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# Diagnosis of Varroa Mite (*Varroa destructor*) and Sustainable Control in Honey Bee (*Apis mellifera*) Colonies—A Review

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**Abstract:** Determining varroa mite infestation levels in honey bee colonies and the proper method and time to perform a diagnosis are important for efficient mite control. Performing a powdered sugar shake or counting mites that drop from combs and bees onto a hive bottom board are two reliable methods for sampling varroa mite to evaluate the efficacy of an acaricide treatment. This overview summarizes studies that examine the efficacy of organic acids and essential oils, mite monitoring, and brood interruption for integrated varroa mite control in organic beekeeping.

**Keywords:** varroa mite detection; diagnosis; infestation; mortality; control; organic treatment

## 1. Introduction

Varroa mites, *Varroa destructor* Anderson and Truemann, are the single most devastating global pests of both adult and immature honey bees, *Apis mellifera* L. Feeding by mites reduces adult bee body weight, life span, and immunity to pathogens [1–4], Adult female varroa mites are physically large (~1.5–2.0 mm wide) parasitic mites that disperse through phoresy by attaching themselves to worker bees and drones. Before worker bees seal comb cells with wax, varroa mites move into brood cells occupied by mature bee larvae where they will eventually feed on the fat bodies and hemolymph of host pupae [5,6]. If mite populations develop undetected, untreated and infested honey bee colonies usually collapse within a year [3]. Although there are multiple factors contributing to colony mortality, in many cases, the most important are varroa parasitism [4] together with the transmission of an extensive set of honey bee viruses [5]. The chemical control of varroa mites presents a paradox. Toxic acaricides must be used to kill mites, but they can also have toxic side-effects on bees and whole colonies. Thus, acaricides must be applied at their lowest effective dose to minimize a build-up of chemical residues and their by-products in bees, honey, and wax [6], as well as to slow the evolution of acaricide resistance [7]. Improved monitoring for mites and the application of less-persistent, low-residual acaricides could help reduce the amount of toxic active products applied each season. Therefore, we review research regarding improvements in mite monitoring and the efficacy of alternative organic acaricides.

### *Evaluation of Colonies Infestation with Varroa Mites*

Researchers and beekeepers employ various standardized or incidental methods to monitor varroa mites infesting adult honey bees and brood [8]. These methods include counting mites on traps and on such colony contents as hive debris on the bottom boards, brood, as well as the bodies of captured worker bees. Sampling such hive contents is crucial for collecting data for optimally timing acaricide applications and effectively controlling mites [9].

Currently, simple field and laboratory diagnostic procedures are available for assessing overall varroa mite infestation within entire honey bee colonies. Beekeepers have been evaluating for decades the efficacy of an acaricide treatment by counting dead mites that drop from brood frames and bees onto the hive bottom board [10,11]. The number of these fallen mites found within a hive's debris correlates well with living mite populations infesting the colony above [12]. In fact, researchers studied the correlation between the natural mite mortality and the total number of mites in honey bee colonies. They found that varroa mite populations in colonies with brood can be approximated by multiplying the daily varroa mite mortality detected on hive bottom boards by 20–40 [13]. After performing mite counts and once infestations of varroa have reached or exceeded economic threshold level, e.g., at 3 mites/100 bees in the colony, control is warranted [13]. However, treatment threshold levels will vary according to colony size, location, management and other stress factors [14]. Therefore, keeping data records of natural mite fall levels within a colony is useful for determining (1) the level of mite infestation before and after acaricidal application and (2) seasonal trends in the size of natural mite populations [15]. Bottom board counts are especially useful for estimating total varroa mites per colony and daily mite mortality, even for very small mite populations [16], because varroa mite populations below 100 mites per colony cannot be detected by examining samples of adult bees or brood [17]. However, mite sampling protocols that rely on bottom board mite counts are time consuming, more costly, and may take several days or weeks to establish a relevant colony infestation level. Varroa mite counts may also vary with levels of mite removal by bees (i.e., hygienic behavior) [18].

A simple non-destructive method for sampling varroa mites living on the bodies of adult honey bees is the "sugar shake". This technique simply involves applying powdered sugar to the bodies of living bees. The powdered sugar will quickly clog the tarsal pads of varroa mites, they lose adhesion, and become permanently dislodged from host bees [19]. The sugar shake method removes 77% to 91% of mites for counting [20,21]. Even in highly infested colonies, 82% recovery of varroa mites is possible using the sugar shake method [22]. The size of mite populations in an entire colony can be extrapolated from counts of mites dislodged from adult bees taken from a single brood frame. Higher precision in estimates of colony infestation simply requires counting dislodged mites from a greater number of captured bees [8]. Hence, the "sugar shake" procedure is a simple and reliable method that beekeepers can use to evaluate the number of varroa mites on adult worker bees, drones, and even throughout an entire colony [22,23]. Sugar shake is also a useful sampling protocol when evaluating different mite controls [24]. There are a few caveats to consider when using the sugar shake method. The efficiency of the powder shake method to extract varroa mites from adult host bees can vary with fluctuations in honey bee population size and environmental conditions. Average effectiveness of the sugar shake method to remove varroa mites from adult honey bees in a hot, humid environment (i.e., 32 °C and 76% RH) is ~66%, a rate below the 94% obtained in drier, cooler conditions (i.e., 26 °C and 71% RH) [25]. Sugar quality is also important for dislodging varroa, with some sugar mixtures outperforming others. For instance, a fine dusting of pure powdered sugar dislodges 70–80% of varroa mites, which greatly exceeds the 50% varroa drop achieved using a mix of powdered sugar and corn starch. Thus, sugar quality combined with a humid environment appears to influence the efficacy of sugar shakes to remove mites [19]. Perhaps, a higher temperature and humidity causes grains to clump, thereby increasing the sugar's coarseness and reducing rates of tarsal clogging. Generally, dusting honey bees with powdered sugar is considered a safe mechanical method for diagnosing colony infestations and in some cases, can be used to reduce varroa mite infestation in field colonies [24].

A similar approach to removing mites from host bees is to use washing kits, often homemade, with water or alcohol (70%) serving as both the washing and collection fluid [26]. Accurate estimates of the number of varroa mites on adults using either the sugar shake method, water wash, or alcohol wash can be performed in colonies without brood for research or practical beekeeping purposes. When using an alcohol wash, approximately 300 bees are sacrificed without any noticeable effect on colony strength or overall health and productivity. However, alcohol washes will kill both mites and the small number of bees selected for sampling. In fact, accurate assessments of colony infestation obtained with this diagnostic test are crucial to ensuring colony survival and productivity [27]. Based on an established number of varroa mites obtained by sugar shake or alcohol wash, applications of miticides or other treatments may be warranted. Therefore, the efficacy of previous control treatments can be assessed using both methods. Varroa mite levels between 3 percent and 5 percent are tolerable for beekeepers. When mite levels are above 5 percent, immediate varroa mite control needs to be performed [28,29]. A similar wide range of economic threshold for varroa mite populations in colonies was established for beekeeping conditions in the United States at levels between 5 and 12 percent and most beekeepers prefer to use the 5 percent [13]. Precise estimation of varroa mite population size is established by surveying mite loads on adult and pupal bees as well as through counts of dead varroa mites within a colony. The population dynamics of varroa mites mainly depend on the reproductive success of varroa females in worker brood cells [30,31]. Furthermore, mite population size may be estimated by a combination of adult bee and brood samples and compared to the number of mites killed by chemical treatment [32].

## 2. Varroa Mite Control with Organic Substances

Proper timing of control is best accomplished by regularly monitoring mite levels inside colonies and comparing varroa mite population trends. Organic acids derived mostly from active components of plant and essential oils are widely used for varroa mite control in conventional and organic honeybee colonies [11,33]. Thymol is one such component, which is a natural constituent of thyme (*Thymus vulgaris*) and a volatile monoterpenoid with acaricidal activity against varroa [28]. The efficacy and environmental safety of several commercial products containing thymol have been evaluated [11,29,30]. It has been established that thymol residues may accumulate in a hive, but they soon dissipate to a natural hive level at or below 1.1 mg thymol/kg honey. Thus, thymol administered to a hive's population does not alter the taste or nutritional quality of harvested honey [31]. Two popular thymol-based acaricides are available for beekeeping. They include ApiGuard® and Thymovar®. ApiGuard is a registered thymol-based fumigant that in its gel form controls high percentages of varroa mites in honeybee colonies. In broodless colonies, ApiGuard can kill 54 percentage points more varroa mites (77% mortality) than does natural mortality alone (23% natural mortality), [14]. Similarly, Fasbinder et al. [33] attained 64% mortality with ApiGuard. The most effective applications of ApiGuard occur in autumn, a time when this acaricide has little to no negative impact on the growth of early spring bee populations [34]. However, like many other acaricides, the effectiveness of ApiGuard treatments varies according to environmental conditions. Thymovar (Andrematt Biocontrol AG) is another thymol-based product formulated as 15 g of thymol impregnated onto a cellulose wafer. This product is typically applied after the last honey harvest in the beekeeping season. In one of our previous experiments, we found that Thymovar kills 54–66% of varroa mites [32] and in another experiment, [35] attained a mean percentage efficacy of 85%.

Other non-persistent organic acids with high acaricidal activity include formic acid (FA), a common defensive compound sprayed by certain arthropods, and oxalic acid (OA), a minor constituent of honey and a plant substance that, in a normal field context, repels or sickens a plant's insect antagonists [30,36]. Another organic acaricide with high activity, derived from hop beta plant acids, is HopGuard®. It is particularly effective at killing varroa mites in colonies with both open and sealed brood. The recommended timing of HopGuard is during the months of June and October in colonies with large brood areas [37]. HopGuard efficacy can vary seasonally and among colonies, but it often induces >80% varroa mite mortality in brood-right colonies. HopGuard works quickly and kills 65% of varroa mites within 24 h of application [38].

FA treatment alone kills 43% of mites on the bodies of adult worker bees, but is less effective in autumn when colonies receive double fumigation with 65% FA [39]. Improved formulations of FA (50 mL and 100 mL at 85%) impregnating thin plates of soft treefiber induces 100% mortality of mother mites with 90% of honey bee brood surviving. Higher bee brood loss did occur with a treatment of 100 mL FA [40]. Absorbent cardboard plates (Illertissen mite plates) soaked with 20 mL of 65% formic acid induces 94% varroa mite mortality in Schwarzwald (Black Forest) colonies [41]. Higher varroa mite control efficiencies of 95–97% using FA impregnated soft fiber plates (Pavatex) depended on both concentration and duration [42]. Formic acid as a volatile organic compound dissipates quickly after treatment and hence, has a very short period of residual activity [43]. At higher temperatures, FA evaporation increases, and if hive ventilation is impeded by bees covering vents with propolis, mite mortality may fall, while brood mortality increases [40].

Oxalic acid (OA) is acutely toxic to varroa mites. Due to this acaricide's high efficacy and low risk of hive contamination, OA has been extensively used by itself and in combination with other mite controls [30,36]. Application typically involves trickling OA in sugar water solution between the frames of a honey bee hive. The recommended application rate per hive is 50 mL of OA solution. Various formulations of OA that have been tested include 3.4% OA dihydrate in 47.6% aqueous sucrose (w/w), 3.7% OA in 27.1% aqueous sucrose, and 3.0% OA in 32.0% aqueous sucrose [44,45]. Oxalic acid can kill 33–69% of varroa mites within the first two days following application, particularly in summer and autumn. From 3 to 11 days post-treatment, OA residual activity drops off significantly to 14–23% after the first 3 and 4 days and to almost 0% after 9 days [46]. Higher concentrations of OA in sugar water does increase varroa mite control but may pose a greater toxicity risk to bees. In fact, honey bees are susceptible to the toxic effects of both conventional and organic acaricides, including OA even at low concentrations [45]. In toxicity tests using caged bees, the highest dosage of OA acaricide is approximately 250 μg per bee, which is a tolerance level 2.5 X higher than previously reported [47]. However, OA is less toxic to honey bees than is the conventional acaricide Apistan®. We suspect that individual bees are killed by direct dermal exposure to OA in both laboratory tests and colony treatments. While some bees are exposed to lethal doses of OA when using the trickling method within colonies, queens have a very low probability of exposure, yet mortality within any caste of honey bee could affect overall colony productivity, unless steps are taken to limit brood and adult exposure during initial acaricide applications [24].

Single applications of OA are the least harmful to bees, but may be insufficient for adequate varroa control. Brødsgaard [48] reported only a 24% efficacy after administering a single OA treatment in the spring using the trickling method. Repeated applications of OA during specific periods of colony development substantially improve varroa mite control. Three consecutive OA treatments in colonies maintained in a temperate environment with brood present reduced varroa mite populations by 39–52%. It seems that colonies with a threshold level less than 1 dead mite found on the hive bottom board per day by the end of July should receive three OA treatments, which will reduce the mite population by approximately 40% [49]. Reducing the mite population ensures a colony's normal development and winter survival. However, OA efficacy rises considerably to 98–99% when colonies lack brood [49,50]. Other researchers reported similar results with brood-right and broodless colonies in different climactic regimes [32,42,44,48]. Additionally, Nanetti et al, [50] and Imdorf et al. [51] eliminated 97.3–99.5% of mites with OA in broodless colonies, whereas Mutinelli et al. [52] achieved 95% mite control with three applications of 5% OA in colonies with capped brood. When using the trickling method in colonies with capped brood during the winter, the cumulative efficacy of four oxalic acid applications ranged from 90% to 100% with a mean value of 98% [53]. In the study by Toufailia and Ratnieks [53], efficacy values may have been overestimated because the experiment included a final Apistan application, which sometimes is less than 100% effective.

As alluded to previously, acaricidal efficacy of repeated OA applications vary during specific periods of colony development. When compared with a relatively low efficacy during the brood season, oxalic acid application results in higher cumulative varroa mite mortalities in overwintering colonies lacking brood. When capped brood is present, ~70% of the phoretic (adult) varroa are killed within two days of OA treatment [46,54]. Three summer applications of 2.9% OA in 31.9% sugar water (w/w) controls 30–52% of varroa mites [50], a range within that discovered by Toufailia et al. [55] (20–93% mite mortality) using similar OA formulations. In brood-right colonies kept in the Mediterranean area (Sardinia, Italy) with brood present, three OA applications in late autumn resulted in 65% varroa mite mortality [54,56]. Higher mite mortalities occur in winter for OA-treated colonies containing brood and in summer, for colonies receiving repeated OA applications. Further research is necessary to assess bee safety and acaricidal effectiveness of oxalic acid in honey bee colonies overwintering in warm temperate climates. A combination of four successive oxalic acid applications during warm temperate winter conditions ensures a substantial reduction in varroa mite populations before colony growth accelerates in spring. The high correlation between the natural mite-mortality and the mite mortality induced after multiple OA treatments during the summer indicates that the continual monitoring of mite drops within bee colonies is important [46].

As we have seen, greater mite control during cooler months indicates that improved varroa mite control is achievable by integrating OA control with a colony's broodless period. Broodless periods may not naturally occur in colonies maintained in warm-temperate to tropical climates where honey bees may reproduce year-round. Modelling seasonal mite population dynamics is needed, however, to determine when to artificially or naturally break brood rearing before applying acaricides like OA [57]. However, in warmer regions where bees tend brood in winter, colonies can be rendered artificially broodless by interrupting brood production through brood removal, particularly drone brood, or queen exclusion [30,58,59]. Coupling screened bottom boards with drone brood removal or queen caging can further interfere with mite reproduction and development [17,29,37]. The removal of brood and the queen also concentrates OA treatment on the phoretic form of varroa mites, reducing the likelihood of OA harming brood and reproductives, and hence, a colony's future productivity. Queen caging combined with a single application of an OA or thymol-based acaricide can increase the efficacy of varroa mite control to 96–97% [60,61]. The temporary caging of the queen, combined with the removal of capped or un-capped brood, has resulted in >93% varroa mite control [62]. The vaporisation OA crystals in colonies without brood is another varroa mites control method. OA crystals are placed into the small cup of the electrically heated applicator inserted under the bee cluster on the hive bottom. Vaporisation 2.25 g of OA per broodless colony induced 97.6% varroa mites mortality with 98% colonies overwinter survival [55]. Experiments were also conducted to test the efficacy of 10 g of OA mixed with 20 mL of glycerin impregnated cellulose strips. The average efficacies in three experimental locations in Argentina were in the rage of 93% to 94% [63].

Colonies with worker bees displaying a propensity to remove varroa mites along with sick brood (bee hygiene) may also be more safely treated with OA. A combination of mite removal by bees and OA applications could be a highly effective method for varroa management, particularly in organic operations where the use of conventional acaricides is prohibited. Comparing all three organic acaricides applied against varroa in colonies containing capped brood, Thymovar is more effective than FA or OA on a per application basis. However, two OA sublimations applied in autumn are as effective, or more effective than Thymol, killing 82% of varroa mites [37]. In contrast, two formic acid treatments did not reach the 85% Thymovar efficacy achieved by [36].

## 3. Integrated Varroa Mite Management

The main objective of integrated varroa management is to reduce or delay the use of chemical treatments in honey bee colonies. There are two options to limit chemical use: (1) removal of varroa from a colony by non-chemical means or (2) slowing varroa population growth [58]. Grooming and hygienic behavior within bee colonies are also important factors slowing varroa population growth [22,64]. Hygienic behavior resulted in reduced varroa mites infestation levels and therefore, this reflects in reducing varroa mites detected using brood trapping techniques [65] or sugar shake as diagnostic c methods [66]. Likewise, varroa reproduction can be further impeded by genetically selecting and maintaining mite-resistant strains of honey bees, [67–69].

One sustainable approach to mite management would be maintaining stocks of genetically resistant bees. Some bee resistance traits show potential for reducing varroa mite populations in colonies and can be used in a selective breeding program. Mite infestation may be reduced by enhancing the ability of bees to hygienically remove varroa mites from brood (i.e., Varroa sensitive hygiene (VSH)) [70,71].

Other useful heritable traits linked to reduced varroa infestation rates are active grooming of phoretic mites, post-capping duration of worker bees [72], suppression of mite reproduction, and the physical damage inflicted upon varroa by bees [68,72,73]. It is evident that proportions of non-reproducing varroa increased by 10–30% in naturally-surviving honey bee populations compared to local susceptible colonies [74]. Breeding for mite resistance is a long-term solution for varroa management in honey bee colonies. In the shorter term, beekeepers maintain strong productive colonies by rearing strong queens, performing diagnostic procedures, and using effective varroa control tactics. Therefore, treatment thresholds have been developed for specific geographic, climatic conditions adopted for specific honey bee breeds [75].

Diagnostic methods, including performing sugar shakes, water washes, and alcohol washes, are essential for evaluating mite densities in honey bee colonies. These same mite density data per adult bee or pupa when run through a life-stage model also help approximate female mite density in a given colony [76]. Assessment methods performed at different times or locations need to be comparable in order to perform effective control [19,26]. It is also evident that differences in the varroa mortality rate may be a function of bee race and climatic conditions. Reduced varroa mite longevity has been reported during winter in temperate climatic conditions [77,78]. Sampling methods including mite removal from adult bees and brood as well as counts of natural varroa mite mortality are important for establishing the level of colony infestation before implementing integrated varroa management.

There are general beekeeping practices to maintain healthy colonies by providing bees with abundant access to protein and carbohydrate. Rich pasture conditions together with regular varroa mite monitoring and organic acaricide applications can produce strong colonies more capable of overwintering and resisting disease [79]. The ultimate aim of accurate monitoring is to keep varroa mite populations well below threshold levels during the spring, summer, and autumn months by means of promoting bee hygienic behavior, drone trapping, and acaricide use [80]. As mite populations begin to rise, commercial pyrethroid-, organophosphate-, or formamidine-based acaricides can be applied [81]. It was found that one Amitraz (formamidine) fumigation was as effective as two applications of Apiguard or Thymovar [82], but efficacy decreased with time because varroa mites have become resistant to acaricides [83,84], so organic treatments are thus more useful. However, varroa populations are evolving resistance to these broad-spectrum products, whether organic or conventional [85,86]. Moreover, these products have residuals that persist in sundry hive products [6] and their formulations are not approved for organic beekeeping. As alternatives, organic substances such as essential oils and organic acids are widespread in nature, some of them occur naturally in honey, and they can be highly effective acaricides [39,55,58].

A variety of integrated varroa mite control management practices are implemented to minimize the use of broad-spectrum organophosphate- and pyrethroid-based acaricides. These practices are suitable for organic and conventional beekeeping and include such active ingredients as organic acids (e.g., formic acid, oxalic acid, and beta plant acids) and the active components of certain essential oils (e.g., thymol) [87,88]. Reliable varroa diagnoses, effective mite control, and healthy colonies are very important issues in apiculture. Therefore, integrated varroa management as a sustainable approach ensures a high quality of honey bee products with prudent use of acaricides to minimize stress on host bees. To achieve sufficient efficacy, these acaricidal substances must be applied at an optimal time to avoid collateral damage to bees and brood. Moreover, specific management practices may enhance the effectiveness of some mite control methods, including seasonal effects on efficacy, treatment protocols, active ingredient rotation, and colony sanitation [64,89]. An attempt has been made by applying 'Good Beekeeping Practice' (GBP) to reduce the use of veterinary medicines in apiculture [90]. The ultimate objective of beekeeping is to produce safe honey bee products [91]. Beekeeper activities including organized varroa control in geographical region in all beekeeping operations, beekeepers trainings and education are crucial for improving honeybee health in the context of the one health approach incorporating designing and implementing programmes, policies, legislation and research in the beekeeping sector [92].

## 4. Conclusions

A variety of diagnostic methods can be used to estimate varroa mite infestation in honey bee colonies, particularly in summer. Summer treatments should be conducted once the natural mite-mortality and the threshold level of the mite infestation have been determined. Differences in climatic and geographic conditions as well as hive management practices will ultimately determine the success of alternative varroa mite controls [93]. Therefore, further experiments need to be conducted in a variety of climatic conditions to determine a colony's development for specific geographical, climatic, and pasture conditions. Accurate threshold infestation levels need to be established using hive debris test, adult bee examination, or brood examination for specific geographic, climatic, and colony conditions. In addition to mite counts on hive bottom boards (mite fall), the sugar shake method (powdered sugar) helps to remove mites physically from bees and is a quick and accurate monitoring tool for assessing colony infestation levels. Coupling the powdered sugar shake method or mite falls with the use of organic acid- and botanical-based acaricides provides alternative methods of integrated varroa mite control in colonies located in both continental or warm temperate climates. Effectiveness of varroa mite control and likelihood of honey bee colonies surviving winter are further improved by using acaricides in conjunction with brood interruption or total brood removal.

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