

## Review

# *Varroa destructor* and Varroosis Control in *Apis mellifera* L.: Current Strategies, Resistance, Residues, and Future Challenges

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## Abstract

*Varroa destructor* A. & T. is widely regarded as the most important ectoparasitic threat to managed *Apis mellifera* L. colonies worldwide and a major contributor to colony losses. Through direct parasitic feeding and the transmission of viral pathogens, particularly deformed wing virus, the mite significantly impairs honey bee health and colony performance. Despite decades of research and the availability of numerous control methods, sustainable management of varroosis remains challenging due to acaricide resistance, residue accumulation, reinvasion dynamics, and environmental factors influencing treatment efficacy. This review synthesizes current knowledge on the biology, epidemiology, pathogenesis, and management of *V. destructor*. based on published scientific literature and regulatory information. Unlike previous reviews that primarily focus on individual control methods, the present review provides an integrated assessment of treatment efficacy, resistance mechanisms, residue-related concerns, regulatory frameworks, emerging RNAi-based technologies, and future challenges affecting the long-term sustainability of *Varroa* management. Current evidence indicates that no single control method provides durable and universally effective suppression of *Varroa* populations. Instead, the most sustainable management outcomes are achieved through integrated pest management programs that combine routine monitoring, treatment rotation, brood-management techniques, and the use of *Varroa*-tolerant bee stocks. The review further demonstrates that resistance development, residue accumulation in hive matrices, and reinvasion from untreated colonies remain the principal factors limiting long-term control success. Addressing these challenges will require coordinated monitoring programs, resistance-informed treatment strategies, and continued development of novel control technologies.



Academic Editor: Renata Bażok

Received: 12 May 2026

Revised: 29 June 2026

Accepted: 30 June 2026

Published: 2 July 2026

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This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.**Keywords:** acaricides; biological control; honey bee health; integrated pest management

## 1. Introduction

The ectoparasitic mite *Varroa destructor* Anderson & Trueman (Acari: Varroidae) is widely recognized as one of the principal biotic factors contributing to mortality in managed *Apis mellifera* Linnaeus (Hymenoptera: Apidae) colonies because it combines direct parasitism with the efficient transmission and amplification of multiple honey bee viruses [1]. Originally associated with the eastern honey bee (*Apis cerana*; Hymenoptera: Apidae),

the parasite shifted to the western honey bee during the twentieth century and subsequently spread across nearly all regions where *A. mellifera* is kept. Because the parasite and the western honey bee did not co-evolve, this host shift resulted in a particularly harmful host–parasite interaction that continues to pose a major challenge for modern apiculture [1].

Our understanding of *Varroa* pathogenesis has changed substantially in recent years. Earlier studies assumed that mites primarily consumed bee hemolymph, but more recent work has demonstrated that *V. destructor* feeds mainly on honey bee fat-body tissue [2]. The fat body is a key metabolic and immunological organ involved in nutrient storage, detoxification processes, and immune regulation. Damage to this tissue helps explain why even relatively moderate mite infestations can destabilize colony health, especially when viral infections and seasonal colony dynamics are taken into account [3].

Beyond the direct physiological damage caused by feeding, *Varroa* mites play a crucial role as vectors and amplifiers of multiple honey bee viruses [4]. Among the most important are Deformed Wing Virus (DWV) and Acute Bee Paralysis Virus (ABPV), which are strongly associated with colony weakening and collapse. Mite infestation increases viral transmission within colonies and contributes to immunosuppression, reduced worker longevity, and impaired colony performance [5]. In temperate climates, untreated colonies with high mite populations often collapse within one to two years as a consequence of these combined effects [5].

Across many regions of the world, annual losses of managed honey bee colonies frequently exceed 20–30%, and numerous studies identify *V. destructor* as the primary biological factor underlying these losses [6,7]. The parasite's global distribution, rapid reproductive cycle, and ability to spread between colonies through drifting and robbing bees make its effective management essential for maintaining colony health.

Despite decades of research, no single method provides consistently reliable control of varroosis. Synthetic acaricides can provide high short-term efficacy, but their long-term usefulness is often limited by the rapid evolution of resistance in *Varroa* populations and by the accumulation of persistent residues in wax and other hive elements, particularly in the case of lipophilic compounds [8]. In contrast, organic acids, essential oils, and biotechnical approaches generally produce fewer residues and lower selection pressure for resistance. However, their effectiveness often depends strongly on colony brood status, ambient temperature, the precision of application, and the availability of labor [9].

For these reasons, integrated pest management (IPM) has become the most widely supported framework for sustainable control of varroosis. Evidence from field experiments and comparative studies shows that effective management programs combine routine quantitative mite monitoring, carefully timed seasonal treatments aimed at protecting winter bees, rotation of treatments with different modes of action to limit resistance development, verification of post-treatment efficacy, and, where possible, the use of brood-cycle manipulation or *Varroa*-tolerant bee stocks [10–12].

Recent developments further highlight the need for an updated synthesis of varroosis management strategies. In particular, increasing reports of resistance to widely used acaricides, including pyrethroids and amitraz, together with advances in the molecular understanding of mite–host interactions and the emergence of new control technologies such as RNA interference (RNAi)-based approaches, are rapidly reshaping the field.

Unlike many previous reviews that focus primarily on individual treatment methods or specific aspects of *Varroa* management, the present review adopts an integrated perspective that combines current evidence on treatment efficacy, resistance mechanisms, residue-related concerns, regulatory frameworks, emerging RNAi-based technologies, reinvasion dynamics, and future environmental challenges. By bringing these diverse but interconnected aspects together, this review provides a comprehensive assessment of the factors influencing the long-term sustainability of *V. destructor* control in modern apiculture.

Therefore, the aim of this review is to critically evaluate current knowledge on the biology, pathogenesis, and management of *V. destructor* and to identify factors affecting the long-term sustainability of control strategies in managed *Apis mellifera* colonies.

To achieve this aim, the review addresses the following objectives:

- (i) To summarize the current understanding of *V. destructor* biology, host–parasite interactions, and their consequences for colony health;
- (ii) To evaluate the efficacy, limitations, and safety of currently available chemical, biotechnical, and biological control strategies;
- (iii) To examine mechanisms of resistance development, residue-related concerns, and factors contributing to treatment failure;
- (iv) To assess emerging technologies, breeding approaches, and future directions for sustainable *Varroa* management.

## 2. Materials and Methods

### 2.1. Literature Search Strategy

A structured literature review was conducted using a predefined search strategy to improve transparency and reproducibility. Literature searches were performed in the Web of Science Core Collection, Scopus, and PubMed databases. These searches were complemented by targeted queries in Google Scholar and relevant regulatory repositories (e.g., EPA, European Commission, and national pesticide registries) in order to capture gray literature and official product documentation.

The search covered publications from January 2000 to March 2026, reflecting the period during which modern *V. destructor* management strategies and resistance monitoring have been extensively studied. Search queries combined controlled vocabulary and free-text terms related to the parasite, host species, and control strategies.

Search terms included combinations of keywords such as: *V. destructor*, varroosis, *Apis mellifera*, honey bee mite, acaricide, amitraz, flumethrin, pyrethroid, coumaphos, oxalic acid, formic acid, thymol, essential oils, integrated pest management, IPM, mite monitoring, resistance, RNA interference, RNAi, biological control, biotechnical control, brood interruption, and hygienic behavior. Data extraction was performed by a single reviewer using predefined extraction criteria to ensure consistency in the collection and interpretation of information across the included sources.

Representative search queries included:

("Varroa destructor" OR varroosis) AND ("Apis mellifera") AND (control OR treatment OR acaricide OR "integrated pest management")

and

("Varroa destructor") AND (resistance OR amitraz OR pyrethroid OR RNAi OR "biotechnical control").

Reference lists of key review articles were additionally screened to identify relevant studies that may not have been captured during the initial database search.

## 2.2. Study Selection and Eligibility Criteria

The database search yielded multiple records across the selected databases. After removing duplicates, titles and abstracts were screened for relevance. Articles that appeared relevant were then assessed in full text according to predefined inclusion criteria.

Studies were included if they reported at least one of the following outcomes:

- Quantitative measures of mite population reduction (absolute infestation levels or percentage treatment efficacy);
- Documented adverse colony outcomes (e.g., queen loss, brood mortality, adult bee toxicity, or reproductive impairment);
- Measurements of chemical residues in hive matrices (wax, honey, or other bee products);
- Confirmed resistance phenotypes or genotypes in *V. destructor* populations.

Preference was given to primary experimental studies, including field trials and laboratory bioassays. Systematic reviews and meta-analyses were used to provide broader context for patterns in treatment efficacy. In addition, regulatory documents and product labels were consulted to verify approved uses, residue limits, and risk assessments.

Because the regulatory approval and practical implementation of control strategies vary substantially between regions, regulatory status was treated as region-specific and time-dependent. Whenever possible, official registries and regulatory guidance documents were used as authoritative sources.

## 2.3. Data Extraction

For each included study, the following information was extracted when available:

- Study type (field trial, laboratory bioassay, review, or regulatory document);
- Geographic location and beekeeping system;
- Treatment type and active compound or intervention method;
- Target mite stage (phoretic or reproductive);
- Treatment efficacy (absolute mite reduction or percentage efficacy);
- Reported adverse colony outcomes;
- Residue measurements in hive products;
- Evidence of resistance phenotypes or genetic mutations.

Data extraction focused on maintaining comparability of outcome measures across studies in order to allow consistent interpretation of treatment performance and its limitations.

## 2.4. Assessment of Evidence Quality

To improve the interpretability of the review, the available evidence was interpreted descriptively according to study design, methodological robustness, and relevance to the objectives of the review.

Greater emphasis was placed on controlled field trials conducted in managed colonies, particularly those employing replicated experimental designs and quantitative mite monitoring. Laboratory bioassays assessing acaricidal toxicity or resistance mechanisms were considered complementary mechanistic evidence. Systematic reviews and meta-analyses were used to identify broader patterns in treatment efficacy across different experimental contexts.

Regulatory documentation was treated as authoritative for treatment approval status, residue limits, and risk assessments, but not as primary evidence of treatment efficacy.

Potential sources of bias, including small sample sizes, lack of untreated control groups, or insufficient reporting of colony conditions, were noted during data extraction and considered when interpreting results.

### 2.5. Evidence Synthesis

Evidence synthesis was conducted using a comparative analytical framework rather than a simple enumeration of reported treatments. Control strategies were evaluated according to four principal dimensions:

1. Biological targetability, distinguishing between treatments targeting phoretic mites on adult bees and those capable of affecting mites within brood cells.
2. Evolutionary robustness, reflecting the likelihood of resistance development based on the mode of action, treatment history, and documented resistance mechanisms.
3. Colony and consumer safety, including potential toxicity to adult bees and brood as well as the accumulation of residues in hive products such as wax and honey.
4. Operational feasibility, encompassing cost, labor requirements, application precision, and suitability for different beekeeping systems.

Because the regulatory approval and practical implementation of treatments vary substantially between geographic regions, regulatory status was treated as region-specific and time-dependent. When possible, official regulatory registries and product labels were used as primary sources of record for treatment approval and permitted uses.

## 3. *Varroa destructor* Biology, Life Cycle, Epidemiology, and Impact

### 3.1. Host Shift and Global Distribution

The ectoparasitic mite *V. destructor* is widely recognized as one of the most destructive parasites of the western honey bee, *Apis mellifera*, and represents a major driver of colony losses in modern apiculture [1]. Originally associated with the eastern honey bee *Apis cerana*, the parasite shifted hosts to *A. mellifera*, which enabled its rapid global spread during the twentieth century. Following its introduction into European honey bee populations in the 1970s and into the Americas in the 1980s, *V. destructor* is now present in most regions where *A. mellifera* is managed. Without effective control measures, colonies in temperate climates typically collapse within two to three years after infestation [1].

### 3.2. Life Cycle and Reproductive Biology

The life cycle of *V. destructor* is closely synchronized with honey bee development and consists of two main phases: a phoretic phase on adult bees and a reproductive phase within sealed brood cells [9]. During the phoretic stage, adult female mites attach to worker bees and are transported throughout the colony. Reproduction occurs exclusively within capped brood cells. Inside the cell, the foundress mite lays a sequence of eggs that typically includes one male followed by several female offspring. These offspring develop and mate within the brood cell before the host bee emerges [1].

Drone brood plays a particularly important role in mite population dynamics because its longer capped period allows greater reproductive success of the parasite. As a result, infestation rates in drone brood are often several times higher than in worker brood. This biological preference is one of the reasons why management strategies such as drone brood removal can reduce mite population growth within colonies [13].

### 3.3. Pathogenesis and Viral Transmission

The pathogenicity of *V. destructor* arises from both direct parasitic feeding and its role as an efficient vector of viral pathogens. Although mites were historically believed to feed mainly on hemolymph, recent evidence indicates that they feed primarily on honey bee fat body tissue, which is a key organ involved in metabolism, detoxification, and immune regulation [3]. Damage to this tissue has significant physiological consequences for the host and contributes to reduced resilience of infested bees. In addition to direct physiological damage, *V. destructor* facilitates the transmission and amplification of several honey bee viruses, including deformed wing virus (DWV) and acute bee paralysis virus (ABPV) [5]. Viral infections associated with mite infestation can lead to developmental abnormalities such as deformed wings, reduced lifespan of adult workers, and impaired flight ability. High viral loads within colonies are therefore considered a major factor contributing to colony weakening and collapse [5].

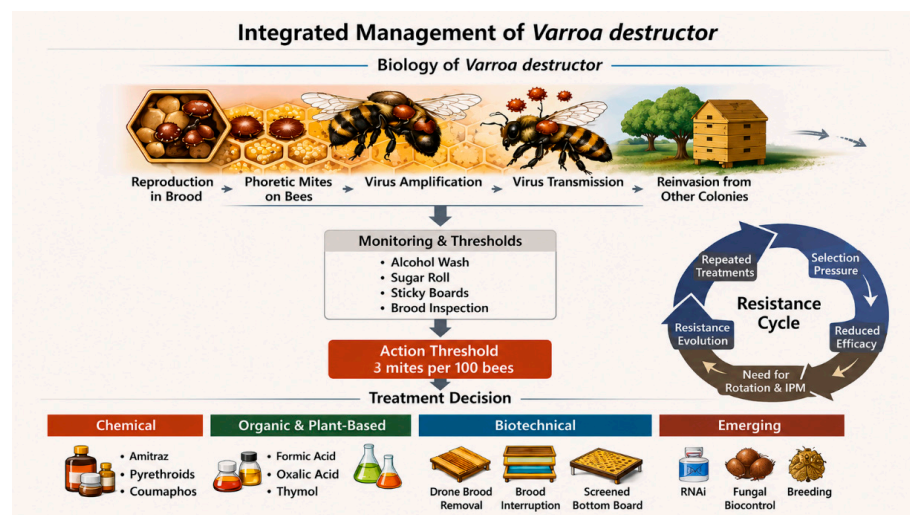
### 3.4. Colony-Level Consequences and Reinvasion Dynamics

At the colony level, the combined effects of parasitic feeding, viral amplification, and immune suppression can lead to progressive colony decline. Infested colonies typically show reduced worker longevity, impaired foraging performance, and disruptions in the production of long-lived winter bees. Consequently, high mite infestations occurring in late summer and early autumn are strongly associated with overwintering colony losses. Mite population dynamics are influenced not only by reproduction within a colony but also by reinvasion from surrounding colonies through drifting or robbing bees. As a result, mite populations can increase rapidly even after treatment, highlighting the importance of regular monitoring and integrated pest management strategies for maintaining sustainable control [14].

## 4. Overview of *Varroa* Control Strategies

The reviewed literature identified several strategies used to control *V. destructor* populations in managed *Apis mellifera* colonies. These approaches can be broadly grouped into four main categories: synthetic acaricides, organic acids and essential oils, biotechnical or brood-management methods, and emerging biological or molecular control strategies. Across the analyzed studies, treatment efficacy, safety for honey bees, and the risk of resistance development varied considerably among these approaches. Synthetic acaricides generally provide the most effective short-term suppression of mite populations, but their long-term use has often been limited by resistance development and the accumulation of residues in hive matrices, particularly wax. In contrast, treatments based on organic acids and essential oils pose a lower risk of persistent residues but often show greater variability in efficacy, depending on colony conditions, environmental factors, and the precision of application. Biotechnical strategies, such as brood interruption and drone brood removal, target the reproductive phase of the mite life cycle and are commonly used as complementary measures within integrated pest management programs. More recently, new approaches have been explored, including RNA interference-based technologies and selective breeding of honey bees with increased resistance to *V. destructor*.

Figure 1 summarizes the principal *V. destructor* control strategies currently used in managed *Apis mellifera* colonies and compares their mechanisms of action, efficacy, major limitations, residue risks, and potential for resistance development. The effectiveness of these interventions depends on several operational factors, including brood status, environmental temperature, infestation intensity, resistance profiles of local mite populations, and the presence of honey supers during treatment.



**Figure 1.** *Varroa destructor* A. & T. control strategies: mechanisms, efficacy, and limitations.

A recent global meta-analysis covering 138 studies conducted in 30 countries reported that synthetic acaricides generally achieve the highest levels of mite suppression under field conditions. However, their long-term effectiveness is increasingly limited by resistance development and concerns about residue accumulation in hive elements [8].

#### 4.1. Comparative Matrix of Major Interventions

Table 1 summarizes the principal *Varroa* control strategies currently used in *Apis mellifera* colonies and provides a comparative overview of their target stage, mechanism of action, efficacy, resistance risk, colony safety, residue potential, and regulatory status.

Biotechnical and biological approaches are typically applied as complementary components of integrated pest management (IPM) rather than as standalone treatments. Reported efficacy ranges represent approximate values derived from field studies and may vary depending on colony strength, brood presence, environmental conditions, reinvasion pressure, and local resistance status.

**Table 1.** *Varroa* control interventions used in *Apis mellifera* L. colonies.

| Control Method                                                | Target Stage                                  | Mechanism                                                                                            | Application                                                                                                                         | Typical Efficacy                                                                       | Resistance                                                                                                               | Colony Risks                                                                                  | Residues                                                                                 | Regulatory Status                                                                                   | References   |
|---------------------------------------------------------------|-----------------------------------------------|------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|--------------|
| Synthetic acaricides                                          |                                               |                                                                                                      |                                                                                                                                     |                                                                                        |                                                                                                                          |                                                                                               |                                                                                          |                                                                                                     |              |
| Amitraz (Apivar strips; related amitraz products).            | Phoretic-dominant, primarily phoretic mites.  | Octopamine receptor agonism; mite octopamine receptors show high sensitivity to amitraz/metabolites. | Impregnated strips placed in brood chamber for several weeks (6 to 8 weeks).                                                        | Historically high; increasingly variable due to emerging resistance.                   | Documented in multiple regions; associated with mutations in mite octopamine receptors.                                  | Generally well tolerated-potential chronic effects on queens and drone reproduction reported. | Amitraz rapidly degrades; metabolites may be detectable in honey after treatment.        | Approved in the United States and many other regions; registration varies by country.               | [15–18]      |
| Pyrethroids (tauf luvalinate, flumethrin; Apistan, Bayvarol). | Phoretic mites.                               | Disruption of voltage-gated sodium channels in the mite nervous system.                              | Impregnated strips typically applied after honey harvest for approximately 6 weeks.                                                 | High in susceptible populations but frequently reduced where resistance is widespread. | Widespread; strongly associated with mutations in the voltage-gated sodium channel (VGSC), particularly at position 925. | Sublethal effects reported; chronic exposure possible due to persistence in hive matrices.    | Lipophilic compounds accumulate in beeswax and are frequently detected in hive products. | Historically widely used; still approved in several regions but often limited by resistance.        | [1,15,19,20] |
| Coumaphos (Check-Mite+).                                      | Phoretic mites.                               | Acetylcholinesterase inhibition; resistance may involve altered metabolic activation.                | Impregnated strips applied in brood chambers, typically without honey supers.                                                       | Highly variable; often low in populations with established resistance.                 | Common in several regions; persistence in wax may maintain selection pressure.                                           | Evidence of impaired queen rearing and behavioral alterations at relevant wax concentrations. | Highly persistent in beeswax; residues may remain for months after treatment.            | Approved in some jurisdictions (e.g., United States) but restricted or discontinued in others.      | [15,21–23]   |
| Organic acids                                                 |                                               |                                                                                                      |                                                                                                                                     |                                                                                        |                                                                                                                          |                                                                                               |                                                                                          |                                                                                                     |              |
| Formic acid (MAQS, Formic Pro; long/short treatments).        | Phoretic mites and mites within capped brood. | Volatile acid disrupting mite respiration and metabolism; capable of penetrating brood caps.         | Short- or long-duration treatments (typically 7–14 days) depending on product formulation; efficacy strongly temperature-dependent. | Often high (commonly 80–95%) under suitable environmental conditions.                  | Resistance considered unlikely or low risk, although monitoring is recommended.                                          | Potential brood mortality and queen loss under unfavorable conditions.                        | Low persistence due to volatility; minimal long-term residues.                           | Widely available in multiple regions; some products permitted during honey flow depending on label. | [1,24,25]    |

Table 1. Cont.

| Control Method                                                                                | Target Stage                                                           | Mechanism                                                                                                       | Application                                                                                                                             | Typical Efficacy                                                                               | Resistance                                                                                                                              | Colony Risks                                                                                       | Residues                                                                                                            | Regulatory Status                                                          | References |
|-----------------------------------------------------------------------------------------------|------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|------------|
| Oxalic acid (trickle/dribble; vaporization; extended-release strips/tablets in some regions). | Primarily phoretic mites; limited effect on mites within capped brood. | Contact toxicity affecting mite physiology; strong selectivity toward mites demonstrated in bioassays.          | Applied by trickling, spraying, or vaporization; most effective during broodless periods.                                               | High during broodless conditions (often $\geq 90\%$ ); reduced efficacy when brood is present. | Field resistance not widely documented; risk currently considered low.                                                                  | Improper dosing may damage brood or increase operator exposure.                                    | Naturally present in honey; properly applied treatments generally do not increase residues above background levels. | Widely used; registration and approved formulations vary by country.       | [14,26]    |
| Lactic acid (spray).                                                                          | Phoretic mites on adult bees.                                          | Contact action impairing mite attachment to the host.                                                           | Spray treatment applied directly to adult bees; labor-intensive and typically used in small apiaries.                                   | Variable and strongly dependent on colony conditions.                                          | Resistance risk considered low.                                                                                                         | Handling stress and colony disturbance during application.                                         | Minimal persistence compared with lipophilic synthetic acaricides.                                                  | Primarily used in some European regions.                                   | [25,27]    |
| Essential oils                                                                                |                                                                        |                                                                                                                 |                                                                                                                                         |                                                                                                |                                                                                                                                         |                                                                                                    |                                                                                                                     |                                                                            |            |
| Thymol (Apiguard, Thymova, Api Life Var).                                                     | Primarily phoretic mites.                                              | Neurophysiological disruption in mites, including modulation of GABA receptors (RDL).                           | Temperature-dependent treatment applied in repeated doses; typically avoided during the main honey flow to prevent honey contamination. | Variable; high in optimized field conditions but moderate in some practical settings.          | Resistance less clearly documented than for synthetic acaricides.                                                                       | Possible disturbance of brood and queens; risk of behavioral changes and honey taint.              | Thymol residues may persist in wax and honey and can influence organoleptic properties.                             | Widely authorized in multiple regions for <i>Varroa</i> control.           | [1,28,29]  |
| Biotechnical methods                                                                          |                                                                        |                                                                                                                 |                                                                                                                                         |                                                                                                |                                                                                                                                         |                                                                                                    |                                                                                                                     |                                                                            |            |
| Hop beta acids HopGuard.                                                                      | Primarily phoretic mites.                                              | Likely contact toxicity affecting mite cuticle and physiology; precise mechanisms remain incompletely resolved. | Impregnated strips placed in brood chambers; some jurisdictions allow use during honey flow.                                            | Moderate to high depending on treatment timing and brood presence.                             | Resistance risk considered low due to primarily physical or contact-based mode of action, although long-term field data remain limited. | Variable efficacy under high brood conditions; possible temporary reduction in colony weight gain. | Low persistence compared with lipophilic synthetic acaricides.                                                      | Registered in the United States and evaluated in Canada and other regions. | [18,30]    |

Table 1. Cont.

| Control Method                                                                                       | Target Stage                                                                                 | Mechanism                                                                                       | Application                                                                         | Typical Efficacy                                                                                                     | Resistance                                                                   | Colony Risks                                                                                         | Residues                                                   | Regulatory Status                                               | References |
|------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|------------------------------------------------------------|-----------------------------------------------------------------|------------|
| Screened bottom boards.                                                                              | Phoretic mites falling from adult bees.                                                      | Mechanical removal of mites through screened floors, reducing the probability of reinfestation. | Permanent hive modification used continuously as a supportive management measure.   | Small to moderate reduction in mite populations; meta-analyses report statistically significant but limited effects. | Not applicable.                                                              | Potential heat loss during winter if poorly managed.                                                 | None.                                                      | Globally available beekeeping equipment.                        | [1,31,32]  |
| Drone brood removal /trapping.                                                                       | Reproductive mites in drone brood.                                                           | Selective removal of brood cells with high mite reproductive success.                           | Repeated removal of capped drone brood during the breeding season.                  | Moderate reduction in mite populations when applied consistently; commonly used as part of IPM programs.             | Not applicable.                                                              | Loss of drone brood; increased labor requirements; potential trade-offs with colony productivity.    | None.                                                      | Globally applicable.                                            | [1,13]     |
| Brood interruption (queen caging, trapping comb, total brood removal) often paired with oxalic acid. | Reproductive mites through temporary brood absence; followed by treatment of phoretic mites. | Interrupts mite reproduction and synchronizes the mite population into the phoretic phase.      | Induction of a temporary broodless period, often followed by oxalic acid treatment. | Often high when correctly implemented.                                                                               | Not applicable.                                                              | Temporary reduction in colony growth; risk to queen performance if poorly managed.                   | Depends on the accompanying treatment (e.g., oxalic acid). | Globally applicable management strategy.                        | [1,33,34]  |
| Hyperthermia devices (thermotherapy).                                                                | Reproductive and, to some extent, phoretic mites.                                            | Exploits higher thermal sensitivity of mites compared with honey bee brood.                     | Device-mediated heating cycles applied to brood frames or entire colonies.          | Promising but variable; results depend on device design and treatment protocol.                                      | Not applicable.                                                              | Potential brood damage; labor and equipment costs.                                                   | None                                                       | Emerging technology with limited commercial availability.       | [35]       |
| Biological                                                                                           |                                                                                              |                                                                                                 |                                                                                     |                                                                                                                      |                                                                              |                                                                                                      |                                                            |                                                                 |            |
| Entomopathogenic fungi (Metarhizium, Beauveria).                                                     | Phoretic and brood-associated mite stages, depending on delivery method.                     | Fungal infection leading to mycosis and mite mortality.                                         | Experimental formulations applied to colonies or hive substrates.                   | Variable; improved strains and formulations show increasing promise in experimental field trials.                    | Resistance considered unlikely in the short term but insufficiently studied. | Potential effects on non-target organisms and hive microbial communities remain under investigation. | No chemical residues expected.                             | Primarily experimental; not widely approved for commercial use. | [1,36]     |

Table 1. Cont.

| Control Method                                 | Target Stage                                        | Mechanism                                                                  | Application                                                              | Typical Efficacy                                                            | Resistance                                                                      | Colony Risks                                                                            | Residues                                                             | Regulatory Status                                                                         | References |
|------------------------------------------------|-----------------------------------------------------|----------------------------------------------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|----------------------------------------------------------------------|-------------------------------------------------------------------------------------------|------------|
| Predatory mites (Stratiolaelaps scimitus).     | Phoretic mites and mites falling to hive debris.    | Predation on <i>Varroa</i> mites within hive substrates.                   | Introduction of predatory mites into hive environments or substrates.    | Mixed evidence; field effectiveness remains uncertain.                      | Not applicable.                                                                 | Ecological interactions and colony-level safety still under evaluation.                 | None.                                                                | Experimental; evaluated in some jurisdictions.                                            | [1,37]     |
| RNAi/dsRNA (e.g., vadesca; emerging products). | Genes essential for mite survival and reproduction. | Sequence-specific gene silencing through double-stranded RNA interference. | Delivery through feeding systems or other product-specific formulations. | Field evidence emerging; early studies indicate promising targeted control. | Long-term resistance possible through sequence evolution or reduced RNA uptake. | Delivery stability and potential off-target effects remain key research considerations. | Minimal chemical residues expected due to biological mode of action. | Recently registered in the United States; regulatory evaluation ongoing in other regions. | [7,38]     |

The comparison presented in Table 1 is based on evidence from peer-reviewed studies and regulatory documentation addressing acaricide efficacy, resistance mechanisms, and colony-level effects, including reports of amitraz resistance [17], pyrethroid resistance associated with voltage-gated sodium channel mutations [19], coumaphos resistance mediated by cytochrome P450-dependent metabolic mechanisms [24], thymol persistence in hive elements [39], the efficacy of screened bottom boards, and brood interruption strategies within IPM programs [34].

Effective *Varroa* management requires routine monitoring of mite populations. Several standardized methods are used in apiculture to estimate infestation levels and determine treatment thresholds (Table 2).

**Table 2.** Major monitoring methods used to estimate *Varroa destructor* A. & T. infestation levels in *Apis mellifera* L. colonies. Action thresholds \* should be considered indicative rather than universally applicable, as they vary according to geographic region, season, colony characteristics, monitoring methodology, and management objectives. Nevertheless, thresholds frequently fall near ~3 mites per 100 adult bees during the active season, with lower thresholds sometimes recommended in late summer to protect winter bee cohorts.

| Monitoring Method                     | Primary Target                    | Principle of Measurement                                                                                                                  | Typical Sampling Protocol                                                                  | Advantages                                                           | Limitations                                                                                         | Typical Action Threshold *                                 |
|---------------------------------------|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|----------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|------------------------------------------------------------|
| Alcohol wash                          | Phoretic mites on adult bees      | Bees are agitated in alcohol to dislodge mites, allowing accurate estimation of phoretic mite infestation relative to the bee sample size | ~300 adult bees collected from brood frames; bees washed in alcohol and mites counted      | Considered the most accurate field method for estimating infestation | Destructive to sampled bees; requires handling equipment                                            | ~2–3 mites per 100 bees during the active season           |
| Powdered sugar roll                   | Phoretic mites on adult bees      | Sugar coating causes mites to lose grip and fall through mesh for counting                                                                | ~300 adult bees dusted with powdered sugar in ventilated jar; mites shaken out and counted | Non-lethal to bees; practical for routine monitoring                 | Slightly lower sensitivity compared with alcohol wash                                               | ~2–3 mites per 100 bees                                    |
| Sticky board mite fall counts         | Naturally falling mites           | Adhesive boards placed beneath screened bottom boards collect mites that fall from the colony                                             | Board inserted for 24–72 h and mites counted                                               | Non-invasive; easy for continuous monitoring                         | Provides only an indirect estimate of colony infestation; influenced by colony size and brood level | Variable; often interpreted relative to seasonal baselines |
| Drone brood uncapping inspection      | Reproductive mites in drone brood | Drone brood cells opened to directly assess mite infestation in capped brood                                                              | Sample of capped drone brood opened and mites counted                                      | Detects reproductive infestation; useful early in the season         | Labor intensive; requires drone brood presence                                                      | No universal threshold; used as qualitative indicator      |
| Visual inspection of symptomatic bees | Virus-associated colony symptoms  | Detection of deformed wings or parasitized brood indicating high mite and viral load                                                      | Visual observation during routine colony inspection                                        | Rapid screening tool                                                 | Very low sensitivity; indicates already severe infestation                                          | Not suitable for quantitative threshold decisions          |

Monitoring results are essential for evidence-based treatment decisions because inappropriate timing of interventions remains one of the major causes of treatment failure in *Varroa* management programs.

#### 4.2. Synthetic Acaricides

Synthetic acaricides have historically formed the backbone of *V. destructor* control programs due to their high initial efficacy and operational simplicity. The most widely

used compounds include amitraz (formamidine class), pyrethroids such as tau-fluvalinate and flumethrin, and organophosphates such as coumaphos. Although these compounds can achieve substantial reductions in mite populations, their long-term effectiveness is increasingly limited by resistance evolution and concerns regarding residue accumulation in hive matrices.

#### 4.2.1. Amitraz (Formamidine Class)

Amitraz remains one of the most widely used miticides globally because of its relatively high efficacy and convenient strip-based application systems. Its acaricidal activity is associated with disruption of octopaminergic signaling pathways in mites, affecting neuromodulation and motor control. The selective toxicity of amitraz is thought to arise from differences between mite and honey bee octopamine receptor sensitivity. Despite its widespread use, resistance to amitraz has been increasingly reported in recent years. Field surveys have documented reduced treatment efficacy in commercial apiaries, with resistant mite populations often associated with mutations affecting octopamine receptor function [17]. These findings indicate a growing continuum between susceptible populations and colonies in which amitraz treatments provide only partial control. Resistance to amitraz is likely multifactorial. In addition to alterations in octopamine receptor function, studies have suggested possible roles of enhanced detoxification pathways and other physiological mechanisms that may reduce treatment susceptibility. Practical factors, including repeated use of amitraz-based products, underdosing, inappropriate treatment timing, and reinvasion from neighboring colonies, may further contribute to apparent treatment failure under field conditions. Application protocols are typically defined by product labels. For example, the United States Environmental Protection Agency label for Apivar specifies removal of honey supers prior to treatment and dosing based on the number of frames covered by bees, with impregnated strips placed in the brood area for several weeks before removal [18]. Residues following amitraz treatment are generally detected as degradation products, including 2,4-dimethylaniline (2,4-DMA) and related metabolites that may occur in honey after treatment [16]. Residue dynamics are particularly relevant in regulatory contexts where honey is the primary monitored matrix and where treatments occur near nectar flows. Potential sublethal effects of amitraz exposure also remain an area of active investigation. Some studies suggest possible impacts on queen reproductive physiology and sperm viability, although the long-term colony-level implications of these effects remain incompletely understood [40].

#### 4.2.2. Pyrethroids (Tau-Fluvalinate, Flumethrin)

Pyrethroid-based miticides were among the first widely adopted chemical controls for *V. destructor*. These compounds act by disrupting voltage-gated sodium channels in the mite nervous system, leading to paralysis and death.

However, extensive use has led to widespread resistance in many regions. Resistance is strongly associated with mutations in the mite voltage-gated sodium channel gene, particularly substitutions at position 925, which significantly reduce pyrethroid sensitivity [19]. As a result, treatment efficacy varies greatly depending on local resistance status. Another important limitation of pyrethroids is their strong lipophilicity. These compounds readily accumulate in beeswax, resulting in prolonged exposure of developing brood and queens and potentially maintaining chronic selection pressure for resistant mite populations [20].

#### 4.2.3. Organophosphates (Coumaphos)

Coumaphos acts as an acetylcholinesterase inhibitor, disrupting neural transmission in mites. Although historically effective, its use has declined in many regions due to both resistance development and concerns regarding residue persistence. Coumaphos

residues can accumulate in beeswax and remain detectable for extended periods after treatment. Studies have shown that concentrations reached in brood combs may approach levels capable of impairing queen development and reproduction [22]. Field studies also demonstrate that substantial residues can persist for months within central brood combs following treatment due to the lipophilicity of the wax [21]. In addition to reproductive effects, behavioral alterations in worker bees have been reported. Experimental studies indicate that coumaphos exposure can affect trophallactic food transfer among workers, suggesting that colony-level impacts may extend beyond direct toxicity [41].

#### 4.3. Organic Acids

Organic acids represent an important group of treatments in *V. destructor* management because they generally present a lower risk of persistent residues and resistance development than many synthetic acaricides. The most widely used compounds include formic acid, oxalic acid, and lactic acid, each with distinct biological targets and operational constraints.

##### 4.3.1. Formic Acid

Formic acid is unique among commonly used treatments because it can penetrate capped brood cells and therefore affect mites during the reproductive phase of the life cycle. This property allows partial control of mites developing within sealed brood (Formic Pro®, NOD Apiary Products Ltd., Frankford, ON, Canada, 2023). Field studies frequently report high levels of mite mortality, often exceeding 90–95% under favorable environmental conditions. However, these benefits are accompanied by important trade-offs. Experimental comparisons of formic acid delivery systems have documented increased brood mortality and elevated rates of queen loss under certain treatment regimens [24]. Although the precise molecular mechanisms of toxicity remain incompletely characterized, proteomic studies suggest that formic acid exposure affects multiple metabolic pathways in the mite [42]. Treatment outcomes are strongly influenced by colony strength and environmental temperature, and guidance documents emphasize strict adherence to recommended temperature ranges and hive ventilation conditions during application [43]. Resistance to formic acid is generally considered unlikely or relatively low compared with single-target synthetic acaricides. Nevertheless, this assumption remains largely inferential, and recent reviews emphasize the importance of continued monitoring for potential tolerance development in *Varroa* populations [25].

##### 4.3.2. Oxalic Acid

Oxalic acid is widely used worldwide, particularly as a treatment during broodless periods. Laboratory bioassays demonstrate strong contact toxicity to *V. destructor* with comparatively lower toxicity to adult honey bees under appropriate dosing conditions [44]. The principal biological limitation of oxalic acid is its low efficacy against mites located within sealed brood cells. Consequently, treatments are most effective when colonies are naturally broodless or when brood interruption strategies are applied. When brood is present, extended-release formulations or repeated vaporization treatments may be required to achieve satisfactory mite suppression [14]. Recently, slow-release oxalic acid formulations have been developed to improve efficacy in colonies containing brood. For example, VarroSan® (Vita Bee Health Limited, Dublin, Ireland) consists of oxalic acid incorporated into cellulose-based strips that are placed within the brood area for several weeks, allowing continuous exposure of phoretic mites throughout multiple brood cycles. Such formulations may be particularly useful for maintaining low mite populations over extended periods and represent an important addition to integrated pest management programs. Application methods vary between regions and products and may include

dribble treatments applied directly to bees, vaporization (sublimation) within the hive, or slow-release strip formulations. Product-specific instructions define dosing protocols based on colony size and hive configuration [26]. Residue interpretation for oxalic acid is complex because the compound naturally occurs in honey. Regulatory product documentation indicates that correct application is not expected to raise oxalic acid levels above natural background concentrations, although transient contamination of bee tissues has been documented following treatment [45].

#### 4.3.3. Lactic Acid

Lactic acid is used as a lower-intensity treatment option but is comparatively labor-intensive because it must be applied directly to adult bees. For this reason, its use is often limited to small-scale apiaries or specific management situations. Recent mechanistic research suggests that lactic acid may impair the ability of mites to attach to bees by altering the structure and function of the mite's arolia, thereby reducing grip and mobility on the host surface [27]. Reported efficacy varies substantially across studies and appears strongly dependent on treatment technique and colony conditions [46]. These findings highlight the importance of verifying treatment effectiveness through post-treatment monitoring.

#### 4.4. Essential Oils and Plant-Derived Products

Plant-derived compounds represent an alternative group of *Varroa* control tools that are generally associated with lower risks of persistent residues and reduced likelihood of resistance development compared with many synthetic acaricides. The most widely used substances include thymol-based formulations and hop beta acids, although several additional biopesticide products have recently entered regulatory approval pathways in some jurisdictions.

##### 4.4.1. Thymol and Thymol-Based Mixtures

Thymol is the most extensively used essential oil component in *Varroa* control strategies. Laboratory studies indicate that thymol can interact with mite  $\gamma$ -aminobutyric acid (GABA) receptors, particularly RDL receptor subtypes, suggesting a neurophysiological mechanism underlying its acaricidal activity [47]. Field efficacy of thymol treatments, however, varies considerably depending on formulation, ambient temperature, and colony conditions. Both early and more recent trials report outcomes ranging from moderate to high mite suppression across different environmental contexts [48]. These variations reflect the volatility of thymol and its sensitivity to temperature-dependent evaporation dynamics. Residue studies demonstrate that thymol can persist in hive elements for extended periods and may influence honey flavor and aroma following treatment, which represents the primary consumer-related concern associated with thymol use rather than toxicological risk to bees [39]. In addition to direct acaricidal activity, thymol may influence honey bee behavior. Experimental studies have shown that thymol exposure can alter hygienic brood removal behavior, potentially interacting with colony traits such as hygienic behavior and complicating interpretation of colony-level outcomes [49].

##### 4.4.2. Hop Beta Acids

Hop beta acids are typically considered contact acaricides with mechanisms that are not yet fully resolved at the molecular level. Regulatory assessments suggest that their primary mode of action may involve physical or membrane-disruptive effects on mites [37]. Experimental and semi-field studies indicate that hop beta acid treatments can achieve substantial mite reductions under appropriate conditions, particularly when applied during periods of reduced brood presence. However, treatment efficacy remains sensitive to application timing and colony brood dynamics [50].

#### 4.4.3. Other Plant-Derived or “Biopesticide” Products

In some jurisdictions, additional products categorized as biopesticides have recently been approved for use against *V. destructor*. Examples include compounds such as sucrose octanoate and glutamic acid-based strips, which are described as acting through physical or biochemical mechanisms with potentially lower selection pressure for resistance. Despite regulatory approval, the peer-reviewed field literature evaluating these products remains relatively limited compared with that available for established treatments such as amitraz, formic acid, oxalic acid, or thymol [30].

### 5. Biotechnical and Physical Methods

Biotechnical and physical control methods target biological vulnerabilities in the life cycle of *V. destructor* and are commonly incorporated into integrated pest management programs. Unlike chemical treatments, these approaches typically rely on manipulation of colony structure, brood dynamics, or physical removal of mites along with the brood. Although their standalone efficacy is usually lower than that of chemical acaricides, they can significantly reduce mite population growth and are often used as complementary strategies. In recent years, biotechnical methods such as drone brood removal, brood interruption, queen caging, and total brood removal have been increasingly incorporated into integrated *Varroa* management programs in several European countries as part of efforts to reduce acaricide use and mitigate resistance development [51–57].

#### 5.1. Screened Bottom Boards

Screened bottom boards are designed to increase natural mite fall and reduce the probability that fallen mites return to the colony. A meta-analysis synthesizing seven studies involving 145 colonies demonstrated a statistically significant reduction in *V. destructor* populations in colonies equipped with screened bottom boards compared with solid floors [58]. Observational studies also suggest that screened bottom boards may be associated with reduced colony loss rates under some conditions. However, their effectiveness depends strongly on management practices. In particular, excessive ventilation during winter may increase the risk of colony starvation if hive insulation and feeding management are inadequate [31]. Consequently, screened bottom boards are generally considered supportive management tools rather than primary control methods.

#### 5.2. Drone Brood Removal and Trapping

Drone brood removal exploits the strong reproductive preference of *V. destructor* for drone brood, which remains capped longer than worker brood and therefore supports higher mite reproductive success. Regular removal of drone brood can reduce the rate of mite population growth within colonies. Field evaluations demonstrate measurable suppression of mite populations when drone brood removal is performed repeatedly throughout the season [13]. Despite its biological rationale, the method has practical limitations. Drone brood removal requires regular colony inspections and repeated brood removal cycles, increasing labor demands. In addition, excessive removal of drone brood may affect colony reproductive dynamics and drone availability.

#### 5.3. Brood Interruption Strategies

Brood interruption techniques are based on the biological requirement of *V. destructor* for capped brood cells to reproduce. Methods such as queen caging, trapping comb systems, and total brood removal temporarily interrupt brood production, thereby exposing mites to treatments that are effective only against phoretic mites. Field studies within integrated pest management frameworks show that brood interruption combined with subsequent

oxalic acid treatment can substantially reduce mite infestations [33]. However, these strategies involve important trade-offs, as temporary reductions in brood production may affect colony productivity, while their implementation requires increased labor and management input. The long-term effects of these interventions on viral dynamics, colony productivity, and overwintering success remain areas of active research. Economic analyses suggest that some brood interruption strategies, particularly total brood removal, can be economically viable under certain beekeeping systems. Nevertheless, their successful implementation requires careful planning, labor investment, and appropriate integration with other management practices [34].

#### 5.4. Hyperthermia (Thermotherapy)

Hyperthermia-based treatments rely on the differential heat tolerance of mites and honey bees. Because *V. destructor* is more sensitive to elevated temperatures than its host, controlled heating of brood areas can suppress mite populations without causing lethal damage to bees when properly applied. Recent studies evaluating device-based thermotherapy systems demonstrate that controlled heating regimens can reduce mite populations in managed colonies. However, treatment efficacy varies considerably depending on the thermotherapy device used, heating protocol, treatment duration, and colony conditions. In addition, these treatments involve important trade-offs, including potential effects on brood survival, colony growth dynamics, and the need for specialized equipment and labor [35].

#### 5.5. Powdered Sugar Mechanical Dislodgement

Mechanical removal of mites using powdered sugar dusting has been used for many years as a simple management technique. Recent experimental work exploring whole-colony treatment approaches suggests that mechanical dislodgement of mites can achieve measurable reductions in infestation levels, particularly when combined with crowding and agitation of treated bees [59]. Nevertheless, the available evidence remains relatively limited compared with that for chemical treatments or brood interruption methods. The scalability of this approach for large commercial operations also remains uncertain.

## 6. Biological Controls, RNAi, and Breeding for Resistance

Biological control strategies aim to reduce *V. destructor* populations through natural antagonists, molecular approaches targeting mite gene expression, or selective breeding of honey bees with increased resistance to infestation. Although these strategies remain less widely implemented than chemical or biotechnological interventions, they represent important directions for reducing long-term dependence on acaricides.

#### 6.1. Entomopathogenic Fungi

Entomopathogenic fungi have long been investigated as potential biological control agents against *V. destructor* A. & T. Among the most extensively studied and promising entomopathogenic fungi are *Beauveria bassiana* B., *Metarhizium anisopliae* M., *Metarhizium brunneum* M., and *Hirsutella thompsonii* F., which have demonstrated varying levels of pathogenicity against *V. destructor* under laboratory and field conditions [19]. A key recent advance involves strain improvement and formulation strategies designed to function under the temperature and humidity conditions present in honey bee colonies. Despite these advances, several challenges remain before fungal biocontrol can be widely implemented. Delivery methods capable of distributing spores effectively within colonies, persistence under hive microclimatic conditions, and consistent performance across multiple seasons are still areas requiring further investigation. In particular, the elevated temperatures maintained within brood nests and fluctuations in colony humidity may

reduce fungal survival, sporulation, and infectivity, thereby limiting field efficacy compared with laboratory conditions. Consequently, further optimization of fungal strains and delivery systems is required before routine application in commercial beekeeping can be recommended.

#### 6.2. Predatory Mites

Predatory mites have also been explored as potential biological control agents. Experimental evaluations of *Stratiolaelaps scimitus* W. have examined both its predation potential against *V. destructor* and its safety for honey bee colonies. Current evidence suggests that while the species can prey on *Varroa* under laboratory conditions, its effectiveness in colonies remains uncertain [60]. Risk assessments conducted in some regulatory contexts have therefore focused primarily on ecological safety rather than recommending widespread deployment. As a result, predatory mites are currently considered a promising research direction rather than a practical control strategy for routine apiary management. Although predatory mites remain a promising research direction, current evidence is insufficient to support their routine use as a field-scale *Varroa* control strategy, and further validation under commercial beekeeping conditions is required.

#### 6.3. RNA Interference (RNAi) and dsRNA-Based Approaches

RNA interference (RNAi) technologies represent one of the most rapidly developing areas in *Varroa* control research. These approaches typically involve delivery of double-stranded RNA (dsRNA) molecules designed to silence essential mite genes, thereby disrupting survival or reproduction. A major regulatory milestone occurred when the United States Environmental Protection Agency issued a final registration decision for a dsRNA-based active ingredient (vadesca) designed to control *V. destructor* through RNAi mechanisms. Regulatory assessments emphasize the high target specificity of dsRNA technologies and the importance of evaluating environmental safety. Experimental studies using dsRNA feeding approaches have reported reductions in mite reproduction and infestation levels under controlled conditions. The high sequence specificity of RNAi-based approaches is considered a major advantage compared with conventional acaricides because it may reduce impacts on non-target organisms and minimize residue concerns. However, treatment efficacy depends on reliable delivery of dsRNA to target mites and the maintenance of molecular stability under field conditions. Several challenges therefore remain before RNAi-based technologies can be routinely implemented in apiculture, including reliable delivery under commercial field conditions, environmental degradation and stability of dsRNA molecules, potential off-target effects on non-target organisms, and the possible long-term evolution of sequence-specific resistance in mite populations. Consequently, further research is needed to optimize delivery systems, assess long-term ecological safety, and establish practical protocols for large-scale field application.

#### 6.4. Breeding for *Varroa*-Resistant Honey Bees

Selective breeding for *Varroa*-resistant honey bee stocks represents one of the most promising long-term strategies for sustainable *Varroa* management. Key traits under investigation include *Varroa*-sensitive hygiene (VSH), mite non-reproduction (MNR), grooming behavior, and mite-biting behavior. Studies evaluating VSH expression in commercial colonies demonstrate that these traits can significantly reduce mite reproduction and colony infestation levels, although their expression often varies widely between colonies and generations [61]. Recent research also highlights the substantial variation observed in breeding programs targeting mite non-reproduction and VSH traits, indicating that effective selection requires long-term evaluation and intensive phenotyping [62]. Policy analyses in Europe further emphasize that the widespread adoption of resistant bee stocks

depends not only on genetic progress but also on coordinated breeding programs, market structures supporting resistant stock production, and effective knowledge transfer to beekeepers [38].

## 7. Treatment Decision Frameworks and IPM Implementation

Effective management of *V. destructor* requires treatment decisions that integrate monitoring data, colony brood status, environmental conditions, and resistance risk. Modern control strategies therefore rely on integrated pest management (IPM) principles that combine regular monitoring, threshold-based intervention, and rotation of treatments with different modes of action.

### 7.1. Monitoring-Based Action Thresholds

Routine monitoring of mite populations is the foundation of evidence-based treatment decisions. Several standardized methods are commonly used to estimate infestation levels, including alcohol wash, powdered sugar roll, and sticky board mite fall counts (see Table 2). Action thresholds vary by region, season, and management system, but many monitoring programs consider approximately three mites per 100 adult bees during the active season as a practical intervention threshold. When infestation levels exceed this range, treatment is generally recommended to prevent exponential growth of mite populations and associated viral amplification.

### 7.2. Treatment Timing and Brood Dynamics

The effectiveness of many treatments is strongly influenced by colony brood status. Oxalic acid, for example, primarily affects phoretic mites and therefore shows the highest efficacy during broodless periods or when combined with brood interruption techniques. In contrast, formic acid can partially penetrate capped brood cells and may therefore be used when brood is present. Seasonal timing is also critical. Treatments applied in late summer or early autumn are particularly important because high mite loads during this period can compromise the production of long-lived winter bees and increase overwintering colony losses.

### 7.3. Rotation of Control Methods and Resistance Management

Repeated use of the same acaricide can create strong selection pressure for resistant mite populations. Consequently, IPM programs emphasize the rotation of treatments with different modes of action and the integration of chemical and non-chemical methods. Combining chemical treatments with biotechnical approaches, such as brood interruption or drone brood removal, can reduce overall treatment frequency and delay resistance development. Post-treatment monitoring is also recommended to verify treatment efficacy and detect early signs of resistance or treatment failure.

### 7.4. Integrated Management in Practical Beekeeping Systems

In practice, sustainable *Varroa* management typically involves combining multiple control strategies throughout the year. Monitoring-based thresholds guide treatment decisions, while seasonal timing, colony condition, and environmental factors influence the selection of specific interventions. Integrated strategies may include chemical treatments during periods of high infestation risk, biotechnical methods to suppress mite population growth, and long-term breeding programs aimed at improving colony resistance. Together, these approaches form the foundation of modern IPM programs designed to maintain mite populations below damaging thresholds while minimizing chemical dependence.

## 8. Resistance Mechanisms, Surveillance, and Resistance Management

### 8.1. Mechanistic Basis of Resistance

The evolution of resistance in *V. destructor* follows a pattern typical of many pesticide-exposed arthropod pests. Repeated exposure to the same compounds generates strong selection pressure, while the mite's relatively short generation time and population dynamics within colonies facilitate the rapid spread of resistant genotypes. In addition, colony-level refugia and reinvasion processes can allow resistant individuals to persist even when specific treatments are temporarily withdrawn [15]. Resistance mechanisms differ among chemical classes. In the case of pyrethroids, resistance is strongly associated with knockdown resistance (kdr) mutations in the voltage-gated sodium channel gene. Substitutions at position 925 are particularly well documented and have been identified in multiple regions, where they correlate with reduced treatment efficacy in field populations [19]. For amitraz, resistance has increasingly been linked to mutations in the mite's  $\beta$ -adrenergic-like octopamine receptor, which is a key molecular target of formamidine compounds. Recent studies demonstrate that specific receptor variants are associated with reduced sensitivity to amitraz and that molecular genotyping can help predict treatment performance in apiaries [15]. Coumaphos resistance appears to involve more complex biochemical mechanisms. Research indicates that resistance can arise through altered activation of the proinsecticide, mediated by reduced activity of specific cytochrome P450 enzymes responsible for converting coumaphos to its toxic metabolite. This finding illustrates that resistance mechanisms may involve modifications of metabolic activation pathways rather than only detoxification processes [23].

### 8.2. Resistance Surveillance Tools

Effective resistance management requires continuous surveillance of mite populations. Monitoring programs typically operate on three complementary levels: field efficacy tracking, laboratory bioassays, and molecular diagnostics. Field efficacy tracking is the most widely implemented approach in commercial beekeeping systems. This method involves quantitative assessment of mite infestation before treatment and repeated sampling after treatment, typically one to two weeks following the exposure period. Monitoring post-treatment resurgence rates provides practical information about treatment performance and helps identify emerging resistance [63]. Some studies also quantify the rate at which mite populations return to intervention thresholds, such as three mites per 100 adult bees. These metrics allow comparisons among treatments and provide information directly relevant to commercial apiary management [64]. Laboratory bioassays provide a more controlled framework for distinguishing true resistance from treatment failures caused by reinvasion, improper application, or environmental variability. Experimental assays using technical amitraz or commercial strip formulations have documented a broad spectrum of susceptibility among mite populations, including confirmed treatment failures in some commercial operations [17]. However, the results of such assays can be influenced by experimental parameters such as temperature, exposure surface, and product age, highlighting the importance of methodological standardization [65]. Molecular diagnostics are currently most advanced for pyrethroid resistance, where mutations in the voltage-gated sodium channel gene provide reliable genetic markers. Diagnostic tools are also being developed for amitraz resistance through detection of variants in the octopamine receptor gene [19]. Large-scale surveillance programs integrating molecular and phenotypic data have revealed substantial regional variation in susceptibility patterns, demonstrating that resistance status can differ markedly even between neighboring apiaries [15].

## 9. Residues, Toxicology, and Environmental Considerations

### 9.1. Residues in Hive Matrices and Their Implications

Residue dynamics of *Varroa* control products differ substantially depending on their chemical properties. Lipophilic acaricides, particularly pyrethroids and coumaphos, readily accumulate in beeswax and can persist through multiple cycles of wax recycling. This creates long-term exposure pathways for developing brood and queens [20]. Large-scale residue surveys in North American apiaries have detected widespread presence of miticides in wax and pollen, frequently including simultaneous occurrence of fluvalinate and coumaphos. These findings demonstrate that honey bee colonies are often exposed to complex mixtures of compounds rather than single substances [20]. Recent research has begun to link such mixture exposures with biological effects. For example, experimental studies indicate that combined exposure to amitraz, fluvalinate, and coumaphos can disrupt the honey bee gut microbiome and increase sensitivity to neonicotinoid toxicity [66]. These results highlight the importance of considering mixture toxicity when evaluating residue risks in hive environments. Coumaphos residues are of particular concern. Field studies have reported high concentrations of coumaphos in brood comb months after a single treatment, with levels reaching mg/kg ranges in wax [21]. These concentrations overlap with experimentally demonstrated toxicity thresholds affecting queen development and queen-cell acceptance. Experimental toxicology models further confirm that coumaphos residues in wax and larval diet can negatively affect larval development, providing dose–response benchmarks relevant for wax recycling and foundation management decisions [21]. Residue dynamics for thymol differ from those of synthetic lipophilic compounds. Thymol may be present at low levels in hive products even before treatment and can persist in honey and wax following application. In most cases, the primary concern relates to sensory effects rather than toxicological risk, as thymol residues may alter honey flavor for several weeks or months after treatment [39]. Amitraz exhibits a different residue profile because the parent compound degrades relatively rapidly. However, its metabolites can be detected in honey following treatment, particularly after fumigation-based applications [16]. For regulatory monitoring purposes, residue analysis therefore typically focuses on marker metabolites rather than the parent compound alone [67].

Organic acids such as oxalic acid and formic acid represent a fundamentally different residue category. Both substances occur naturally in the environment and are relatively unstable in hive conditions. Regulatory product information for oxalic acid treatments indicates that correct application is not expected to increase honey residues above natural background levels, although transient exposure of bees and hive components may occur after treatment [45]. Consequently, consumer residue risk for these compounds differs substantially from that associated with wax-accumulating synthetic acaricides.

### 9.2. Effects on Bees Castes and Brood

A consistent finding in the literature is that colony-level toxicity cannot be assessed solely through adult worker survival. Sublethal effects on reproduction and brood development may significantly affect colony performance. Coumaphos exposure, for example, can strongly impair queen rearing when residues accumulate in brood comb. Experimental studies demonstrate reduced queen development and lower queen weight at sublethal exposure levels [22]. Similarly, formic acid treatments can result in brood mortality and occasional queen loss under certain delivery systems or environmental conditions, despite achieving high mite control [24]. Oxalic acid treatments can also affect brood if applied incorrectly or during periods of active brood production. Regulatory guidance therefore emphasizes that oxalic acid is most appropriate for broodless conditions or for use within carefully controlled treatment regimens [63]. Reproductive endpoints are increasingly used

in toxicological evaluations of *Varroa* control products. Studies investigating chronic exposure to miticides and mixtures of acaricides have documented effects on sperm viability, larval development, and colony reproductive performance [68].

### 9.3. Environmental Toxicity and Non-Target Risks

Environmental considerations are increasingly incorporated into regulatory assessments of *Varroa* control products. Some acaricides used within hives carry explicit environmental warnings regarding aquatic toxicity or waste disposal. Improper disposal of treated wax, equipment washing, or contaminated hive materials may allow these compounds to enter environmental pathways [38]. Newer biopesticide classes are frequently promoted as having lower environmental risks compared with conventional acaricides. However, the ecological safety of these products depends on multiple factors, including their degradation dynamics, exposure pathways, and interactions with non-target arthropods inhabiting apiary environments [26].

## 10. Regulation, Adoption Economics, and Research Priorities

### 10.1. Regulatory Status and Approved Treatments by Region

Regulatory frameworks governing *V. destructor* control differ substantially between jurisdictions. In the United States, most in-hive *Varroa* treatments are regulated as pesticides requiring federal registration. In contrast, many European countries regulate these products as veterinary medicinal products, subject to harmonized requirements concerning efficacy, residue safety, and target-animal protection. Consequently, treatment availability, labeling requirements, and approval pathways differ across regions.

#### 10.1.1. United States

In the United States, *Varroa* treatments are regulated primarily through the Environmental Protection Agency (EPA). The EPA maintains an updated list of registered products and active ingredients approved for use in honey bee colonies. These include organic acids such as oxalic acid and formic acid, thymol-based products, hop beta acids, synthetic acaricides such as amitraz, and more recently RNA interference-based technologies, including dsRNA-active ingredients such as vadesca [30].

#### 10.1.2. European Union and Europe-Wide Regulatory Framework

Within the European Union, many *Varroa* treatments are regulated as veterinary medicinal products. The European Medicines Agency (EMA) provides guidance on data requirements for efficacy, safety for bees, and residue considerations in products intended to control *V. destructor* [69]. Authorized products are documented in the Union Product Database, which serves as a centralized repository for veterinary medicines approved across EU and EEA member states [70]. Nevertheless, product availability can vary between countries depending on national authorization procedures. For example, VarroMed® (BeeVital GmbH, Seeham, Austria), a combination product containing oxalic acid dihydrate and formic acid, has a European Public Assessment Report documenting its authorization history and updates through recent regulatory reviews [28].

#### 10.1.3. United Kingdom

Following regulatory divergence after Brexit, veterinary medicines for honey bees in the United Kingdom are administered by the Veterinary Medicines Directorate. The agency maintains an online database listing currently authorized treatments, including thymol-based gels, oxalic acid solutions and powders, and pyrethroid-based strips. Guidance

issued by the National Bee Unit emphasizes the importance of using authorized products to prevent contamination of honey and other hive products [32].

#### 10.1.4. Canada

In Canada, regulatory oversight of *Varroa* control products is primarily coordinated through the Pest Management Regulatory Agency of Health Canada. Provincial extension programs frequently refer to federal regulatory documents when providing treatment recommendations to beekeepers. Practical management guides commonly list amitraz, pyrethroids, formic acid products, thymol formulations, hop beta acids, and oxalic acid as available options, while emphasizing rotation of treatments and residue management [71].

#### 10.1.5. Australia

Australia detected *V. destructor* for the first time in 2022, and regulatory responses have therefore developed rapidly. Current management relies on emergency permits and approved treatment lists coordinated by government agencies and industry organizations. Guidance emphasizes threshold-based treatment decisions, legal compliance with permitted products, and careful selection of treatments compatible with temperature conditions and number of honey supers [72].

#### 10.1.6. New Zealand

In New Zealand, the Ministry for Primary Industries conducts regular national colony loss surveys that provide insight into treatment practices and management trends. Survey results indicate that beekeepers commonly apply treatments in spring and autumn, often using combinations of flumethrin and amitraz. These patterns highlight the importance of resistance monitoring, as repeated use of the same compounds can contribute to selection pressure in mite populations [73].

## 11. Adoption Barriers, Economics, and Research Priorities

### 11.1. Practicality, Cost, and Adoption Barriers

A persistent gap exists between treatment efficacy demonstrated under experimental conditions and the effectiveness of *Varroa* control strategies in routine beekeeping practice. Several practical factors contribute to this “efficacy-in-practice” gap, including labor availability, equipment costs (such as vaporizers or protective equipment), temperature constraints affecting volatile treatments, limited colony access in migratory beekeeping systems, and beekeeper risk perception [72]. Survey-based studies illustrate these constraints clearly. For example, research in Australia found that although many beekeepers expressed interest in non-chemical control strategies, most relied primarily on chemical treatments because of concerns about labor demands and operational practicality [68]. Similar patterns are observed in Europe, where large-scale survey analyses reveal geographically clustered “treatment cultures,” such as reliance on amitraz strips, long-duration formic acid treatments, or amitraz fumigation. These patterns indicate that adoption of control strategies is influenced not only by biological efficacy but also by advisory networks, supply chains, local traditions, and regulatory frameworks [74]. Economic analyses of biotechnical methods further illustrate this complexity. Techniques such as total brood removal can be economically viable within certain management systems, but their implementation requires careful planning and sufficient labor resources [34]. Similarly, breeding programs aimed at developing *Varroa*-resistant honey bee stocks are widely regarded as an important long-term strategy, yet their adoption remains constrained by structural factors, including limited breeding infrastructure, fragmented markets for resistant stock, and incomplete evidence for performance across diverse environments [38].

### 11.2. Data Gaps and Research Priorities

Despite extensive research on *V. destructor*, several key knowledge gaps continue to limit the development of sustainable control strategies.

#### 11.2.1. Standardization of Efficacy and Resistance Testing

Variation in bioassay methodologies, exposure conditions, and reporting metrics complicates comparison between studies and may obscure emerging resistance patterns. Developing harmonized protocols that link field treatment failures with validated phenotypic and genotypic resistance markers remains a high priority [65]. In addition, the absence of standardized resistance thresholds and diagnostic criteria limits early detection of resistance and hinders reliable comparisons among regions and treatment systems. Establishing internationally accepted monitoring and testing frameworks would improve resistance surveillance, facilitate data integration across studies, and strengthen evidence-based decision-making in *Varroa* management.

#### 11.2.2. Brood, Queen, and Drone Endpoints in Safety Assessment

Evidence indicates that reproductive parameters such as queen development, sperm viability, brood survival, and drone fertility can be highly sensitive to pesticide exposure. However, these endpoints remain inconsistently evaluated in both laboratory and field studies, despite their direct relevance to colony performance and long-term viability. Their routine inclusion in safety assessments would provide a more realistic evaluation of treatment-associated risks and improve the ability to detect sublethal effects that may not be apparent from short-term measures of colony survival or mite control efficacy [22].

#### 11.2.3. Residue Ecology and Mixture Toxicology

Because lipophilic acaricides accumulate in beeswax and can persist through repeated wax recycling, future research should establish practical thresholds for safe wax reuse and evaluate the biological consequences of common residue mixtures on brood development, immune function, and detoxification capacity [20]. Particular attention should be given to chronic low-dose exposure, as colonies are often exposed simultaneously to multiple acaricide residues and environmental contaminants rather than single compounds in isolation. A better understanding of mixture toxicity and long-term sublethal effects is essential for improving residue risk assessment and developing sustainable wax management practices.

#### 11.2.4. Field-Scalable Biological Control Strategies

Entomopathogenic fungi and predatory mites show promising mechanistic potential, but their practical use requires reliable delivery systems and consistent efficacy under commercial beekeeping conditions across multiple seasons [37]. Current evidence is largely derived from experimental or small-scale studies, whereas validation under diverse climatic conditions and management systems remains limited. Future research should focus on optimizing formulation stability, delivery efficiency, and integration with existing integrated pest management programs to determine whether these approaches can provide economically viable and operationally practical alternatives to conventional acaricides.

#### 11.2.5. Durability and Governance of RNAi Technologies

With dsRNA-based products now entering regulatory approval pathways, research priorities include improving delivery reliability under field conditions, assessing potential off-target ecological effects, and evaluating the long-term evolution of sequence-based resistance [75]. Additional attention should be directed toward the environmental persistence of dsRNA molecules, their interactions with non-target organisms, and the development of regulatory frameworks that balance innovation with ecological safety. Addressing these

challenges will be essential for ensuring the long-term effectiveness, public acceptance, and sustainable implementation of RNAi-based *Varroa* control technologies.

#### 11.2.6. Resistance-Reducing Management Systems

Emerging evidence suggests that operational management patterns, not only individual compounds, strongly influence resistance evolution. Future studies should therefore evaluate integrated system-level strategies that combine treatment rotation with brood interruption, comb replacement, and coordinated regional management approaches [17]. Particular emphasis should be placed on identifying management practices that reduce selection pressure while maintaining effective mite control over multiple seasons. A better understanding of how treatment timing, monitoring-based interventions, and landscape-scale coordination influence resistance dynamics will be essential for developing sustainable long-term *Varroa* management programs.

### 12. Reinvasion Ecology and Landscape-Level Dynamics

Reinvasion of *V. destructor* following treatment is increasingly recognized as a landscape-level ecological process. Mites can be transferred between colonies through drifting workers, robbing behavior, and the movement of foragers from collapsing colonies with high infestation levels. These processes can produce sudden increases in mite loads in previously treated colonies, particularly during late summer and autumn when colony density and foraging interactions are high [29]. Recent observational and modeling studies further suggest that reinvasion risk depends on several interacting factors, including infestation pressure in neighboring colonies, brood availability in recipient colonies, and apiary configuration, with linear hive arrangements potentially facilitating greater bee drift and parasite transmission [75]. Implication for IPM: Effective varroosis management therefore requires coordination at the landscape or regional level, including synchronized monitoring and treatment among neighboring beekeeping operations.

### 13. Climate Change and Implications for Varroa Control

Climate change is expected to influence both *V. destructor* population dynamics and the efficacy of several control strategies. Elevated seasonal temperatures may increase mite infestation levels during autumn by promoting extended brood production and larger worker populations, thereby providing additional reproductive opportunities for mites [76].

Temperature also plays a critical role in the performance and safety of several commonly used treatments, particularly those based on volatile organic acids and essential oils. Multi-regional field trials have shown that extreme temperature conditions may alter treatment efficacy and increase the risk of adverse colony effects, including brood mortality or queen loss [77]. Treatment timing and product selection should increasingly account for local climatic conditions, colony brood status, and short-term weather variability.

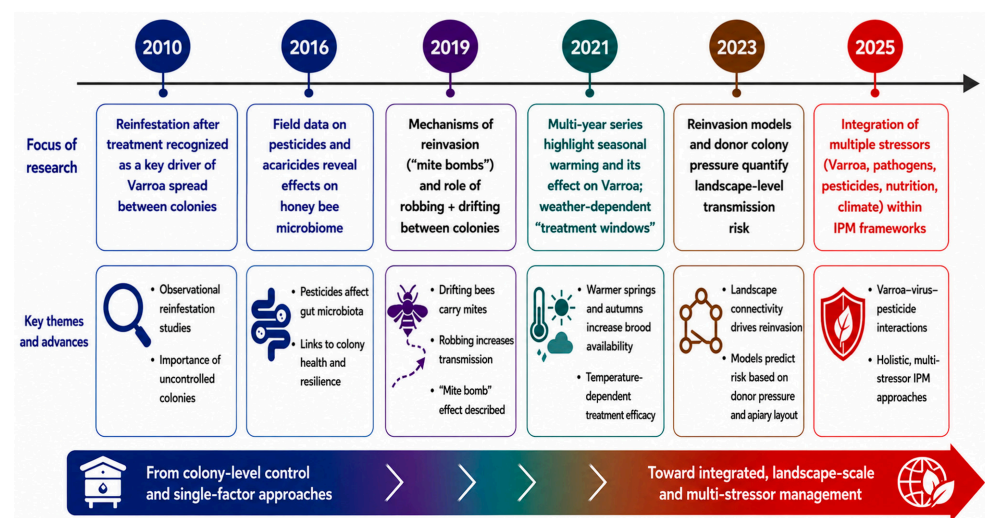
### 14. Colony Microbiome Interactions

The role of the honey bee microbiome has recently emerged as an important dimension of colony health in the context of *Varroa* infestation and chemical control. Residues of pesticides and acaricides within hive matrices may alter the composition and stability of the honey bee gut microbiome. Field studies have demonstrated that exposure to commonly used in-hive chemicals such as coumaphos and tau-fluvalinate can significantly modify bacterial community structure in worker bee intestines [78]. Interactions between parasitism and chemical stressors may further exacerbate colony vulnerability. Experimental studies indicate that *Varroa* infestation can increase susceptibility to agrochemicals such as imidacloprid while simultaneously reducing gut microbial diversity in developing bees [79].

These findings suggest that parasite pressure, chemical exposure, and microbial dysbiosis may interact synergistically to influence colony resilience. Strategies that minimize long-term chemical accumulation in wax and avoid overlapping treatments may help preserve microbial stability and improve colony health outcomes.

## 15. Future Perspectives

As illustrated in Figure 2, research on *V. destructor* has progressively shifted from colony-level chemical control toward integrated frameworks incorporating reinvasion dynamics, environmental drivers, and multi-stressor interactions. Despite decades of research and the availability of multiple treatment options, sustainable control of *V. destructor* remains a major challenge in modern apiculture. Increasing evidence indicates that colony decline is rarely driven by a single factor, but rather by complex interactions among *Varroa* infestation, viral pathogens, pesticides, nutrition, and environmental stressors. A better understanding of these synergistic interactions will be essential for improving risk assessment and designing more effective management strategies. Future progress will require long-term international and multidisciplinary research integrating bee pathology, toxicology, genetics, ecology, and practical beekeeping management. Such coordinated efforts are necessary to improve resistance monitoring, standardize experimental protocols, and support the development of more robust and context-dependent control strategies.



**Figure 2.** Evolution of research priorities in *Varroa destructor* A. & T. management (2010–2025). This figure represents a conceptual synthesis of major research trends rather than a strict chronological sequence of discoveries.

## 16. Limitations of the Review

Several limitations of the present review should be acknowledged. Although a structured literature search strategy was applied, the review was not conducted as a formal systematic review or meta-analysis. Consequently, no formal study quality scoring system or quantitative risk-of-bias assessment was performed. In addition, the review relied primarily on peer-reviewed literature indexed in major scientific databases and selected regulatory documents, which may have introduced publication bias. Finally, although efforts were made to identify relevant international literature, some studies published in languages other than English may not have been captured.

## 17. Conclusions

The evidence reviewed in this study demonstrates that no single intervention provides durable and universally effective control of *V. destructor*. Instead, successful management depends on integrating multiple complementary strategies that account for mite biology, colony condition, environmental factors, and local resistance patterns.

Future progress will depend on coordinated, multidisciplinary research integrating bee pathology, toxicology, genetics, ecology, and practical beekeeping management. Such efforts are essential for improving resistance monitoring, standardizing experimental approaches, and developing more robust and adaptable control strategies. Given that no single method currently fulfills all criteria of efficacy, safety, resistance durability, and operational feasibility, integrated pest management remains the most scientifically supported framework for sustainable control of *V. destructor* in *Apis mellifera* colonies.

**Author Contributions:** Conceptualization, M.Z., E.T. and G.B.; data curation, G.B., E.T., A.B. and M.Z.; writing—original draft preparation, G.B., E.T. and M.Z.; writing—review and editing, G.B., A.B. and E.T. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Data Availability Statement:** The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

**Acknowledgments:** The authors would like to thank the anonymous reviewers for their kind suggestions and constructive comments.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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