Chapter 14

Comparison of the use of Essential Oils for the Control of Varroa (*Varroa destructor*) in *Apis mellifera*

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ABSTRACT

Varroasis in *Apis mellifera* is today the most serious problem in beekeeping, since it affects the brood and the adult bee since it feeds mainly on the fat of its host, causing malformations, reducing the lifespan of the bee adult and the mite is considered a transmitter of viral diseases of bees, such as bag brood and acute paralysis. Different alternatives have been sought for its control, among them are: bees genetically resistant to varroa, use of acaricides that can leave chemical residues in the wax and honey, mainly affecting the safety of beekeeping by-products, in addition to generating resistance in the parasite due to the misuse of said chemicals. Therefore, an alternative is the use of natural products of plant origin that have been shown to have significant potential for the control of varroa and to be an alternative to the use of chemical products, so the objective of this study was a systematic review of articles scientists in search engines such as: Google Scholar, Scopus or Web of Science, on the comparative use of essential oils, recommended for the control of Varroa based on previous research.

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INTRODUCTION

Beekeeping is an economic activity of great importance in several regions of our country, since in addition to generating quality products such as honey, royal jelly and propolis, it provides services of undoubted value for the maintenance of ecosystems, since Bees are one of the most important pollinators, which is why it is necessary to keep bees healthy, preventing diseases and parasites from damaging their work (Moreno, 2022).

One of the main parasites of bees is Varroa destructor, a mite that is considered one of the most important dangers for agriculture. It is an external parasite that attacks both adult and baby bees without distinction and whose life cycle is adapted to that of the bee.

Varroa destructor adheres to the body of adult bees, affecting their work and reducing their lifespan. It consumes the fat reserves of the offspring, in addition to causing malformations and transmitting viral diseases, in such a way that the colony sees its population and honey production decimated and this means that, if the presence of this parasite is not attended to in time, production is seriously affected, causing severe economic damage to the beekeeper (Guerra et al., 2010).

The most common treatments are based on chemical substances that eliminate the mite, affecting its development and killing it, but also favoring the appearance of resistance in different parts of the world. Some of the most used compounds to attack this parasite are Amitraz, oxalic acid, formic acid, coumaphos, thymol and pyrethroids (flumethrin and Tau-fluvalinate) (Medici, 2021).

The use of these commercial products which can be purchased without problems, and whose main drawback is that most of these chemical substances can leave residues contaminating the honey and wax, generating problems for the beekeeper, so alternatives must be sought to eliminate this parasite without damaging collaterals to bee products. The bibliography consulted to carry out this research suggests the use of essential oils, which are natural products that do not affect production and are instead harmful to Varroa destructor. Essential oils do not have organoleptic effects on honey and do not affect bees or their products, therefore they can be an alternative for Varroa control (Reyna et al., 2021).

Importance of Varroa in Beekeeping

Varroa Destroyer

Until 1957, Varroa destructor developed the ability to parasitize the western bee Apis mellifera, since then it spread very rapidly in much of the world (Oldroyd, 1999). The species V. destructor was confused with V. jacobsoni until Anderson and Trueman, (2000) verified that they are different species. V. destructor is attributed with the loss of hundreds of colonies and billions of dollars in relation to the benefit of the agriculture, (Guerra Jr. et al., 2010; Padilla-Álvarez and Flores-Serrano, 2011; Hamiduzzaman et al., 2012; Sanabria et al., 2015).

It has been held responsible for the hive depopulation syndrome, which has been occurring worldwide and has not yet been completely explained (Bacci, 2007). No other pathogen, like this mite, has caused as much impact on bees in the entire history of beekeeping (Le Conte et al., 2007; Seeley, 2007; Rosenkranz et al., 2010; Evans and Cook, 2018), having Keep in mind that the losses currently are incalculable.

When the varroa mite interacts with the bee, there is a close relationship between the biological cycle of both species; The life cycle of the mite comprises two distinct phases (phoretic phase and reproductive phase), the phoretic phase occurs on the adult bee and the reproductive phase occurs only within the sealed brood cell (Rosenkranz et al., 2010).

The mite has evolved in such a way that when it invades bee larval cells to reproduce, they avoid being eliminated from them by worker bees thanks to a kairomonal strategy (chemical substance (pheromone) released by one species that is 'detected' by another species that uses it for its own benefit (Traynor et al., 2020). These pheromones help it camouflage itself between larvae and adult bees by capturing chemical signals that help it avoid the bees' defense mechanisms (Nazzi and Le Conte, 2016; Airahuacho and Rubina, 2021).

The parasitized brood become adult bees that spend less time caring for the brood in the hive (Zanni et al., 2018), and will be a possible vector for varroa dispersal to new colonies (Brosi et al., 2017). The first egg deposited that is male will be the one that fertilizes the females (Evans and Cook, 2018). Only mites that have reached maturity (females) survive, males and immature mites die when uncapping occurs.

The varroa females, which just emerged after their cycle in eight days; They exhibit chelicerae as non-functional stumps with mouthparts that allow them to adhere to adult bees. Fertile adult varroas and some juveniles remain attached to the bees, their mouthparts and the varroa digestive system are structured to feed on semi-solid tissue through extraoral salivary digestion (Ramsey et al., 2018).

Ramsey et al., (2019) showed that this parasite not only consumes hemolymph, but also damages host bees by consuming body fat, a tissue more or less analogous to the liver of mammals. Years ago it was mistakenly thought that the mite fed only on hemolymph. However, the mites fed with body fat survived longer and produced more eggs than those fed with hemolymph. After this, the adult varroae detach from the body of the worker and invade a new capped cell. In females, sexual maturity is reached after 24 hours. Males die shortly after mating, so it is difficult to find them outside the cell.

Varroa infestations in cold environments can vary depending on the climatic environment and the size and strength of the colonies. In cold climates, when the availability of brood in weak colonies decreases, there is a greater incidence of mites in the phoretic state, increasing the proportion of varroa in brood as in adult bees, which decimates their metabolic rate and thus the activity productive (Vásquez, et al., 2000).

Negative Effects of the use of Chemicals in Beekeeping

Acaricides can leave chemical residues in honey and mainly in wax (Lensky and Slabeski, 1981; Cruz, 2007; Lanzelotti, 2007) on the other hand, some results could suggest the participation of pesticides as a cause of colony collapse (Vargas-V et al., 2020). There is evidence in Spain that beeswax contaminated by the application of coumaphos and amitraz in hives can contaminate honey and offspring in contact with it, although the levels found by these researchers did not reach the LD50 for brood nor the MRLs (Maximum Residue Limits) for honey, except for amitraz in December in some hives (Albero et al., 2023). The negative effect on bees is the combination of pyrethroids (fluvalinate and flumethrin) and/or other Neonicotinoids can have a synergistic potentiating effect that makes them 100 times more toxic than that of any pesticide individually, for example, fluvalinate and coumaphos (more toxic effect). Toxic multiplier (Medici, 2021).

Chemical Effects of the use of Chemicals in the Presence of Varroa

The fight against varroa began in the 19th century, developing various techniques such as mechanical, chemical and natural procedures to prevent colony losses (Masry et al., 2020, Spivak and Danka, 2021). Unfortunately, beekeepers continue to apply intensively and often without veterinary control a small group of active materials, fundamentally three groups of pesticides: pyrethroids (taufluvalinate and flumethrin), organophosphates (coumaphos) or amidines (amitraz). Mites have developed resistance to previous pesticides (Milani, 1995; Elzen and Westervelt, 2002; Pettis, 2004; Evans and Cook, 2018). The mechanisms responsible for acaricide resistance involved both metabolic changes and target site mutations in Varroa mite populations worldwide (González-Cabrera et al., 2013; Dmitryjuk et al., 2014). Tau-fluvalinate Resistance to tau-fluvalinate has been established with splice variants and mutations in the voltage-gated sodium channel that can potentially lead to a reduction in the interaction of the acaricide with this protein (Wang et al., 2002). Resistance to coumaphos has also been reported in Varroa mites (Elzen and Westervelt, 2002; Pettis, 2004), but the resistance mechanisms are not clear in the mites.

However, esterase-mediated detoxification may be involved (Sammataro et al., 2005). Resistance to coumaphos has also been reported in Varroa mites, but the resistance mechanisms are not clear in the mites. However, esterase-mediated detoxification may be involved. Although the mechanisms of resistance to Coumaphos by the mite are not completely clear, it is known that this product interferes with the activity of acetylcholinesterase that intervenes with nerve signaling. Therefore, they make these acaricides ineffective (Elzen and Westervelt, 2002; Pettis, 2004).

Use of Essential Oils against Varroa

To prevent the development of resistance to chemical acaricides, as well as environmental contamination, the option of developing and using acaricides from organic substances, therefore, natural and ecological, is sought (Karimi et al., 2022). Some of the most outstanding research on the subject is described.

Sublethal effects may be useful in varroa control; Any effect on this mite that interferes with its ability to locate its host may have practical value as a control method. They developed a school in the search for sublethal effects to control bee parasites and proposed a protocol to evaluate plant products.

Table 1: Comparison of the use of essential products and oils and the decrease in the presence of varroa destructor

Name of the product/ author	Mechanism of action	Advantages of use	Disadvantages of use	Application and recommended dosage
Lemon oil Cymbopongon citratus (Sabahi et al., 2018)	Contains properties to kill mosquitoes and mites	acaricidal activity	Low toxicity in adult bees	5 ml It is placed on the interior walls of the hive.
Oregano oil (Origanum Vulgare) (Chambi and Condori, 2016)	Fungicide, acaricide and disinfectant properties.	Low toxicity for bees Parasite death due to water stress or asphyxiation.	Oil volatilization. Oil application temperature.	30 ml of essential oregano oil Spraying in hives
Garlic oil (Allium sativum) (Reyna et al., 2021)	acaricidal activity	Does not damage the hive Low cost	High doses negative effect on bees resulting in mortality in mites and bees	10 ml In hives
Wild thyme (Acantholippia seriphioides) (Ruffinengo et al., 2005)	Antibacterial and antifungal properties, repellent effect on varroa	Low risk for bees and the environment Leaves no residue in honey	May require high concentrations to be effective May be expensive	0.25; 05 y 1 gram Microencapsulation and evaporation.
Eucalyptus oil (Eucaliyptus radiata) (Ahumada et al, 2022)	Insecticidal and repellent properties	Low risk to bees Effective against varroa and other parasites Low cost	May require frequent applications possible impact on honey flavor	20 ml It is placed on the interior walls of the hive.
formic acid (ácido orgánico) (Calderón et al., 2014)	Varroa irritation, pH alteration in brood cells	Effective against varroa in the pupal stage Low resistance of varroa Decomposition into oxalic acid, leaves no residue	- Potentially corrosive and dangerous to handle Requires special application equipment May cause harm to bees in high concentrations	150 gr Organic gel that is placed on the frames of the breeding chamber.
Thymol gel (timol es componente del aceite de tomillo) (Calderón et al., 2014)	acaricidal effect	Low toxicity Low environmental impact	Toxic when using high concentrations	25 gr Gradual release gel
Neem oil (Azadirachta indica) (Gonzalez et al., 2006)	acaricidal effect	Non-toxic to bees It is used on pests in flowering crops.	In low doses they do not cause parasite mortality.	1-2 mg Spray application
Oxalic acid (Vásquez, 2006)	Direct toxic damage to varroa	- Effective against varroa in the pupal state and adult mites Does not leave residues in the wax Low cost	- Requires repeated application for effective control Potentially toxic to bees at high concentrations	50 ml in sugar solution
Peppermint oil (Mentha Piperita) (Cueto and Estevez, 2020)	Repellent and toxic effect on varroa	- Low risk to bees Lower likelihood of varroa resistance Does not leave toxic residues in honey	- May require frequent applications for effective control Possible impact on honey flavor	9.27 ml Infusions

Source: self-made

Different tests were carried out to determine through which method the elimination and/or control of varroa could be more effective, which were: Acute lethality test in varroa and bees (Application of distilled water plus emulsifier, in concentrations corresponding to those used for each dilution treatment), repellency test with choice (If all the varroas remain on zone zero or there is an equitable distribution on both zones, the values of A will counteract those of B; if all the mites remain on the pupae of zone B, the values of A will be zero and the values of B), repellency test without choice (In this case the pupae that were placed in zone Ac and Bc were treated with the corresponding products and concentrations, as well like the witnesses described). In conclusion, it is postulated that the death of varroa females is due to starvation, caused by the inability of the mites to feed on the offered pupae.

Sabahi et al., (Sabahi et al., 2018), carried out a study which evaluated the relative toxicity and selectivity of anethole and lemongrass oil (*Cymbopogon citratus)* and Sweet marigold on Varroa mites and on larvae and adult honey bees. In which the essential oil was obtained from plant tissues by steam distillation. The methodology consisted of six concentrations of the essential oil prepared in 1.5 ml of acetone, using 280 mites in eight replications, under controlled conditions of temperature 26° and 26% relative humidity. The solution was spread on the inner walls of the vials with the mites, covering after 10', the results were read 4 hours post treatment. With the help of a needle, the mites were touched; if they did not move, they were considered dead. It was obtained that anethole and Cymbopogon oil showed significant effects on mites at the same time, high selectivity and safety margins for the honey bee.

Chambi and Condori (Chambi and Condori, 2016) carried out a study, which used oregano essential oil (Origanum vulgare) at 40, 45, 50, 55 and 60% analyzing volatilization in oasis sponge in three surface support areas. It was decided to use the 40% concentration since it contains a greater amount of ethyl alcohol and volatilization increases, although the amount of active product is lower, there is no significant difference with the other concentrations. This concentration was compared against a commercial acaricide, resulting in the use of oregano oil being 23% more efficient than the commercial one.

(Table 1) identifies the main substances and essential oils used to eliminate Varroa in bees. Considering lemon, eucalyptus, oregano, garlic and thyme oil for its low toxicity effect on bees and honey, this being a harmless product for human consumption.

The oils can be applied in different ways such as evaporation, gel or directly to the hive depending on the active substance of each oil, in addition to the fact that some require the use of another component (ethyl alcohol) for their activation and to have the effect of eliminating Varroa. In addition, each oil has a varied action time that can range from hours to weeks where they will be most effective on the parasite (González- Gómez et al., 2006).

As for formic acid, its effectiveness can be in combination with thymol, which is a component of thyme oil, considering other factors such as temperature, ventilation so that the steam is distributed evenly over the hive, its effectiveness can be from the first day. of application until the 30th.

Conclusions

Varroasis is a complex problem for the beekeeping industry worldwide. Adequate varroa control planning should first include the restricted use of chemicals, and second, management techniques that include low levels of parasite infestation. The current trend in animal production lies in the use of practices and use of noble and natural substances that guarantee animal well-being, thus the safety of the product, thus being the bases of food safety. There are many studies related to the use and applications of essential oils on the control of varroa destructor in *Apis mellifera*, however those presented here are for isolated use, that is, they do not combine more than one oil with another to identify if there is a synergistic action. It is proposed to search or carry out studies where two or more essential oils are combined. Likewise, identify through laboratory studies if there is a residual of the active substances of essential oils in honey, thereby detecting if safety is affected or a product with added value can be obtained. The present study offers recommendations for beekeepers interested in using essential oils to control varroa and as a result reduce the use of acaricides that leave residues in the wax and honey and cause resistance in the mite.

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