasites. These include a broad range of chemotherapeutic compounds that have been tested for their ability to control honey bee diseases. Most of these products were promising in terms of controlling pathogen growth either in culture or in bee colonies. Unfortunately none of the tested compounds achieved a complete control of the diseases that could irradiate them (Lodesani and Costa, 2005).

Facing these challenges of partially active molecules, a series of alternative strategies has been developed and implemented to control honey bee diseases. These methods include the use disease resistant bee lines that are currently being deployed by many breeding programs. In recent years, the recourse to biological control agents has been gaining ground and seems to be promising. This relies on the exploration of beneficial microorganisms that antagonize with honey bee pathogens and/or the eco-friendly natural products.

Antibiotics, fungicides and anti-mites: One of the earliest strategies that have been implemented to control pathogens and parasites threatening honeybees was the use of antibiotic and fungicide molecules. Over the course of its development, this strategy faced numerous limitations including the low number of available molecules, their lack of specificity in terms of action and the quick development of resistance.

The first pesticides released for use by beekeepers included pyrethroids and organophosphates (Milani and Barbattini, 1988). These molecules were used to control hive parasites such as mites. Their use has substantially increased the cost of production whilst never provided a complete control of the parasites. In addition, mites developed resistance over time reducing more and more the efficacy of the used molecules. At present, Apistan, a product containing the synthetic pyrethroid fluvalinate, completely lost its effectiveness for the control of *V. destructor* due the parasite evolvement of resistance. It has been replaced with plastic strips containing the organophosphate coumophos, the latter efficacy did not last long (Trouiller, 1998; Elzen *et al*., 1999) and has currently been substituted by Amitraz, a triazapentadiene compound of an unknown action. To guarantee efficacy, beekeepers tend to increase doses and rates and to use various product mixtures. Some chemicals, particularly fluvalinate, may accumulate in wax, exposing honey bees to levels of chemical residues that are harmful to worker bees longevity (Chauzat *et al*., 2009; Johnson *et al*., 2009) and may pollute the honey and other hive products (Martel *et al*., 2007).

Tetracycline antibiotics such as oxytetracycline hydrochloride (OTC) have been used to control beehive diseases such as AFB. In recent years, tetracycline resistant strains have been emerging in many countries including USA, Canada and Argentina (Cox, 2000; Miyagi *et al*., 2000; Alippi *et al*., 2007). Hives treatment with oxytetracyline hydrochloride may mask disease signs for several months while *P. larvae* spores may be still found in the honey. The OTCs were also used against *M. plutonius* infection. The efficacy of other antibiotics, such as tylosin and lincomycin and their derivatives have also been tested against AFB (Fedlaufer *et al*., 2001; Kochansky *et al*., 2001; Elzen *et al*., 2002; Alippi *et al*., 2005; Pettis and Feldlaufer, 2005). Among these Tilmicosin, a semi-synthetic macrolide antibiotic synthesized by a chemical modification of a tylosin and exclusively developed for veterinary use (Reynaldi *et al*., 2008). There is still a lack of information regarding its use although, it has been approved to control causal agents of respiratory diseases in farm animals, including Gram-positive bacteria, mycoplasma and some Gram-negative bacteria (Shryock *et al*., 2002). Likewise, the fumagillin fungicide was used against *N. apis* and was shown to suppress infection when applied at doses ranging from 0.005 to 0.03 mg mL^{-1}, without detectable side-effects.

Genetic breeding: In the early 1990s several breeding programs have been implemented to develop honeybee population with resistance/tolerance to major diseases and/or with the hygienic behavior (Spivak and Gilliam, 1998). The hygienic behavior consists of selecting and/or training bred bees to early detect and discard diseased larvae before they become infectious towards the whole colony. Hygienic behavior in bees is defined as the ability of bees to detect and remove diseased or parasitized brood. It is considered the primary mechanism of honey bee resistance to a variety of brood diseases (Spivak and Gilliam, 1993; Spivak, 1996). Hygienic bees have an acute sense of smell and able to detect affected cells very soon after infection and to remove them before large numbers of spores have been produced (Spivak *et al.*, 2003). The importance of this behavior in breeding comes from the fact that bees are able to detect infected brood better than any beekeeper specialist, hence preventing the spread of the disease to the colony healthy member bees.

The genetic analysis of the hygienic behavior conducted in the early 1960s (Rothenbuhler, 1964) and in recent years (Spivak and Reuter, 2001; Lapidge *et al.*, 2002) revealed that this trait is recessive and under a complex genetic control and involves a number of genes whose products interact in a complex way and demonstrated that increased genetic diversity in bees may have an important function in reducing the likelihood of outbreaks of the disease (Tarpy, 2003; Evans and Spivak, 2010).

Sanitation practices: Management and sanitation strategies are directed toward helping bees defend against infection or avoiding infection in the first place. These practices include supplemental feeding to improve the nutritional and health of bees, keeping hives clean and well ventilated (Gochnauer *et al.*, 1975), replacing storage and brood combs annually and avoiding transfer of combs between colonies (Malonova and Titera, 1995; Flores *et al.*, 2005). Several different sterilization methods have been tested in attempts to reduce spores load in beehives. Fumigation of beehive equipment using various chemicals was performed by Gochnauer and Margetts (1980) and Faucon *et al.* (1982), but these were not widely accepted due to residuals found in both wood and wax. Some beekeepers have also tried fumigation with lactic, formic and oxalic acid (Calderone, 1999; Higes *et al.*, 1999; Dodologlu and Emsen, 2009) to combat the mite. Although, these approaches do not replace insecticides in the colonies, they are somewhat less effective in controlling mites and can directly be toxic to the bees. Lactic acid (15%, v/v) is sprayed on combs covered with bees results in 90% decrease in mites population (Rendall, 1996), while formic acid has been used successfully in Germany by soaking an absorbent pad held in a cradle over the brood nest (Rendall, 1996). Formic acid is also used on soaked pads in Italy with an efficacy of up to 98.8%, when used every third day for three weeks (Mutinelli *et al.*, 1994).

Gamma irradiation from a Cobalt-60 source was effectively used to sterilize contaminated beekeeping equipment (Hornitzky and Willis, 1983). Irradiation was also tested to sterilize wax and honey (Wooton *et al.*, 1985). At the optimum level of radiation there were no negative effects detected on wax composition; though some physico-chemical alterations were observed in honey, including decreases in enzymatic activities and a change in color (Baggio *et al.*, 2005). However, the accessibility of radiation facilities is the limiting factor of this technology. Likewise, sterilization of honey using heat, although efficient shows several limitations. Current research efforts are focused on other alternative methods such as microwave radiation, infrared heating, ultra-sonication and ultra-filtration to preserve honey quality (Subramanian *et al.*, 2007).

Biological Control Agents (BCA): Considering the worldwide spread of honey bee diseases and the lack of registered and effective molecules to fight them, there is a great interest in developing alternative control methods. Non-contaminating natural biocides produced by biological control agents, although still a great challenge will help manage bee disease and improve the quality of honey and other by-products while avoiding the presence of undesirable residues.

Antagonistic bacteria and fungi: A broad range of bacteria have been tested on *P. larvae* in culture, most of which were antagonistic and exhibited and antibacterial activity against pathogen found in the gut of *A. mellifera*. Most of these bacteria belonged to *Bacillus* species (Evans and Armstrong, 2005, 2006). The bacterial communities, occurring in the digestive tract of the Japanese honeybee, *Apis cerana japonica* were also assessed and investigated by *in vitro* inhibition assays and their ability to inhibit *Paenibacillus larvae* was determined (Yoshiyama and Kimura, 2009). Alippi and Reynaldi (2006) investigated the potential use of aerobic spore-forming bacteria isolated from honey samples and other apiarian sources as biocontrol agents against *P. larvae*. Interestingly, species of *Bacillus*, *Paenibacillus* and *Brevibacillus*, frequently isolated from apiarian sources (Gilliam and Prest, 1978) have been reported to be effective biocontrols by producing antibiotics and antifungal metabolites (Nielsen and Sorensen, 1997). Most of the isolates exhibiting very strong activity against the honeybee pathogens were identified as bacillus sp., (*B. megaterium*, *B. licheniformis* and *B. cereus*). Furthermore, a new bacterial isolate identified as *Paenibacillus polymyxa* showed a high level of antimicrobial activity against *P. larvae* sp. (Lee *et al*., 2009). Recently, a new study demonstrated the presence of actinomycetes associated with hives of bees from the genus *Apis* and *Trigona*. These actinomycetes were able to produce antibacterial activity against bee pathogens *P. larvae* and *M. plutonius*, which provides a new source of microorganisms able to produce novel antibiotics to combat bee diseases in the beekeeping industry (Promnuan *et al*., 2009). Likewise, many other microbes associated with honey bees, such as certain *Penicillum* and *Aspergillus*, showed inhibiting effects on growth of *A. apis* in culture (Gilliam *et al*., 1988).

Natural products: Many natural compounds have been tested in honey bee colonies and on the pathogens in culture in an attempt to control chalkbrood and American foulbrood (Heath, 1982). Some of them include natural plant-derived antimicrobial/antifungal compounds (Aronstein and Hayes, 2004; Mourad *et al*., 2005; Gende *et al*., 2008). Essential oils containing citral and geraniol, were reported to have the best inhibiting effect on fungal growth *in vitro* (Calderone *et al*., 1994). Bailac *et al*. (2006) showed that oils whose composition has mainly benzenic compounds such as cinnamon oil *(Cinnamomum zeylanicum)* presented a good antimicrobial activity against strains of *Paenibacillus larvae* subsp. *larvae*.

Propolis, another natural product, derived from plant resins and produced by honeybees to seal the walls and entrance of the hive, contributes to the protection of the colony against different pathogens and has antimicrobial properties (Ghisalberti, 1979). Propolis has empirically been used in apiculture for the prevention of AFB and other honeybee diseases for years (Mlagan and Sulimanovic, 1982) and it is well known for its strong inhibitory effects against Gram-positive and negative bacteria in laboratory cultures (Drago *et al*., 2000; Sforcin *et al*., 2000; Bastos *et al*., 2008).

The antibacterial activity of propolis could be related to the chemical composition of propolis, which includes phenolic compounds (flavonoids and aromatic acids) (Arfaoui *et al*., 2007), terpenes and essential oils among others (Marcucci, 1995; Kumazawa *et al*., 2002). Phenolics are well known as antimicrobial compounds with antibacterial and antifungal activity. The *in vitro* challenge of many fungi with these compounds revealed their effectiveness in reducing fungal growth (Arfaoui *et al*., 2006).

MECHANISMS OF ACTION OF BIOLOGICAL CONTROL AGENTS

If biological control is to become a viable alternative for honey bees' protection, a large number of Biological Control Agents (BCAs) with various modes of action must be isolated and identified.

Recent studies showed the deployment of several BCAs with effective methods for the selection of antagonistic microorganisms, as well as the establishment of *in vitro* and *in vivo* disease challenge experiments using adult bees and larvae (Flores *et al.*, 2004; Bastos *et al.*, 2008).

Multiple modes of action were determined. These include the production of antibiotics, antibiotic-like compounds, bacteriocins, antifungal metabolites, (Alippi and Reynaldi, 2006), the stimulation of bee's immune system (Evans and Lopez, 2004) and the enhancement of the defense response of honeybees (Antúnez *et al.*, 2008). Other factor may be implicated in the interaction: Honey bee-Biological control agent-Pathogen.

Antibiosis: Many bacterial species have been shown to inhibit the growth of bee's pathogens *in vitro* as well as to reduce disease symptoms *in vivo* through the production of secondary metabolites, which are used in direct antagonism with pathogens and pests (Alippi and Reynaldi, 2006; Lee *et al.*, 2009; Yoshiyama and Kimura, 2009). The ability to produce multiple classes of antibiotics, that differentially inhibit bee pathogens, is likely to enhance biological control. Several biocontrol strains are known to produce multiple antibiotics which can suppress one or more pathogens. For example, many *Bacillus* species inhibiting the growth of *P. larvae* and *A. apis* by producing a number of antibiotics were identified (Nielsen and Sorensen, 1997). These species are known for their high production of a variety of secondary metabolites such as antibiotics, bioinsecticides, enzymes and lipopeptides (Phister *et al.*, 2004). Sabate *et al.* (2009) were able to isolate three different *B. subtilis* strains that can inhibit two important honeybee pathogens by two different mechanisms; that is surfactin synthesis and fungicide or cell-to-cell interaction.

More recently, a *Paenibacillus polymyxa* with a high antibacterial activity against *P. larvae* was isolated from honey (Lee *et al.*, 2009). This species has been known to produce polymyxins with broad spectra of activity that includes Gram negative and positive pathogenic bacteria (Storm *et al.*, 1977). Polymyxins are classified as peptide antibiotics synthesized by multienzyme complexes. More than 15 polymyxins have been identified which are known to bind to lipid A of the bacterial cell membrane, resulting in membrane disruption and cell death (Martin *et al.*, 2003). Actinomycetes, known for their secondary metabolites, which have been successfully used as drugs in human and veterinary medicines, have been shown to inhibit the growth of *P. larvae in vitro* by producing antimicrobial compounds (Promnuan *et al.*, 2009) yet to be identified. Over more than 43,000 known bioactives natural products, almost ¼ are produced by actinomycetes. (Lazzarini *et al.*, 2000). The most important genus is *Streptomyces* with over 500 described species producing many important antibiotics, including Streptomycin. Other antibiotics produced by *Streptomyces* sp., include spectinomycin, neomycin, tetracyclines, nystatin, erythromycin and chloramphenicol.

Stimulation of the bee immune system: Besides their group strategies to combat disease such as grooming, nest hygiene and other behavioral traits which can reduce the impacts of many parasites and pathogens (Spivak and Reuter, 2001), honey bees possess also individual defense mechanisms, including immune responses toward pathogens (Evans, 2004). These involve diverse set of actions including the secretion of antimicrobial peptides, phagocytosis, melanization and the enzymatic degradation of pathogens (Hoffmann, 2003).

Most research on honey bee immune traits has focused on responses toward bacterial threats, toward which both larval and adult bees are vulnerable (Casteels *et al.*, 1990; Evans *et al.*, 2006). Evans and Lopez (2004) showed that non pathogenic bacteria can stimulate the immune response of honey bee larvae, helping bees survive exposure to pathogens. They also found that non-pathogenic bacteria can generate a sustained increase in levels of the antibacterial peptide abaecin and defensin. The authors proposed then that the mixture of non-pathogenic bacteria could be presented as a potential surrogate for assaying the immune responses of different honey bee lineages and that non-pathogenic bacteria can be used as a probiotic to enhance honey bee immunity, helping bee larvae and other life stages, survive attacks from pathogens in the field.

Antúnez *et al*. (2008) proposed a possible indirect effect of the propolis such as the stimulation of the bee immune system as the same product was able to enhance innate and adaptive immune responses of mouse, bovines and humans. *In vitro* and *in vivo* assays demonstrated that propolis activates macrophages, increasing their microbiocidal activity, enhances the lytic activity of natural killer cells and stimulates antibodies production (Sforcin, 2007).

CONCLUSION

With the increasing demand on organic honey and the reduction of increased dependence on synthetic pesticides and antimicrobial compounds that often lead to a general deterioration of honey bee colonies and the environment, it is quite obvious that an Integrated Pest Management (IPM) approach is needed to guarantee the sustainability of the beekeeping industry. Several steps are required for the settlement of such a successful approach, including an appropriate management and sanitation of the hives and the use of honey bee disease-resistant lineage. The ultimate step will be to deploy biocontrol agents, introduced to honey bees through various method of application such as spraying, feeding, evaporating or fumigating, to reduce disease levels and prevent the development and spread of pathogens. Recent findings gathered using biocontrol agents such as propolis, essential oils, probiotic bacteria, gut microbial communities, seem promising and would be conceivable to combine them within an integrated IPM strategy to manage honey bee pathogens and pests. Nevertheless, more research is still needed to examine the mechanisms governing their mechanisms of action and guarantee their safe deployment.

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