



***Varroa destructor* (Acari: Parasitiformes: Varroidae) a dangerous parasite of honey bees (Hymenoptera: Apidae)**

Adjlane Noureddine^{1,2} and Haddad Nizar²

¹Department of Agronomy, Faculty of Science, University of M'hamed Bougara, Boumerdes 35000, Algeria; ²National Agricultural Research Center, P.O.Box 639-Baq'a' 19381, Jordan.

Email: adjlanenoureddine@hotmail.com

ABSTRACT: The honeybee is an essential element of environmental balance in the world, particularly for its role in the pollination of many plant species. It also has other interests such as the production of honey, propolis, royal jelly and wax. Among several diseases on honey bees, the most dangerous is varroosis and threaten different species of honeybee population. Varroosis is caused by an external parasitic mite, *Varroa destructor* which parasites both bees and brood. It causes enormous damage to the colony and is a gateway to other viral and bacterial diseases. Information on the influence of this disease on colonies, symptoms and pathogenic actions, reproduction, development cycle and treatment methods viz chemical, natural, biological and biotechnical against *Varroa* are discussed.

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KEY WORDS: *Varroa destructor*, hematophagous mite, *Apis* spp., parasitosis, varroosis

INTRODUCTION

Honeybees, along with other wild pollinators, are essential to maintaining the diversity of plants and our food resources. However, for the past 20 years, the beekeeping sector has faced a general weakening of the colonies, leading to a sharp increase in bee mortality rates worldwide. The winter loss rate is 10%, which considered normal; it currently stands at 20% on average that adds losses during the seasons of around 10%. Among the causes of these mortalities, there is varroosis which is considered as the first risk factor, it is the main health hazard of honey bees (Adjlane *et al.*, 2013, 2016; Van Der Zee *et al.*, 2015; Thoms *et al.*, 2016; Molineri *et al.*, 2018; Adjlane and Haddad, 2017, 2018). According to Anderson and

Trueman (2000) varroosis is a parasitosis of the adult bee and its brood, caused by an external parasitic hematophagous mite, *Varroa destructor* (Parasitiformes: Varroidae). *Varroa* has been responsible for an epidemic in *Apis mellifera* L. (Apidae) since it has been transferred from the Asian bee, *Apis cerana* (Colin, 1999). It is present in almost all countries around the world (Fig. 1).

The pathogenic role was ignored while the varroosis spread with extreme rapidity from 1964 all over the world, leaving no area unscathed to date and causing the death of colonies. The development of the transhumance of the colonies as well as the commercial exchanges allowed a contact between the two species of *A. cerana* and the European bee *A. mellifera* then the passage of the *Varroa*

* Author for correspondence

on the latter. This subsequently caused the spread of ectoparasitosis on all continents (Colin, 1999). Knowledge of the biology of the mite, population dynamics in a well-defined region and the race of bees are necessary for the programming of an integrated varroosis control strategy. This article elucidates on varroosis, its influence on the colony and the means of fighting the disease.

1. Systematics

The genus *Varroa* belongs to the subfamily Varroinae, family of Varroidae and the genus *Varroa* has four clearly identified species (De Guzman and Rinderer, 1999) (Fig. 2). The varroa mite was collected for the first time by entomologist Edward Jacobson from bees of the island of Java of the species *Apis cerana*. Oudemans, a Dutch acarologist first described it in 1904 and gave it the name of *Varroa jacobsoni* in homage to its discoverer. The existing host-parasite relationship between the bee *A. cerana* and the mite is currently in a state of equilibrium, so that *V. jacobsoni* does not presently constitute a threat for *A. cerana* (Donzé et al., 1998). The passage of *Varroa* from its original host *A. cerana* to its new host *A. mellifera* probably took place during the 1940s. The importation of colonies of *A. mellifera* bees into Asia where they were not present in the years 1930, gave the opportunity to pass on this freshly arrived host (Donzé and Guerin, 1994).

The first observation of *Varroa* in the brood of *A. mellifera* is thought to have occurred in Korea in the 1950s (Topolska, 2001). It was not until 1966 that the danger and potential damage to beekeeping caused by the spread of the parasite was officially reported. The distribution of *Varroa* in beehives has therefore become, according to international exchanges of bees (colonies, queens), gradually global. Anderson and Truemann (2000) separated the species mite originally known as *V. jacobsoni* into two distinct species. The name of the species which groups together the mites infesting the honeybee *Apis mellifera* is now *V. destructor*.

2. Adaptation of *Varroa* to *Apis mellifera*

Like all parasites, *Varroa destructor* has properties that allow it to adapt to its host. Morphologically,

the flattened shape of the *Varroa* allows it to be applied to the body of the bee and to escape the movements of watering the latter. Its legs end in suction cups, its palms in claws allowing attachment.

- The varroa's life cycle follows to that of the bee (does not make sense), which allows it to reproduce and feed in the brood (Ritter, 1981), Compared to the original *A. cerana* host.
- The duration of the brooding of *A. mellifera* brood (12 days for workers and 15 days for males) is longer than that in *A. cerana* which gives more chance for immature (Donzé et al., 1998).
- The temperature regulation in *A. mellifera* makes the brood more favorable than that of *A. cerana*.
- The cleaning behavior in *A. mellifera* is not as frequent as in *A. cerana* (Naumann, 1991).

3. Biology of the mite

Varroa destructor has a remarkable sexual dimorphism (Martin, 2003). The male mite differs from the female by its small size, white color, globular body and legs stretched forward. It only exists in the alveoli at the time of reproduction, for this, its chelicerae are modified to inject spermatophores (Rosenkranz et al., 2009).

The life cycle of *Varroa* is strictly linked to the bee. It has two phases: phoretic on the adult bee, and reproductive in the cells of the brooded brood of males and workers (Fries, 2005). The *Varroa*'s reproductive phase lasts from the seal to the emergence of the bee. The so-called founding varroa female enters a brood cell a few hours before sealing and immerses herself in the larval food (Ifantidis, 1988). After sealing, it perforates the integuments of the nymph creating a site of nourishment, stimulates its oogenesis and begins its laying. The first egg, haploid, will give a male; the other diploids will give females through the following stages: egg, larva, protonymph and deutonymph. Mating takes place in the socket, in the area of faecal accumulation. When the adult bee emerges, the founding female and the mature female exit

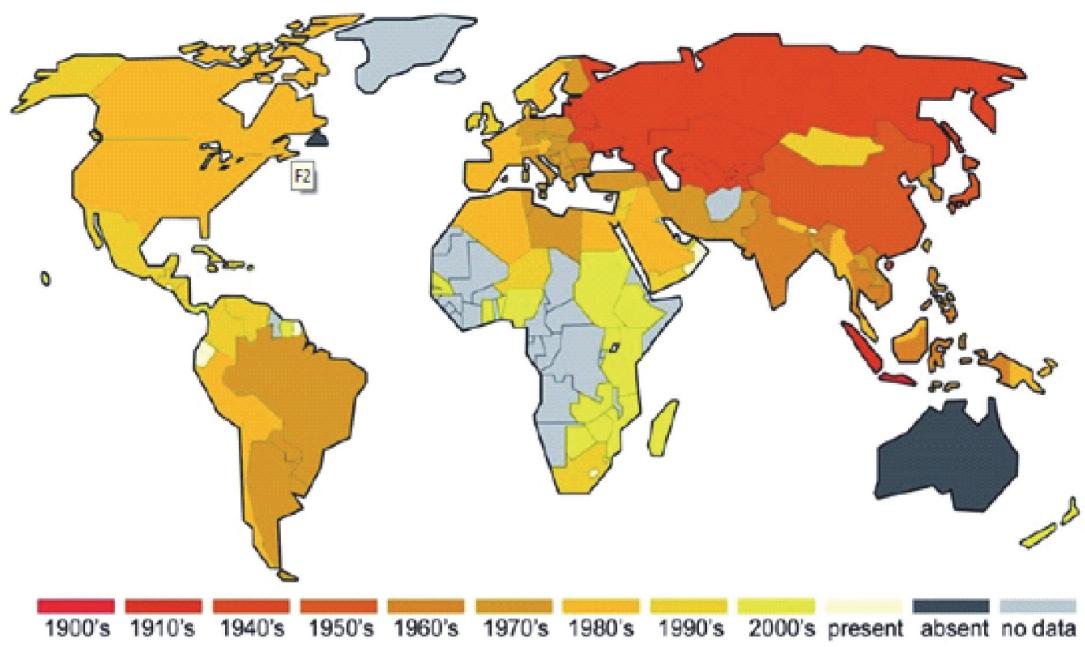


Fig. 1 Dispersal of *V. destructor* worldwide (Wilfert *et al.*, 2016)

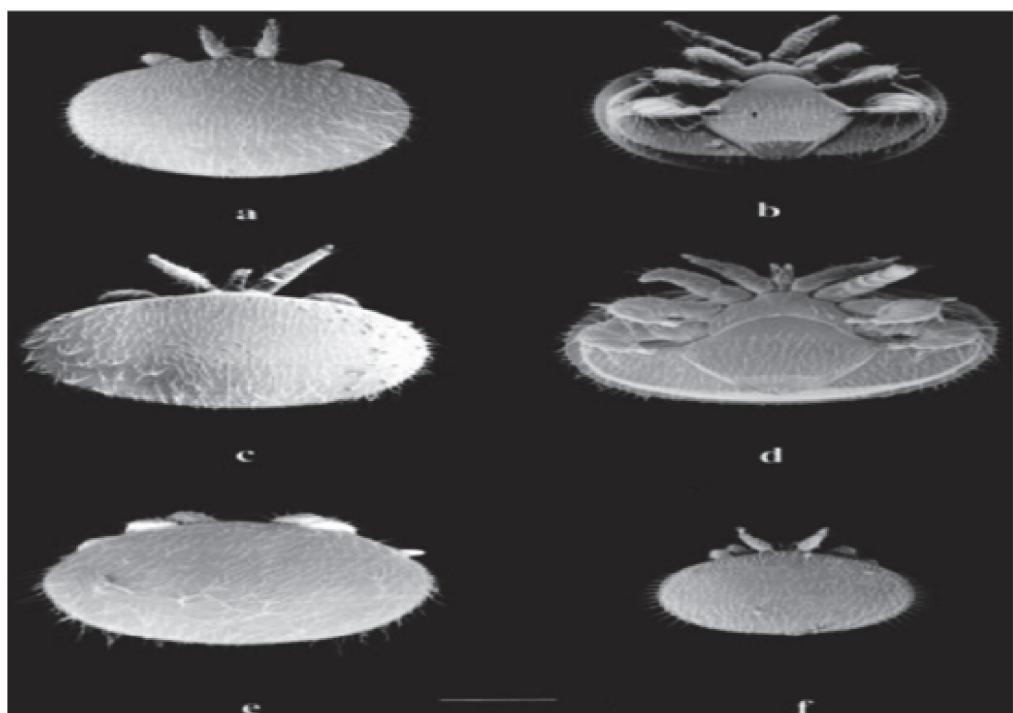


Fig. 2 Dorsal and ventral faces of adult females in electron microscopy of (a) and (b) *V. jacobsoni* (Java haplotype), (c) and (d) *V. destructor* (haplotype K), (e) *V. rindereri* and (f) *V. underwoodi*. The scale size is 500 μm (Illustration from Anderson and Trueman, 2000)

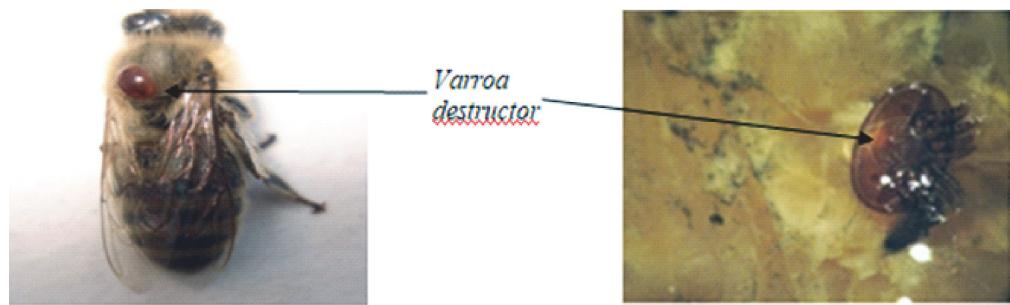


Fig. 3 Parasitic mite *Varroa destructor* of the honey bee

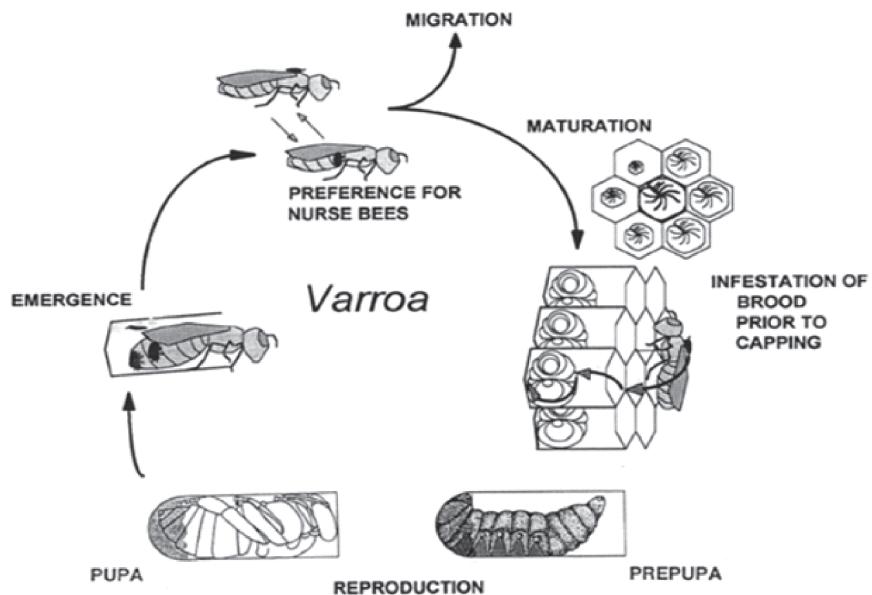


Fig. 4 *Varroa*'s biological cycle (Donzé *et al.*, 1996)



Fig. 5 Dead bees with deformed and atrophied wings

the socket while the male dies with the immature (Faucon, 2003). The phoresia phase corresponds to the period between the exit of the varroa from the cell and its entry into another cell (Martin, 2003).

The duration of the life cycle is seven to eight days for females, and six to seven days for males. Females have four to five cycles in their life (De Vaublanc, 2004). The egg is white, ovoid and small in size ($230 \mu\text{m} \times 300 \mu\text{m}$). Then the female varroa lays eggs approximately every 30 hours: between 26 and 32 hours at a temperature of 34-35° C. The female varroa generally lays five eggs, six rarely and seven exceptionally and has a laying potential of 18 to 30 eggs (Rosenkranz *et al.*, 2009). The development time from egg to adult through the two larval stages (proto then deutonymphe) is between 5.8 and 6.6 days (Donzé and Guerin, 1994) (Fig. 4).

5 Factors influencing the entry of the female *Varroa* founder in the brood

Several authors have highlighted the influence of certain factors on the founder's entry into the brood.

Mechanical factors:

The size of the brood cells and the distance between the larva and the edge of the cell significantly influence the infestation (Calis *et al.*, 2006). As a result, the founder shows a clear preference for the brood of the male (Le Conte and Arnold, 1988).

Ethological factors:

In order to enter the brood, the founder must be only a few millimeters from the cell. To do this, the female prefers nurse bees, which are in contact with the brood (Krauss *et al.*, 1986). On the other hand, the bumblebee brood cells are more attractive than those of workers (Calis *et al.*, 2006).

Chemical factors:

Four fatty acid methyl esters (methyl palmitate, methyl oleate, methyl linoleate, and methyl linolenate) trigger the capping of the worker cells by the adult bees (Le Conte *et al.*, 1989). These compounds are secreted by the worker larvae a few hours before the cell is closed and are present in great

amounts on the larval surface during the capping period. They disappear during the following days (Trouiller *et al.*, 1991)

Thermal factors:

The thermo-referendum of the varroa, which is between 31.3 and 34.2 °C, corresponds well to the temperature of the bee brood and to the temperature of the body of the workers (32.4 °C on the thorax and 31 °C on the abdomen). On the other hand, very high temperatures inhibit its reproduction (Le Conte *et al.*, 1990).

6. Development of the *Varroa* population

The population-wide cycle of *Varroa* is dependent on that of the colony. During the summer period, the mite infestation increases in parallel with the bee brood. The number of parasites present in the colony at the start of the summer phase remains a determining parameter for the evolution of the infestation rate during the season. A study of 35 colonies suggests a 100-fold increase in the *Varroa* population over the course of summer (Garcia-Fernandez *et al.*, 1995).

Martin (1997) proposed a mathematical model including multiple factors to describe the dynamics of *Varroa* population in bee colonies in a temperate region with a continental climate. These factors are: the total number of workers and brood (eggs, larvae and pupae) during the season, the total number of mites, the rate of *Varroa* invasion in brood cells, the proportion of *Varroa* having a viable reproduction, the density of the *Varroa* population and the mortality of *Varroa*'s at emergence. This model predicts that approximately 65% of the mite population is found in the brood at all times (Martin, 1997).

7. Propagation factors

Varroasis has spread rapidly and inexorably from bee to bee, from beehive, and even from one apiary to another. This is due to several factors, either natural or beekeeping. Natural factors: varroasis can spread naturally by foraging drift, swarming and desertion, by looting and theft of males that change colonies. Beekeeping factors, in addition to

natural factors, the beekeeper's manipulations can contribute to the spread of the disease. These include transhumance, the concentration of colonies in the same region as well as commercial activities (trade in queens and swarms) (Faucon, 2003).

8. Pathological actions

The parasitism of *V. destructor* acts on adult bees and on the brood in three actions: spoiler, mechanical and vector.

Spoliatory action:

Varroa infestation is associated with a decrease in the total number of hemocytes in nurses, as well as for all life stages of drones (Salem *et al.*, 2006). At the gene expression level, infestation involves less expression of the gene encoding phenol oxidase and genes encoding antimicrobial peptides (Yang *et al.*, 2005). Fat reduction has also been observed (Drescher and Schneider, 1987); all of these data suggest an overall decline in the immune competence of infested bees. Ramsey *et al.* (2019) have shown that *Varroa* does not consume hemolymph, as has been admitted, but damages the host bees by consuming the fatty substance, the drop in total protein fluctuates between 10 and 50% in parasitized nymphs (Dandeu *et al.*, 1991).

Mechanical action:

The presence of the parasite in the adult bee alters its behavior to the detriment of its usual tasks (Faucon, 2003). The parasitism leads to malformations and weakness of the young worker. A heavy infestation causes the death of nymphs before the emergence and birth of mutilated bees (Boecking and Genersch, 2008). According to Schneider and Drescher (1987), the survival rate of adult bees beyond 25 days, under laboratory conditions, is around 50% if the bees are from healthy larvae, but it is reduced 25% if the larvae are contaminated with three varroa. In the internal organs, a reduction of 10% in the size of the acini of the hypopharyngeal glands is observed in born parasitized bees. De Jong *et al.* (1982) reported that 6% of the parasitized infant bees have a shortening of the abdomen and localized deformations, especially in the wings.

Vector action:

The role of the mite in the transmission and pathogenesis of certain viruses appears to be twofold. On the one hand, *Varroa*, through its role as a vector, injects the viruses which it carries directly into the hemolymph of the bee. On the other hand, an activating role through the bite of *Varroa* allows the activation of certain viruses, present in the latent state in the hemolymph of the bee (Tentcheva *et al.*, 2004). The parasite is capable of transmitting a certain number of viruses: the acute paralysis virus (Acute Bee Paralysis Virus - ABPV), the Black Queen Cell Virus (BQCV), the wing virus Deformed Wings Virus (DWV), the Israel Acute Paralysis Virus (IAPV) or the Kashmeer Bee Virus (KBV) (De Miranda *et al.*, 2013; Reyes-Quintana *et al.*, 2019; Posada-Florez *et al.*, 2019).

9. Symptoms

Varroasis clinical symptoms include brood and bee disorders (Charriere *et al.*, 2011). One of the main signs of the disease is the presence of an irregular or lacunar brood with atrophied dead wings under the operculum. Symptoms in adult bees are mainly related to the presence of workers with deformed wings, trailing and dead bees (Fig. 5).

According to Faucon (2003), varroasis shows no sign of disease up to a critical level where the colony is difficult to recover. When the pressure of the parasite increases, the following symptoms appear at the level of adult bees: Trailing bees, walking in disorderly directions and dead bees, bees with deformed wings, sometimes black, spread apart, or asymmetrical and atrophied bees and nymphs. At the brood level, decrease in the laying of the queen, mosaic brood and nymphs alive but atrophied under the cover, or dead under the cover.

10. Screening

Varroa mite screening allows beekeepers to estimate the population of mites parasitizing a colony in order to apply the control strategy best suited to their situation. This is an essential step in pest control in beekeeping, which allows, in particular, to know the level of parasitism in a colony before

and after a treatment. Thus, precise monitoring and good knowledge of the levels of infestation in a beekeeping herd are the basis of an adequate integrated pest management strategy. Several methods are available and each one has a level of sensitivity (Dietemann *et al.*, 2013; Calderone and Turcotie, 1998; Macedo *et al.*, 2002).

Examination of the brood:

This method consists of removing mites, which are in the cells of the brooded brood (preferably those of the males). This method gives an idea of the brood parasitism rate or the brood infestation rate.

Bee examination:

This method allows us to assess the infestation rate of adult bees. It consists of taking sample of bees (about 200 bees), placing them in a jar containing 70% to 80 % alcohol or water with detergent added. After shaking well, we count the fallen mites, their percentage in relation to the bees collected tells us about the degree of infestation in the colony (De Jong *et al.*, 1982).

The natural fall by placing swaddles:

The laying of greased swaddles covered with a grid on the floors of the hive for a few days, their reading and replacement allow estimating the daily mortality of the mite.

11. Management of varroosis

The fight against varroosis aims to keep the infestation below the harmful threshold.

Use of acaricide treatments:

Since the appearance of the varroa mite, several chemical molecules have been applied in several countries around the world. The most applied are based on Fluvalinate (Apistan®, Klartan®), Amitraz (Apivar®), Flumethrin (Bayvarol®) and Coumaphos (Perizin®). The single and repeated use of an active ingredient resulted in the development of resistance acquired by *V. destructor*. Thus, the effectiveness of most of the

chemical acaricides used varies between 60 to 95% (Rosenkranz *et al.*, 2010).

The phenomenon of resistance to several chemical molecules has been reported by several authors (Lodesani *et al.*, 1995; Vandame *et al.*, 1995; Londzin and Sledzinsky, 1996; Elzen *et al.*, 1988; Mozes *et al.*, 2000; Milani and Della Vedova, 2002; Garcia-Salinas *et al.*, 2006). This has forced beekeepers to move towards natural control based primarily on the use of oxalic acid, formic and thymol. In addition, it has been observed that certain acaricide residues and certain metabolites resulting from the degradation of these molecules accumulate in the wax (Bogdanov *et al.*, 1988), and sometimes they even contaminate the products of the hive (Bogdanov, 2006).

Natural treatments:

These treatments are based on essential oils and organic acids, which can act on mites (Rosenkranz *et al.*, 2009). Formic acid can be used in different forms, either at a concentration of 65% (w/w) for the fumigation of colonies ("MiteWipe" or "Flash" methods) or in the form of commercial MAQS strips ® 46.7%. This acid organic kills mites by inhibiting their mitochondrial respiration (Johnson, 2015). In fact, formic acid is the only organic acaricide that has the ability to kill the mites inside brood cells (Fries *et al.*, 1994). Lactic acid spray in aqueous solution is very effective in the absence of brood. The presence of the latter causes the drop in efficiency from 80! to 40! (Rosenkranz *et al.*, 2009). Oxalic acid has been used in the fight against varroosis for several years as an additional treatment as part of the integrated control plan (Barbançon and Monod, 2005). At first, it was used in the form of a spray, which involved removing each frame from the hive. Then, beekeepers used oxalic acid by dripping on bees in the back alleys of the frames. Applied by spraying, oxalic acid has proven itself for such a fall treatment. The efficacy is very high against mites and bees tolerate this treatment well (Toomemaa, 2019; Jack *et al.*, 2020). Spraying application on the other hand has the disadvantage of being laborious (Chariere *et al.*, 1998).

Essential oils:

Imdorf *et al* (1999) tested more than 150 essential oils by screening in the laboratory and in situ to assess their toxicity, repellency, attractiveness, as well as their effects on the reproduction of varroa mites. Among all the components tested, thymol had the best result in practical beekeeping. Thymol, camphor and other oils have shown effectiveness against varroasis with less risk on bees and on bee products (Rosenkranz *et al.*, 2009; De Jesús May-Itzá and Medina, 2019; Tlak Gajger *et al.*, 2020). Thymol provides an efficiency comparable to that of formic acid. It is a volatile monoterpenoid naturally present in thyme, *Thymus vulgaris*. Thymol acts on the nervous system of varroamite by interacting with GABA receptors involved in neurotransmission in animals (Johnson, 2015).

Biological control:

Entomopathogenic fungi seem to present the most promising future. Several isolates from different species (*Verticillium lecanii*, *Hirsutella* spp., *Paecilomyces* spp., *Beauveria bassiana*, *Metarhizium anisopliae* and *Tolypocladium* spp.) have shown an interesting varroacid effect (Shaw *et al.*, 2002). Field tests with *M. anisopliae* (Metschnikoff, Hypocreales: Clavicipitaceae) indicated efficacy comparable to that of Apistan. The entomopathogenic fungi *Beauveria bassiana*, *M. anisopliae*, *Clonostachys rosea* and *Hirsutella thompsonii* have also demonstrated certain degrees of control against *V. destructor* in *in vivo* tests (Kanga *et al.*, 2006). However, these fungi are also found to be pathogenic for bees and can interfere with the development of brood, among other things (Meikle *et al.*, 2012).

Biomechanical control:

Removing the brood of males - technique consists in placing a frame of cells of false drones so that it is rebuilt by the workers in cells of false drones at the edge of the brood chamber. The queen will lay male eggs (unfertilized) and, knowing that the mites prefer this type of brood, the latter will enter it. Once the cells are sealed, these frames are removed and destroyed, trapping a significant number of parasites (Wantuch and Tarpy, 2009).

Selection of bees tolerant or resistant to *Varroa destructor*:

Selection is based on the behaviors and genetic characteristics of bees contributing to resistance. The most studied are the attraction of brood for varroa mites, the duration of brooding time and the hygienic and de-husking behavior.

Varroosis is a very dangerous disease that attacks the honeybee. It causes enormous damage to bee colonies. Secondary infections caused by viruses are one of the causes of colony collapse. Knowledge of the biology of the mite, population dynamics in a well-defined region and the race of bees are necessary for the programming of an integrated varroasis control strategy.

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