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CONTINUOUS MANAGEMENT OF *VARROA* MITE IN HONEY BEE, *APIS MELLIFERA,* COLONIES

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ABSTRACT: *Varroa* mite (*Varroa destructor*) is the major challenge for beekeeping worldwide. Therefore, various methods and materials for *Varroa* control have been suggested and tested including; plant extracts, essential oils, biological agents, mechanical methods and some chemicals. This paper aims to present the available options for *Varroa* control and to survey the performed comparative studies among these options. This paper can be considered as a good guide for researchers during their studies on *Varroa* control. The presented review highlights that still more comparative studies among *Varroa* control options under different ecological conditions are strongly required. Basically the performed researches have been concentrated on testing chemical materials and essential oils while relatively few studies have been done on the other control options. It is concluded that the *Varroa* mite should be continuously (monthly) managed within honey bee colonies using mechanical methods or treatment with essential oils (mainly thymol). In severe cases, and especially during the fall and not during honey seasons, the use of chemical materials can be done with preference to oxalic or formic acid.

KEY WORDS: honey bees, mite, ectoparasite, control, *Varroa destructor*

INTRODUCTION

The *Varroa* mite has many deleterious impacts on honey bee colonies. These impacts are well known and include, for example, the weakness of honey bees by feeding on the haemolymph (Rosenkranz et al. 2010), transportation of viruses to host bees including Kashmir bee virus (Chen et al. 2004), suppresses the immunity of honey bees (Shen et al. 2005), impact bee wing Shape (Cakmak et al. 2011) and can impact virgin queen's acceptance and mating success (Rateb et al. 2010). Due to the importance of the *Varroa* as a major pest to honey bee colonies, various studies have been done worldwide on this mite to investigate its biology, behavior and means of control. Different reviews were presented to cover the wide range of topics related to *Varroa*. Tolerance of honey bees in South America to *Varroa* mite was reviewed by Rosenkranz (1999). Imdorf et al. (1999) reviewed the use of essential oils as a control method while pathogen based biopesticides were reviewed by Meikle et al. (2012). Calis et al. (1999a) reviewed mite control based on using trapping in honey bee brood. The biology and control methods of *Varroa* were reviewed by Rosenkranz et al. (2010) while Dietemann et al. (2012) presented a review about *Varroa* mite followed by a suggested short and long term plans towards *Varroa* control. Czirják and Monica (2013) reviewed the different options available for the control of *Varroa* mite in Romania. Recently, Dietemann et al. (2013) presented standard methods for *Varroa* research.

This paper aims to present a guide to the available *Varroa* control options and to review the performed comparative studies within and among options to highlight the gaps in the previous investigations.

METHODS OF CONTROL

1. Plant extracts and essential oils

The effectiveness of the essential oils against a wide range of pests and plant pathogens is well known (Koul et al. 2008). Many comparative studies were done on the effectiveness of essential oils and plant extracts as possible *Varroa* control materials. The recent comparative studies during 2000s are presented in Table 1. While studies till 1999 were reviewed by Imdorf et al. 1999. The other studies which were mentioned in any recent review are not presented here to avoid unnecessary repetitions.

In Ruffinengo et al. (2001) study, the highest *Varroa* mortality (63%) was to *H. latifolia*. Black cumin oil in Allam and Zakaria (2009) investigations caused the highest dead *Varroa* fall number (with total of 1209 *Varroa*) while in Refaei (2011) study it was to thymol gel (apiguard) which caused fall of 151.3 *Varroa* mites. In Al-Zarog and El-Bassiouny (2013) experiments, thyme showed the highest efficacy causing *Varroa* reduction rate of 61.7% while garlic oil cacused 75.03% reduction of *Varroa* population in investigations of Goswami and Khan (2013). Neem oil caused high *Varroa* reduction (59%) from the brood (Qayyoum et al. 2013). The highest effectiveness was to coriander oil in Omran et al. (2011) study and to marjoram oil in Kraus et al. (1994) study. Hop beta acids had the ability to control *Varroa* in honey bee colonies with no impacts on bees or queens (DeGrandi-

Table 1.
Comparative studies on plant extracts and essential oils.

Studied plant extracts and essential oils / experiment type	Author (year)
Heterotheca latifolia and *Tagetes minuta* essential oils / Laboratory	Ruffinengo et al. (2001)
Spearmint, eucalyptus, thyme, marjoram, basil, cumin, garlic, orange, geranium, and eugenol menthol / Laboratory and Field	Ismail et al. (2006)
Black cumin oil, *Nigella sativa*, in comparison with a mixture of black cumin oil, lemon oil and jasmine oil, *Jasminum grandiflorum* / Field	Allam and Zakaria (2009)
Marjoram, coriander, peppermint and black cumin / Field	Omran et al. (2011)
Basil oils, camphor (*Eucalyptus* spp.),apiguard (thymol gel) and thyme (*Thymus vulgaris*) / Field	Refaei (2011)
Neem seed, *Azadirachta indica* / Laboratory	Gonzalez-Gomez et al. (2012)
Beta acids from hops plant, *Humulus lupulus* / Laboratory and Field	DeGrandi-Hoffman et al. (2012)
Lemon grass, *Cymbopagon flexuosus*; thyme, *Thymus vulgaris*; cinnamon, *Cinnamomum zeylanicum*; anise, *Pimpinella anisum* / Field	Abd El-Wahab et al. (2012)
Oils of common rue, eucalyptus, mint and thymol / Field	Castagnino and Orsi (2012)
Plant extract of fenugreek, santonica, mixture of fenugreek and santonica, and thyme / Field	Al-Zarog and El-Bassiouny (2013)
Tobacco oil, mixture (neem, garlic and tobacco oils) and neem oil / Laboratory	Qayyoum et al. (2013)
Oils of garlic, turmeric, tulsi, ajwain, clove and cinnamon / Field	Goswami and Khan (2013)
Eucalyptus, cinnamon and menthol oils in combination with other control methods / Field	Kotwal and Abrol (2013)
Thymol powder / Field	Ahmad et al. (2013)

Hoffman et al. 2012). Kotwal and Abrol (2013) found varying results according to the season and the best results, during February to April, were to queen caging practice and cinnamon oil. In general, the performed studies on the use of these oils against *Varroa* mite either in vitro or within colonies showed high degree of efficacy. It is clear that different oils were used through studies with lacking of wide comparisons among them.

2. Biological methods

2.1. Entomopathogenic fungi. Various entomopathogenic fungi were tested as possible control agents to *Varroa*. These fungi were placed in comparative experiments as follows; laboratory experiments using *M. anisopliae* and *Beauveria bassiana* (Rodríguez et al. 2009), and using *B. bassiana*, *M. anisopliae* and *Clonostachys rosea* (Hamiduzzaman et al. 2012), and field experiments using *Lecanicillium lecanii* and *Metarhizium anisopliae* (Gerritsen and Cornelissen 2006), and using *B. bassiana* and *M. anisopliae* (Ahmed and Abd-Elhady 2013).

The efficacy of the entomopathogenic fungi against *Varroa* is not constant (Meikle et al. 2012). Gerritsen and Cornelissen (2006) found that *M. anisopliae* and *L. lecanii* were not effective against *Varroa* within beehives while Hamiduzzaman et al. (2012) found a good ability of the entomopathogenic fungi to reduce *Varroa* damages to honey bees. In Rodríguez et al. (2009) study, *M. anisopliae* had high pathogenic capacity against *Varroa* with mortality rate of 85% and with good ability to tolerate beehive temperature. Similarly, Ahmed and Abd-Elhady (2013) found good results to *M. anisopliae*. The impact of these fungi on honey bee is rather low (Hamiduzzaman et al. 2012). A comparative study between all the previously mentioned fungi is required under laboratory and field conditions, and by using different formulations. These fungi should be tested on different honey bee subspecies. Temperature within beehives should be taken into account due to its importance for fungi (Davidson et al. 2012). It could be said that *M. anisopliae* is the best *Varroa* control option than other entomopathogenic fungi.

2.2. Mite predator. The use of mite predator (i.e. pseudoscorpions; Arachnida: Pseudoscorpionida) was tested. Fagan et al. (2012) found possibility of using pseudoscorpions as *Varroa* predator with no harmful impacts on honey bee larvae. For a single beehive, they expected about 25 *Varroa* predators can manage *Varroa* population. Still more investigations on the *Varroa* predators are strongly required. Comparative studies between possible biological control agents against *Varroa* should be also done.

3. Oxalic and formic acid

3.1. Oxalic acid. Under field conditions, Mutinelli et al. (1997) found the efficacy of sprayed 5% (w/v) oxalic acid is higher than topical application with oxalic acid. Higes et al. (1999) found the efficacy of 3 % sprayed oxalic acid was 94 % and 73% in autumn and spring, respectively with losing three queens within the treated colonies. Gregorc and Planinc (2004) found significant increase in mite mortality after the treatment with Oxalic acid (2.9%). Akyol and Yeninar (2009) found efficacy of oxalic acid with mean 93.40% in the fall with no harmful impacts on queens, brood or bees. A water solution of 0.5% sprayed oxalic acid gave effective control of the mite in autumn and was not toxic to bees than higher concentrations of 1.0 and 1.5% (Toomemaa et al. 2010). The concentration is very important for good effectiveness (Rashid et al. 2012). The treatment with oxalic acid also reduced *Varroa* mite infestation in adult bees by 87.4% (Castagnino and Orsi 2012).On the contrary, oxalic acid treatment in Spring did not protect honey bees from *Varroa* spread (Pileckas et al. 2012). It could be said that oxalic acid is an effective *Varroa* control treatment in honey bee colonies.

3.2. Formic acid. The effectiveness of formic acid is well known for sealed and unsealed brood, but its results are impacted by temperature (Czirják and Monica 2013). Under field conditions, Lupo and Gerling (1990) found good results for formic acid treatment against *Varroa* in June and August. Bahreini et al. (2001) concluded that formic acid had significant effectiveness against *Varroa* for honey bee colonies in Iran. Cornelissen and Gerritsen (2006) found more effectiveness of 3% oxalic acid sprayed on the bees by 1% than formic acid against *Varroa* in combination with swarm prevention technique (hive splitting into two) in Spring. The effect of the oxalic acid treatment was higher than that of the formic acid with less harmful impacts on honey bees (Pileckas et al. 2012). In line with this finding, Mahmood et al. (2012) found 3.2% oxalic acid treatment recorded higher effectiveness in controlling *Varroa* than formic acid and flumethrin strip (Bayvarol). Thus, oxalic acid is better than formic acid as mite control treatment.

4. Acaricides

4.1. Studied acaricides. Under field conditions, the efficacy of various acaricides against *Varroa* mite was investigated. Marchetti and Barbattini (1984) compared the efficacy of apiakaridim, folbex VA, thymol crystals and taktic. Kraus and Berg (1994) studied the efficacy of lactic acid, Perizin with lactic acid, and Perizin. Bahreini et al. (2001) studied fluvalinate (Apistan ®) and formic acid 65% while Girisgin and Aydin (2010) studied flumethrin (Varostop®). Apiguard, thymovar, oxalic acid, and amitraz fumigation were compared in a study by Gregorc and Planinc (2012). Adjlane et al. (2013) tested bayvarol, apivar, apistan, amitraz and tau-fluvalinate (Mavrik) while Semkiw et al. (2013) studied the effectiveness of amitraz strips as 500 mg active substance/strip.

4.2. Efficacy of acaricides. Marchetti and Barbattini (1984) found lower effectiveness of thymol crystals while folbex VA, apiakaridim and taktic showed insignificant results. The mean efficacy of amitraz was from 90.6% to 94.6% (Kraus and Berg 1994) and for flumethrin was 94.9 % in fall season (Girisgin and Aydin 2010). The lactic acid showed potential to be *Varroa* mite control compound with some limitations (Kraus and Berg 1994). The results of Gregorc and Planinc (2012) showed oxalic acid, thymovar, apiguard or amitraz fumigations were of limited use during the brood periods. In Adjlane et al. (2013) study, bayvarol had the highest efficiency rate (91.62%), followed by apivar (86.50%). Amitraz efficacy did not change over time although the problem of mite resistance to miticides (Semkiw et al. 2013). Great variability in chemical efficacy is found and it could be said that amitraz is good option for *Varroa* control in honey bee colonies.

5. Resistance of honey bee subspecies to *Varroa* mite

Honey bee genotypes differ in their ability to resist the *Varroa* mite and this ability is related to the intensity of grooming behavior in these genotypes (Guzman-Novoa et al. 2012). An example of these tolerance genotypes is the African honey bees which have more tolerance ability than European honey bee races (Moretto and de Mello 1999). Honey bee races could be resistance to *Varroa* mite through grooming behavior, Varroa Sensitive Hygiene (Villa et al. 2009) or hygienic behavior (e.g. Ibrahim and Spivak 2006). These behaviors could be impacted by some factors, including temperature and humidity (Tahmasbi 2009). On the contrary, Bak et al. (2013) found no impact of the honey bee subspecies on *Varroa* population growth. NajiKhoei et al. (2011) found no relationship between colony strength as the number of workers within colonies and resistance against *Varroa*. Obtaining a resistance bee race to *Varroa* mite is a good option towards sustainable mite control.

6. Mechanical and heating methods

Various mechanical methods can be used for *Varroa* control. Not all of them are well documented, including smoking practice over honey bees using plant leaves or using pollen traps to dislodge *Varroa* from forager bees.

6.1. Dusting method. Ellis et al. (2009) found insignificant *Varroa* control after dusting colonies with powdered sugar. Bak et al. (2009) found no statistical differences between shaking with the powdered sugar and flotation method of diagnosing the level of infestation with *Varroa*. Dusting with powdered sugar could be considered as a weak tool for *Varroa* control (Berry et al. 2012) while honey bee sprinkling with sugar syrup increased mite drop 2.5–2.7 times compared with the natural mite drop (Pileckas et al. 2012).

6.2. Modified beehives. Another method the use of the modified beehive bottoms which have also benefit in controlling small hive beetle (Keshlaf and Spooner-Hart 2013). Using modified beehive bottoms filled with water was not found to spread disease and *Varroa* in the colonies (Abou-Shaara et al. 2013).

6.3. Drone comb method. Drone comb trapping significantly reduced infestation of honey bee colonies (Pileckas et al. 2012). Calderone (2005) found that the drone brood removal technique showed efficacy for *Varroa* control with enhanced honey production in treated colonies. The efficacy of this technique depends on the available number of drone cells for *Varroa* trapping (Calis et al. 1999b). This technique depends on the preference of *Varroa* mite to drone cells with large size than worker cells with small size. However, combs with small cells can not reduce *Varroa* infestation (Berry et al. 2010). It worth to mention that queen caging to reduce brood area is another method for controlling *Varroa* (Kotwal and Abrol 2013).

6.4. Heating method. Heating also can be used as an option towards mite control. Harbo (2000) found that 40°C is the lowest temperature at which all mites can be removed from caged bees over 48 hours. Huang (2001) invented a device “mitezapper” for *Varroa* control depends on heating for killing the mite. All these methods can be considered as a safe way towards *Varroa* control and should be applied in combination with other methods.

7. Compounds derived from honey bee and *Varroa*

Different compounds can be applied for *Varroa* control either by repelling it or by disturbing its normal behaviors. Hoppe and Ritter (1988) found that Nasonov pheromone (mainly geraniol) has repellent effect on *Varroa* mite. Another repellent effect was found by Kraus (1990) to alarm pheromone (mainly 1-octanol). Newly molted females are more attractive to *Varroa* male than older ones (Ziegelmann et al. 2013a). Disrupting the *Varroa* mating behavior could be done using the female sex pheromone (Ziegelmann et al. 2013b). No much studies were done on these points, however, it could be expected that using such materials as control method is not ease. May be mixing repelling compounds with female sex pheromone in one blend could be more effective against *Varroa*.

COMPARISONS AMONG CONTROL OPTIONS

Some comparisons were done among *Varroa* control options to identify the best ones. Abd El-Wahab et al. (2012) found effective control results to formic acid and the highest concentration (100%) of essential oils; lemon grass, cinnamon, thyme and anise while garlic oil had better results than formic acid in study by Goswami and Khan (2013). In another investigation, Kotwal and Abrol (2013) found the best results of *Varroa* control were to formic acid and cinnamon oil for treated colonies during August to October while during November to January was to thymol + oxalic acid, and to sulphur dust and oxalic acid during May to July. The best results were to oxalic acid and common rue oil in Castagnino and Orsi (2012) study. The highest effectiveness against *Varroa* mite was to thymol powder with 73.72% mite mortality followed by formic acid with 72.23% and fluvalinate strips with 69.21% (Ahmad et al. 2013). Shoukry et al. (2013) found formic acid 60% had less impact on the mating ability of honey bee drones than oxalic acid, fluvalinate, thymol and amitraz. Most of the performed studies concentrated on the comparison between essential oils and chemical materials. Still more studies are required to present comparisons among biological methods, chemical materials, essential oils, genetic traits and mechanical methods to recommend the best effective method.

CONCLUSION

Still more comparative studies are required to be performed under laboratory and field conditions on the *Varroa* control options. These studies should be done on a wide scale and under different ecological conditions. All possible factors impacting treatment efficacy should be considered during studies (e.g. *Varroa* mite population and brood rearing activity). It worth to mention that it is too hard to compare results of different studies as researchers use various ways to assess treatment efficacy. Thus, using percent of dead *Varroa* after treatment application is advisable to be used as a standard indicator for treatment efficacy at any study on *Varroa* control. That to facility results comparison between different treatments or experiments. The *Varroa* mite can be considered as a continuous problem for beekeepers especially since there are various dispersal methods for the *Varroa*. Due to the fundamental role of bee foraging behavior in *Varroa* mite prevalence (Abou-Shaara 2014) beekeepers can not easily control *Varroa* mite by 100% in their colonies. It could be said that the required task is the continuous management of *Varroa* mite within colonies. In the light of the reviewed studies, beekeepers can monthly do one practice to manage *Varroa* mite in their colonies using mechanical methods (e.g. sprinkling honey bees with sugar syrup) or treatment with essential oils (mainly thymol). In severe cases, and especially during the fall and not during honey seasons, the use of chemical materials can be done with preference to oxalic or formic acid. It could be expected that the continuous management of *Varroa* will reduce its damages greatly and will save honey bee colonies from losing.

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