Uludag Bee Journal
Uludag Bee J.
e-ISSN: 2687-5594
ISSN 1303-0248
https://dergipark.org.tr/en/pub/uluaricilik
https://uadubj.uludag.edu.tr/

A review of the mechanisms of acaricide resistance in *Varroa destructor* affecting honey bees

Shah ALAM[1*], Muhammad Asif AZIZ[1], Muhammad SHEHZAD[1], Sumaira MAQSOOD[2]

[1]Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan
Corresponding author E-mail: shahalamkhanrabbani@gmail.com
[2]Department of Environmental Sciences, Kohsar University, Murree, Pakistan

Received: 03.03.2026 Accepted: 28.04.2026 Published: 04.06.2026

Abstract

Honey bees (*Apis mellifera*) are crucial pollinators for economically significant crops. However, their colonies are increasingly threatened by a wide range of pathogens and parasites. Among these, the ectoparasitic mite, *Varroa destructor*, poses the most serious threat to bees worldwide. To combat Varroa mite infestations, beekeepers use a range of synthetic and organic acaricides. Nonetheless, the continuous use of these acaricides has led to the development of resistance in *Varroa* mites, thereby complicating control efforts. Key resistance mechanisms include enhanced detoxification and target-site insensitivity, which are influenced by genetic, anthropogenic, and ecological factors. While resistance mechanisms to pyrethroids are well documented, those related to organophosphates and formamidines remain less well understood. This review summarizes the current knowledge of *Varroa* mites' resistance mechanisms to pyrethroids, organophosphates, and formamidines. It highlights recent advancements in non-chemical mite management approaches in the context of integrated pest management, and suggests future research directions to develop effective and sustainable mite control strategies.

Keywords: *Varroa destructor*, *Apis mellifera*, Acaricide resistance, Pyrethroids, Formamidines, Detoxification mechanisms

INTRODUCTION

Honeybees are vital pollinators of both wildflowers and agricultural crops (Genersch 2010) and contribute more than €153 billion euros in the worlds' economy annually (Gallai 2009, Puranik et al. 2023). However, multiple stressors affect honeybees, including infections, pests, and habitat degradation (Giacobino et al. 2018). Varroa mites, scientifically known as *Varroa destructor* (Trueman & Anderson 2000), are detrimental parasites with a substantial global impact, particularly on the European honey bee (*Apis mellifera* Linnaeus 1756), the economically most important bee species (Morawetz et al. 2019, Traynor et al. 2020). These mites primarily invade fat bodies and hemolymph of honey bees during their reproductive phase to get nutrition, impairing the immune system of their hosts through the toxins they inject (Ramsey et al. 2019, Han et al. 2024). In addition to the aforementioned aspects, Varroa mites are vectors for several pathogens that cause severe viral infections within bee colonies (Giacobino et al. 2018, Hubert et al. 2015, Wilfert et al. 2016). To manage Varroa during its global spread, synthetic pesticides have been a vital tool for beekeepers. Chemical agents commonly used against this parasite include formamidines, organophosphates, and pyrethroids. Beekeepers have a natural tendency towards these chemicals due to their ease of use and historical efficacy (Evans & Cook 2018, Kamler et al., 2016).

A thorough review of literature indicates 23-31% of beekeepers in New Zealand exclusively employed either amitraz or flumethrin, while in the United Kingdom, 18% of beekeepers used amitraz exclusively (Stahlmann-Brown et al. 2022, Valentine & Martin 2023). However, due to their rapid reproductive potential, short developmental period, and frequent exposure to acaricides, resistance has evolved in these mites, rendering control measures inefficient in many cases (Zhan et al. 2015, Boi et al. 2016, Erban et al. 2017). Pesticide resistance is a relative decrease in a pest population's susceptibility to a particular pesticide (Siddiqui et al. 2023). It has become an important issue to understand the status of acaricide resistance and mechanisms involved in resistance development so that new, eco-friendly and effective control measures could be formulated for this arthropod. To provide deep insights into

effective preventive and control strategies, this review focuses on the resistance status and potential mechanisms of Varroa mites to acaricides.

Literature review and data analysis

To investigate the mechanisms of various acaricide resistance in *V. destructor*, an ectoparasitic mite of the honey bee *Apis mellifera*, a systematic search of peer-reviewed literature was conducted across Google Scholar, PubMed, Science Direct, Scopus, and Research Gate, for articles published between 1998 and 2025, following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines (Page et al. 2021). The search employed the following keywords: *A. mellifera,* European honeybee, pyrethroids, organo-phosphates, amitraz, formamidines, target-site mutation, detoxification enzymes, metabolic resistance, resistance, esterases, glutathione S-transferases, mechanisms, and management.

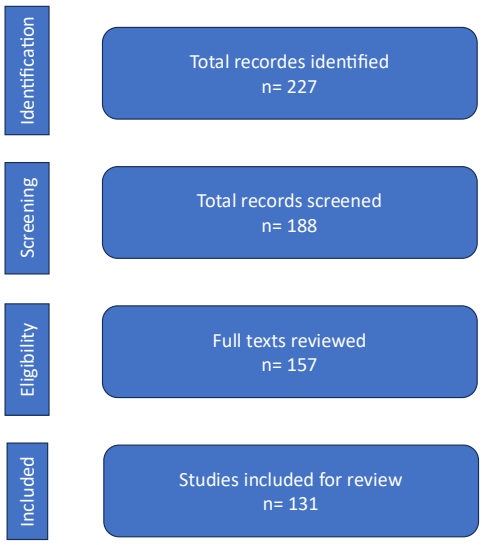


Fig. 1. PRISMA flowchart depicting literature selection

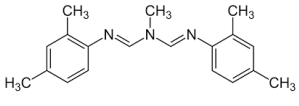
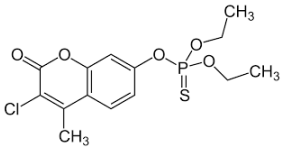
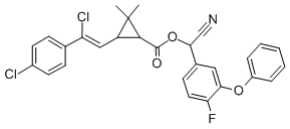
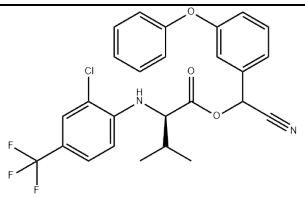
Articles that were theoretical and commentaries were excluded. Studies focusing on *A. ceranae* or other mite host relationships were also excluded. Only original articles investigating the biochemical and genetic mechanisms underlying acaricide resistance in *V. destructor* infesting *A. mellifera* were retained. After screening, a total of 188 articles were assessed for eligibility; 131 were retained, reviewed in detail, and synthesized for this review (Fig 1). Each identified article was examined to determine whether it was an original article describing pyrethroids, organophosphates, amitraz, formamidines, target-site mutations, detoxification enzymes, metabolic resistance, esterases, glutathione S-transferases, mechanisms, and management. All citations of previous resistance in Varroa or of reviews that might include citations of previously reported works were noted, and these works were added to the list of articles to be reviewed.

Global status of *Varroa* Mite Resistance to acaricides

The development of acaricide resistance has emerged as a persistent and escalating issue, and has become an unabated phenomenon over the course of the last few decades. As a result, many management strategies do not yield effective mite suppression, and ultimately, mites cause significant economic losses to apiculture worldwide (De Rouck et al. 2023). Acaricide resistance in Varroa mite populations has been continuously documented across multiple regions of the world (Table 1), posing a significant challenge to controlling this parasite (Higes et al. 2020). The empirical evidence indicates that mite resistance has developed to repeatedly applied acaricides, particularly those belonging to the organophosphate and pyrethroid groups (Marsky et al. 2024, Celikkol & Dogac 2025). The evolution of resistance has been driven by strong selection pressure from repeated exposure of a single group and is often accompanied by cross-resistance to other groups having a similar mode of action or chemical nature (Rodriguez-Vivas et al., 2018). This has complicated the mite management strategies and reduced the effectiveness of alternative treatments within the same chemical group.

A comparative analysis of studies highlights the severity of resistance levels across regions. An increase of 34-39 fold resistance against amitraz has been reported in Argentina and Uruguay, respectively, from field-collected populations as compared to reference populations (Maggi et al. 2010, Mitton et al. 2016). In contrast, Kamler et al. (2016) reported resistance not only to amitraz but also to tau-fluvalinate and acrinathrin, suggesting broad-spectrum resistance in mites from Czech bee colonies. Such regional differences may reflect the influence of local beekeeping practices, acaricide application frequency, alternate treatments rotation strategies on the development of resistance. Several reports highlight the diverse cross-resistance in mites, further complicating the control efforts. A detailed overview of resistance status across different regions is presented in Table 1.

Table 1. Chemical structure of acaricides used against *Varroa destructor* and resistance reports in different regions of the globe

Acaricides	Chemical Structure	Countries	References
Amitraz		Yugoslavia United States of America France, México, Argentina, Croatia Canada	(Dujin et al. 1991) (Elzen et al. 1999) (Mathieu & Faucon 2000) (Rodríguez-Dehaibes et al. 2005) (Maggi et al. 2010) (Tlak Gajger et al. 2019) Bahreini et al. 2025
Coumaphos		Italy Argentina United States of America Uruguay	(Spreafico et al. 2001) (Sammataro et al. 2005) (Maggi et al. 2009) (Maggi et al. 2011) (Kanga et al. 2016)
Flumethrin		Italy, France, Poland, Germany, Belgium, Austria, Hungary, Finland United Kingdom, United States of America, Argentina, México, New Zealand, Uruguay, Finland	(Lodesani et al. 1995) (Thompson et al. 2002) (Sanford 2001) (Goodwin et al. 2005) (Mitton et al. 2016)
tau-Fluvalinatelinate		Italy France Finland United Kingdom United States	(Fernandez & Omar 1997) (Mozes-Koch et al. 2000) (Rodríguez-Dehaibes et al. 2005)

Genetic Factors Leading to the Development of Resistance in Mites

Genetic factors play a critical role in the development and spread of acaricide resistance in different mite populations. Key factors include the number of dominant resistant genes, their frequency within populations, genetic diversity, and adaptive capacity of the resistant populations (Georghiou & Taylor, 1977). Initially, upon the introduction of novel acaricides, resistance-related genes in the Varroa population are typically present at low frequencies. However, their repeated and sometimes indiscriminate exposure imposed strong selection pressure, resulting in a rapid increase in the frequency of resistance-conferring genes (González-Cabrera et al. 2018).

Comparative genetic studies provide important insights into the dynamics of resistance development. For example, González-Cabrera et al. (2016, 2018) demonstrated that resistance to pyrethroids such as tau-fluvalinate and flumethrin is linked with a marked increase in the frequency of resistant homozygotes in mite populations. Such studies also reveal that selection pressure not only increases the frequency of resistance alleles but also drives their fixation in certain populations. Conversely, Beaurepaire et al. (2017) argued that the haplodiploid reproductive nature and frequent inbreeding are the reasons for the overall low genetic variability in Varroa mites. As a result, resistant homozygosity is promoted, thereby facilitating the expression of resistance phenotypes even in small mite populations. Both studies suggest that intrinsic genetic structure, in combination with selection pressure, shapes resistance trajectories.

The resistance to pyrethroids is strongly linked to the mutations in the voltage-gated sodium channel proteins (VGSC) gene, which is a very important component of the mite's nervous system. Almecija et al. (2022) suggested that insensitivity to tau-fluvalinate is associated with the structural alterations in the VGSC proteins. More specifically, Millán-Leiva et al. (2021) identified mutations that replace the amino acid at position 925 in VGSC, which are documented as key markers conferring

resistance to pyrethroids in *V. destructor* populations. Although these findings provide strong evidence of target-site resistance mechanisms, their distribution and prevalence vary across regions, suggesting localized evolutionary patterns.

Despite substantial research on identifying resistance-associated mutations and understanding genetic markers, considerable areas still require investigation. Thus, include studies on metabolic resistance mechanisms, long-term population-level genetic monitoring to explore temporal dynamics of resistance allele frequencies, and the interaction between genetic resistance and environmental factors. Moreover, the pace of translating research findings on genetic resistance into apicultural management practices remains relatively slow.

Human Operating Factors

Furthermore, human operational factors have played a critical role in the development of acaricide resistance. These include selection of acaricide compound, its properties, application techniques, frequency and timing of treatments (Obaid et al. 2022). Over-reliance on a specific group of acaricides with a similar mode of action exerts stronger selection pressure. This practice eventually, rapidly eliminates the susceptible members, and replaces them with resistant and difficult to manage ones. When resistant parasites outweigh susceptible ones, the acaricides lose their efficacy significantly (Sparks et al. 2021). Empirical evidence from several studies supports such impacts. As reported by Johnson et al. (2013), Mozes-Koch et al. (2000), and Thompson et al. (2002), these trends have been observed with repeated and prolonged use of a single acaricide treatment. To overcome resistance, acaricide rotation is widely recommended as a key component of the integrated resistance management (IRM) strategy. However, the robust empirical validation of this approach for the long-term effectiveness of Varroa mites is still needed.

The method of application may significantly influence acaricide efficacy by affecting the dosage. Santiago et al. (2000) observed variations in median lethal concentrations (LC50) when three acaricides were applied via different routes. Such variability may result in suboptimal dosing, repeated treatments, and, consequently, increased selection pressure.

Miscellaneous Factors

Several other factors related to the host-environment interactions, like bee activity, brood population, disease infection level in the colony, and the hive architecture, can impact the effectiveness of acaricides, thereby contributing to the selection for resistance in mites (Gracia et al. 2017, de Mattos et al. 2017) (Fig. 2). While systematic research on these factors is lacking, and currently they serve only as potential indicators of resistance development. However, future evaluation of acaricide efficacy should incorporate on-site studies to quantify the influence of these variables on acaricide exposure and the resulting selection pressure.

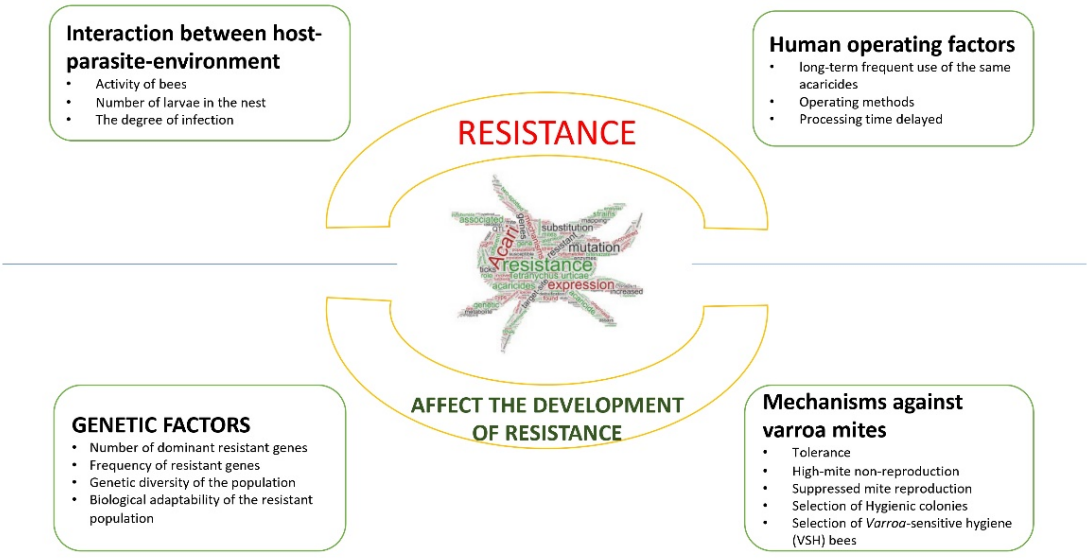


Fig. 2. Schematic diagram of different factors leading the way toward development of resistance in the Varroa mite; human-operated factors, genetic factors and associated aspects

Mechanism of Resistance Evolution in *Varroa* mite to Acaricides

Several mechanisms are involved in the development of resistance in insects including *V. destructor*. These include cuticular resistance, metabolic resistance, and target site insensitivity (Abbott 2006, Le Conte et al. 2015, Sammataro et al. 2005). The process underlying cuticular resistance remains relatively underexplored, a number of studies present information about resistance, metabolic, and target-site development in different groups of arthropods (Balabanidou et al. 2018, Shehzad et al. 2023, Strachecka et al. 2015).

Target Site Resistance

Target site resistance arises from the gene's structural modifications or point mutations encoding the target proteins that interact directly with the acaricides (Casida & Durkin 2013). Resistance to pyrethroid acaricides is most commonly associated with alterations in the voltage-gated sodium channel (VGSC), a critical transmembrane protein that initiates and propagates action potentials in neuronal membranes (Dong et al. 2014, Vu 2016). Synthetic pyrethroid acaricides can modify the function of these sodium ion channels by binding to specific sites, leading to continuous electrical discharges in the insect nervous system and ultimately causing paralysis and death (Liu 2015). Diverse VGSC mutation sites are linked with pyrethroid resistance. For instance, Wang et al. (2002) identified four novel mutation sites, F1528L (IIIS6), L1595P (IIIS6-IVS1 linker), I1752V (IVS5), and M1823I (IVS6), associated with pyrethroid resistance (Fig. 3).

In contrast, other studies indicate that mutations in the S5 segment of domain II of the VGSC are associated with pyrethroid resistance (Cheng et al. 2019). Similarly, L925V substitution has been reported as a key factor conferring resistance in pyrethroid-treated colonies (González-Cabrera et al. 2013). Comparatively, although a broader set of potential mutation sites has been reported (Wang et al. 2003), only a limited subset appears to be more consistent and evolutionarily stable across different geographical regions (González-Cabrera et al. 2013, Hubert et al. 2014).

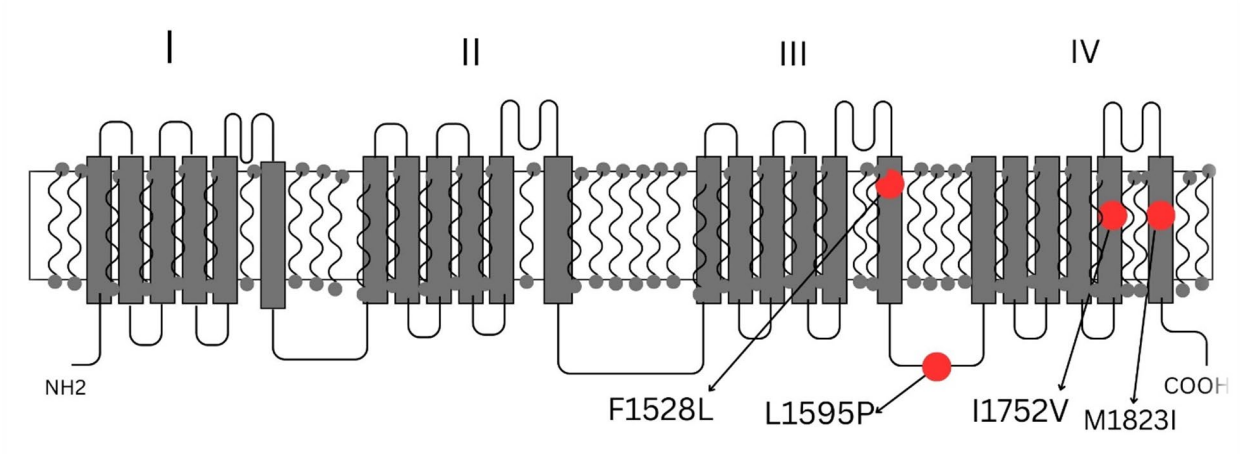


Fig. 3. Amino acid changes on the sodium channel in four different locations. Red circles indicate mutations in *V. destructor* that confer pyrethroid resistance (Wang et al. 2003).

Gonzalez-Cabrera et al. (2013) investigated resistance in UK *Varroa* mite samples through PCR amplification and sequencing. In the IIS5 helix of the sodium channel protein, they identified a leucine-to-valine substitution (L925V) as a hotspot for resistance. This mutation, proposed to be involved in pyrethroid (tau-fluvaliante) resistance, was present in 100% resistant mites as compared to 8% in the control mites. Strachecka et al. (2015) used the Polymerase chain reaction-single-strand conformational polymorphism (PCR-SSCP) and reported lower global DNA methylation levels in the resistant populations compared to the sensitive ones.

This study provided insight into a previously undescribed mechanism of resistance: epigenetic regulation as a contributing factor. Millán-Leiva et al. (2018) introduced a novel PCR-RFLP-based genotyping assay to discriminate among known mutations at amino acid position 925 (L925V, L925I, L925M) of the VGSC for rapid differentiation between sensitive and resistant strains. As a cheap and easy-to-perform assay in the lab, this assay also offered accuracy and robustness comparable to TaqMan®. Stara et al. (2019) applied the in vitro Vail test through the PCR-RFLP method to detect the L925 knockdown resistance (kdr) mutations in the sodium channel gene (*Na$_V$Ch*), at codon L925 (L925V/I/M). The resistant mites showed higher resistance values (0.400) than the susceptible ones (0.00783). During viability tests, 97% of susceptible phenotypes died; however, 63% of resistant phenotypes carried the L925V mutation, highlighting the test's reliability.

Comparative studies across different geographical regions (Alissandrakis et al. 2017, Cabrera et al. 2016, Farjamfar et al. 2018, Gonzalez-Cabrera et al. 2013, González- Hubert et al. 2014, Stara et al.

2019) indicate both shared and region-specific mutation patterns, suggesting convergent and localized evolutionary responses to acaricide selection pressure. While target-site resistance to erythroid is relatively well understood, there is a need for deeper investigation to gain further insights into the interactions among multiple mutations (epistatic effects) and how they influence resistant phenotypes.

Metabolic Detoxification Mechanisms

Metabolic detoxification is an important mechanism for explaining acaricide resistance in Varroa mites, driven by altered expression or activity of detoxification enzymes (Fig. 4). Overexpression or enhanced activity of these enzymes modulates detoxification, allowing xenobiotic compounds to be rapidly broken down and eliminated from the body (Sammataro et al. 2005). It is believed that alterations in the regulation of three major enzyme families, viz. esterases, glutathione-S transferases, and P450 monooxygenases in arthropods, are primarily responsible for resistance by modulating both the rate and efficacy of metabolism (Namountougou et al. 2012).

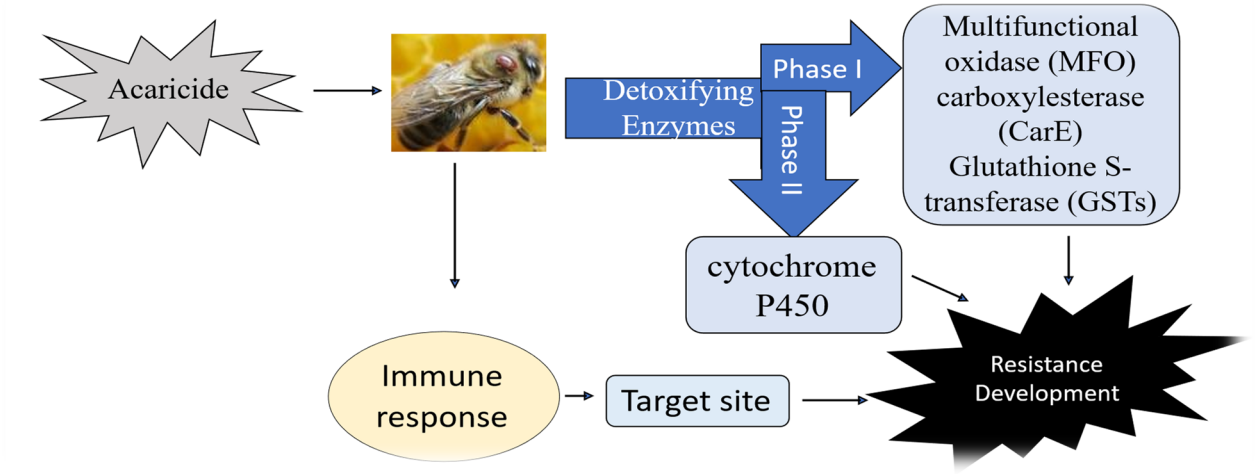


Fig. 4. The metabolic detoxification process in the Varroa mite. This is depicted alongside the roles of several detoxifying enzymes, including glutathione S-transferase (GST), carboxylesterase (CarE), and multifunctional oxidase (MFO). These enzymes, which are mediated by cytochrome P450 via Phase I and Phase II pathways, aid in the development of resistance and in the mites' immunological response before the acaricide reaches its target site.

Previous studies have shown variability in the enzymatic responses to different groups of acaricides. For example, a coumahos-resistant Varroa population was reported to down-regulate the metabolic enzyme, P450 monooxygenase (Vlogiannitis et al. 2021). Similarly, Roth et al. (2021) reported no significant fluctuation in the activity of three primary detoxification enzymes: glutathione S-transferases (GSTs), cytochrome monooxygenases (P450s), and esterases, which have been observed frequently in the development of resistance against different acaricides in Varroa mites. These findings indicate that metabolic resistance is not always the sole mechanism conferring resistance in any particular mite population, and that other mechanisms may also be involved. The following sections describe reports from different geographical regions that document enzymatic variation and its association with acaricide resistance in the *Varroa* mite.

Cytochrome P450 Monooxygenases (P450s)

Cytochrome P450 monooxygenases comprise a diverse group of enzymes involved in the oxidative metabolism of xenobiotics into less toxic compounds, thereby reducing their efficacy, and hence play a key role in the detoxification processes in insects and arachnids (Lu et al. 2021).

From the perspective of Varroa mites, information reflecting quantitative fluctuation in cytochrome P450s is somewhat limited. Johnson et al. (2010) reported the role of cytochrome P450 enzymes in the detoxification of tau-fluvalinate and coumaphos in honey bees. Given that both bees and *Varroa* experience the same chemical exposure within the hive, it is plausible that similar detoxification processes occur in mites. Accordingly, P450 metabolic pathways are likely to be relevant to both organisms. Genath et al. (2020) demonstrated the differential expression of P450 genes in mites exposed to formic acid, with five genes upregulated and one deregulated, indicating a complex gene specific response in the context of detoxification. Furthermore, Vlogiannitis et al. (2021) demonstrated reduced bio-activation of coumaphos in Varroa mites, which must be converted into an active metabolite, coroxon by cytochrome P450 enzymes.

The resistant mites exhibited reduced metabolic activation of coumaphos. Since the mite genome contains 26 cytochromes P450 (CYP) genes, resistant mite populations showed overexpression of one CYP gene and underexpression of two CYP genes, which are thought to be associated with this kind of mite resistance. Collectively, most studies indicate the importance of P450 enzymes in detoxification; however, studies also highlight their

role in reduced bio-activation, suggesting that their function is not straightforward. This phenomenon underscores the need for further research to investigate gene regulation and its interactions with other resistance-conferring mechanisms.

Esterases

Esterases are key enzymes involved in the detoxification of xenobiotics and are widely associated with pesticide resistance in arthropods, including *Varroa* mites (Cao et al. 2008; Muthusamy et al. 2014). These enzymes contain nonspecific carboxylesterases and cholinesterases, which reduce the toxicity by hydrolyzing ester bonds in chemical compounds (Dang et al. 2017). The organophosphate acaricide resistance, is closely linked to acetylcholinesterase (ACHE), which is responsible for terminating the nerve impulse through acetylcholine hydrolysis (de Mattos et al. 2017). Hence, it is used to monitor resistance in *V. destructor*, mainly against organophosphates (Sammataro et al. 2005).

Mutations in the AChE gene in mites frequently lead to organophosphate resistance by attenuating the enzyme's susceptibility to inhibition by these chemicals (French-Constant et al. 2004, Hernández-Rodríguez et al. 2022). Dmitryjuk et al. (2014) observed ten-fold higher esterase activity in relation to acaricide-resistant individuals. Hillesheim et al. (2019) found that the aforementioned enzyme played a negligible role in the development of resistance. These findings suggest that esterase activity is population- or compound-dependent rather than universally associated with resistance. According to a recent study by Kim et al. (2022), *V. destructor* releases a cocktail of acetylcholinesterase enzymes. One of these isoforms, VdAChE2, was found to be predominantly active in the legs and the cuticle, suggesting that esterases may help mites defend against acaricide penetration.

Glutathione S-Transferases (GSTs)

The glutathione S-transferases (GSTs) are an important class of enzymes involved in the detoxification of xenobiotic compounds and play a central role in the metabolism of acaricides. GSTs catalyze the conjugation of reduced glutathione to toxic compounds, increasing their solubility, reducing toxicity, and facilitating excretion. This study by Gerson et al. (1991) showed that GST activity is much greater in pyrethroid-resistant mites than in susceptible populations. GSTs enable the conjugation process to neutralize the toxic effects of acaricides, allowing mites to detoxify and remove these chemicals with high efficiency.

Formamidine Resistance

Formamidines, with Amitraz as a representative, are a leading group of acaricides widely used in the treatment of animal ectoparasites, including Varroa (Pohorecka et al. 2018). Unlike pyrethroids, formamidines exert their toxic effects by blocking octopamine receptors, which are essential for neurotransmission in arthropods (Casida & Durkin 2013). Overstimulation of these receptors can provoke tremors and convulsions, affecting both juvenile and adult mites (Mendes et al. 2013, Prullage et al. 2011). Although a case of formamidine resistance was identified in mites during a field trial in Minnesota in 2000, reports of resistance have been scarce, and the acaricide continues to be utilized globally for mite management (Rinkevich 2020).

Studies have demonstrated the efficacy of formamidine and formic acid in controlling mite infestations in bee colonies during the spring and fall seasons (Vandervalk et al. 2014). In 2018, a comparative analysis of five synthetic acaricides revealed their capacity to control mite populations in hives, with formamidine (amitraz) and phosphorus-based chemicals showing promising results (Bakar et al. 2018). Hernandez-Rodriguez et al. (2021) conducted a 2-year assessment of various acaricides in Spain and found that formamidines were highly effective against female *Varroa* mites. However, Corly et al. (2013) conducted an experiment on ticks and reported increased mutation frequency in the B-epinephrine octopamine receptor (RmBAOR) following formamidine exposure, leading to resistance.

A recent study suggested that the (F290L) mutation in octopamine receptors in Varroa mites may occur following formamidine exposure, leading to resistance (Hernández-Rodríguez et al. 2025). Collectively, these studies indicate the high effectiveness of formamidine as a cornerstone of Varroa control. But their long-term sustainability remains uncertain due to emerging evidence of target-site mutations. Therefore, proactive resistance monitoring and integrated management are emphasized rather than relying solely on formamidine-based control.

Emerging Technologies and Research Trends

Currently, new research directions and advanced technologies are the focus. Advancements in molecular technology are among the new opportunities for researchers in the fight against *Varroa* mites. Such advanced technology methods include RNA interference (RNAi), microbiome manipulation, CRISPR-Cas9 gene editing, and the Internet of Things (IoT).

RNA interference (RNAi)

RNA interference (RNAi) is an advanced and emerging tool to manage *Varroa* mite populations. RNAi plays an important role in regulating protein production. During the synthesis of a particular protein from messenger RNA (mRNA), RNAi targets double-stranded RNA (dsRNA), leading to the degradation of the corresponding mRNA before it can direct protein synthesis, thereby temporarily inhibiting protein production (Kim & Rossi 2008).

When introduced into mites, these molecules induce the breakdown of the target mRNA, thereby inhibiting the synthesis of essential proteins. RNAi has been successfully used to investigate gene functions in a wide range of invertebrate species (Campbell et al. 2009, Minakuchi et al. 2009, Shakesby et al. 2009, Walshe et al. 2009). Campbell et al. (2010) demonstrated the otential of RNAi-based tactics to disrupt mite development and reproduction after intra-haemocoelic injection of dsRNA targeting Vd GST-mu1, suggesting their potential as a sustainable control strategy. Similarly, Garbian et al. (2012) reported that dsRNA ingested by honey bees can be transmitted to the parasitic mite *V. destructor,* which feeds on the honey hemolymph and fat bodies, resulting in up to 60% reduction in mite population.

Gene editing with CRISPR-Cas9

By making precise changes to the genome of *V. destructor*, CRISPR-Cas9 could generate mites that are less fit or more susceptible to acaricides. CRISPR-based approaches can target the genes associated with resistance mechanisms, such as those involved in target-site insensitivity or metabolic detoxification pathways. For example, Inak et al. (2024) used CRISPR-Cas9 to produce gene-edited mites that showed reduced survival under acaricide exposure, suggesting a possible avenue for mite management strategies. Although CRISPR-Cas9 is a powerful research tool, its field application for Varroa control faces some practical, environmental, and ethical challenges and requires further validation.

Microbiome Modulation

The microbiota of honey bees *(A. mellifera)* and their parasites can influence host health and resistance dynamics. The use of probiotic treatments to modulate the bee microbiome has been proposed as a strategy to promote overall health and resilience against Varroa mites and other stressors. Anderson et al. (2011) demonstrated that alterations in the bee microbiome can influence interactions with Varroa mites, potentially moderating their effects. This suggests that a balanced, healthy microbial community in bees can enhance immune responses and reduce the negative impacts of mite infestations on bee health. Microbiome modulating is less invasive than genetic modification; however, it is influenced by a variety of factors, including diet, pesticides, and climate, which can affect its stability. Large-scale field trials are required to confirm whether microbiome–based intervention can produce reliable results under diverse beekeeping conditions.

Remote sensing and Internet of Things (IoT)

Remote sensing and Internet of Things (IoT) technologies are increasingly used to monitor the hive conditions and the extent of *Varroa* infestation. Smart hive sensor devices enable continuous, data-driven analysis and monitoring, and can even generate real-time alerts to support timely management interventions. For example, Ferrari et al. (2007) highlighted the potential of sensor-based systems to monitor mite infestation and hive activity, including acoustic signals such as buzzing and swarming sounds, which may indicate colony health and stress levels. Although these technologies offer clear advantages in precision and early detection, they also have practical limitations, including cost, technical complexity, and the need for reliable connectivity in remote apiaries. However, these technologies can be used to complement the traditional inspection methods until their effectiveness is fully validated.

Integrated Pest Management (IPM)

Integrated pest management (IPM) is a well-established approach that combines multiple methods to control *V. destructor* while minimizing harm to the bees and the environment. Nowadays, Varroa mite resistance represents a major and escalating challenge in the apicultural industry. Many researchers have proposed temporarily discontinuing certain acaricides for a few years to reverse mite resistance (Farjamfar et al. 2018). Although this approach could restore the susceptibility, it is not without practical implications. Effective management increasingly relies on IPM tactics aimed at reducing the frequency of resistance alleles in mite populations by using chemicals with different modes of action (Gonzalea-Cabrera et al. 2018, Vu et al. 2020).

Alternatives to conventional chemical control, such as oxalic acid and formic acid, have shown considerable efficacy in reducing mite populations (Papežíková et al. 2017, Pietropaoli and Formato 2018). Despite their effectiveness, these treatments are highly dependent on environmental and brood conditions, which can limit their reliability. Additionally, several biocontrol agents, such as *Metarhizium anisopliae, Beauveria bassiana, Stratiolaelaps scimitus, and Coccidia albicans* (Hamiduzzaman et al. 2012, Rangel and Ward 2018, Reinbacher et al., 2018, Pusceddu et al. 2019, Steenberg et al. 2010) offer novel and environmentally friendly alternatives. Nevertheless, their field-level effectiveness remains inconsistent, and large-scale validation is still limited.

Nazzi & Le-Conte (2016) used pheromone-based traps that mimic the chemical signals of bee brood, thereby reducing *Varroa* mite infestation without harming the bees. Similarly, Emsen et al. (2020) demonstrated that essential oils such as camphor and eucalyptol exhibit acaricidal activity against the Varroa mite, offering alternatives to synthetic insecticides and helping reduce acaricide residues in honey. The extracts of sandalwood and thyme also offer miticidal properties (Leza et al. 2015, Lin et al. 2020). Given that no single method provides an effective solution, a more holistic, adaptive approach is needed that integrates multiple control methods and continuously monitors resistance dynamics.

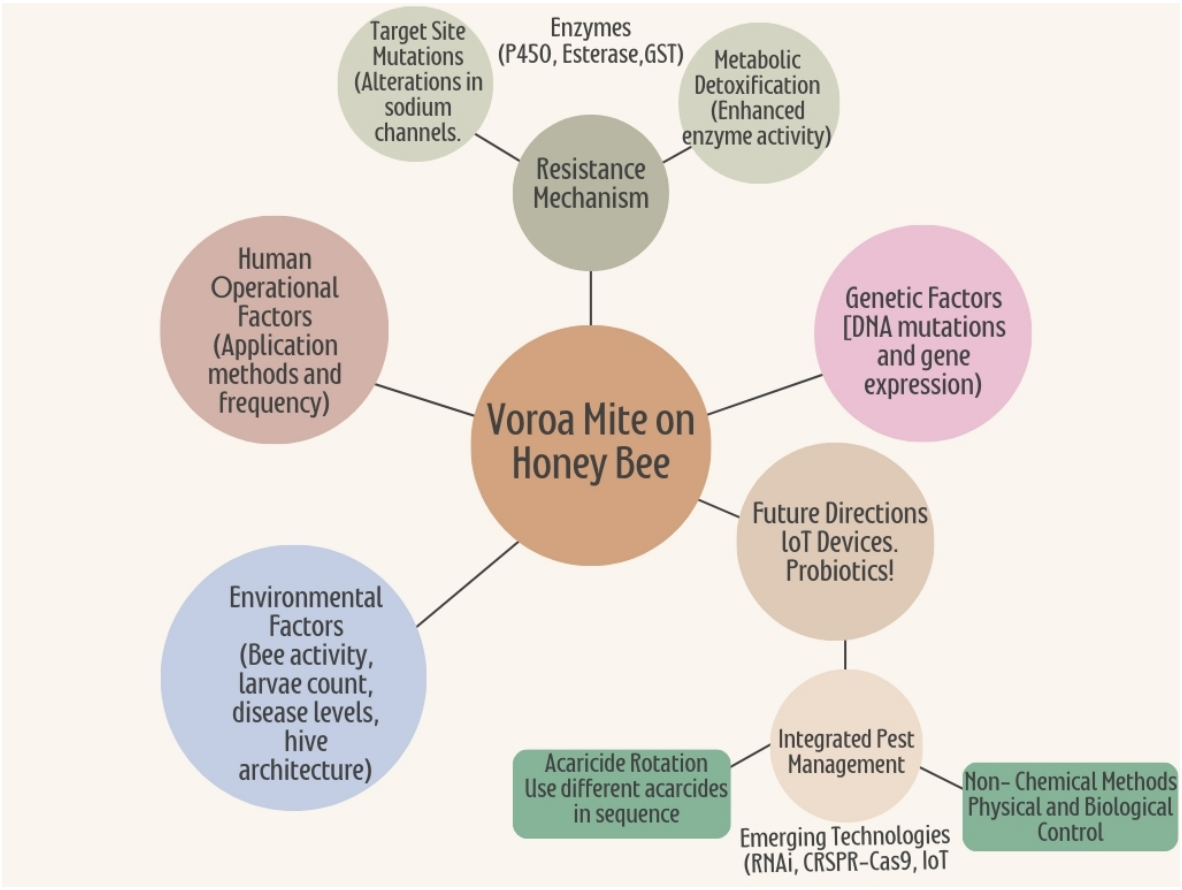


Fig 5. A diagram illustrates the mechanisms of acaricide resistance in *Varroa* mites and the management strategies used to combat them. At the center, a *Varroa* mite on a honey bee is surrounded by key factors: genetic influences such as DNA mutations, resistance mechanisms such as target-site mutations and metabolic detoxification and human operational factors such as application methods and frequency.

Possible resistance and control strategies for effective mite control under contemporary situations are illustrated in Fig. 5.

Environmental factors, including bee activity and hive conditions, also play a role. Integrated Pest Management (IPM) strategies encompass acaricide rotation, non-chemical methods, and emerging technologies like RNAi and CRISPR-Cas9. Future directions highlight IoT devices for monitoring and probiotic treatments for bees. Arrows and labels connect these elements, illustrating their interactions and providing a comprehensive view of the multifaceted approach needed to manage Varroa mite resistance effectively.

Case studies and real-world applications

Case studies from different geographical regions provide practical insights into the effectiveness of IPM strategies. Pettis & Delaplane (2010) conducted an experiment in commercial apiaries in California, implementing drone brood removal, screened bottom boards, and organic acid treatments. These measures resulted in significant reductions in the Varroa mite population and improved colony health. In Europe, particularly in Germany, beekeepers have adopted IPM practices, such as alternating between natural and synthetic acaricides and using Varroa-resistant bee strains, resulting in sustainable control of mite infestations and reduced colony losses (Rosenkranz et al. 2010).

Advantages and Limitations of IPM

The IPM framework by categorizing treatments into cultural mechanical, organic chemical, and synthetic chemical options, allowing beekeepers to match intervention to their colony actual varroa levels. It promotes resistance management by discouraging synthetic chemical treatments when mite loads fall below 3 mites/100 bee threshold, reducing unnecessary acaricide selection pressure. The tool respects beekeeper autonomy by not mandating specific products within a category; instead, it directs users to supplementary tables for efficacy and regional availability data. An IPM strategy that prioritizes sustainable integrated control over routine chemical application.

In IPM strategies, the beekeepers independently determine economic feasibility, a critical gap given that cost often drives treatment compliance. It offers no specific guidance on selecting among multiple options within the same IPM category, requiring users to interpret efficacy data without comparative recommendations. A less experienced beekeeper may struggle to weigh the trade-offs between different acaricides, particularly given documented resistance profiles for fluvalinate and amitraz. Finally, the exclusion of biocontrol means the

framework cannot incorporate emerging biocontrol agents if they become commercially available in the future.

Analysis of Economic and Environmental Impact

Infestations of Varroa mites have significant economic and environmental **consequences**. They contribute to colony losses and reduced honey production, increasing costs associated with treatments and management. Van-Engelsdorp et al. (2012) emphasized that cost-benefit analysis of different control strategies can assist beekeepers in selecting economically viable and environmentally sustainable options. Reliance on chemical acaricides can lead to environmental contamination and the development of resistance in Varroa mite populations. Therefore, there is an increasing need to explore and implement natural treatments and biocontrol tactics in combination with chemical treatments to reduce environmental risks (Ellis et al. 2019). Although alternative approaches require higher initial investments and greater management efforts, they are generally more environmentally sustainable and can yield long-term benefits. However, ongoing research is needed to make alternatives easier to apply, cost-effective, and accessible, thereby enabling wider adoption among beekeepers.

Regulations framework and policies

A strong regulatory framework and coherent policies are essential for the effective management of *Varroa* mites. International organizations such as the World Organization of Animal Health (OIE) and the Food and Agriculture Organization (FAO) provide guidelines for controlling honeybee diseases and parasitic infestations, emphasizing the careful use of acaricides and the adoption of Integrated Pest Management (FAO 2014). At the national level, policies vary; however, many countries promote sustainable beekeeping practices and support research into innovative alternative control methods. For example, the European Commission (2013) restricted the use of certain neonicotinoids, encouraging the development of safer, more sustainable pest management approaches, particularly for *Varroa* control.

Despite international guidelines, implementation in developing countries remains inconsistent due to weak national policies, limited regulatory capacity, poor enforcement, and limited access to approved treatments. This highlights the need for locally adapted, evidence-based regulations that balance agricultural productivity with pollination health and environmental sustainability.

Research Gaps and Future Directions

Although significant progress has been made in controlling *Varroa* mites, important research gaps remain. A deeper understanding of the genetics and biochemical mechanisms underlying acaricide resistance is needed to support the development of more effective and targeted control strategies (Johnson et al. 2013). Furthermore, improving the precision and reliability of IPM techniques remains a priority. More research is required to evaluate the long-term effectiveness and adaptability across different environmental conditions and beekeeping systems (Petties & Delaplane 2010). Moreover, instead of fragmented research, there is a need for large-scale, longitudinal studies that integrate the Internet of Things (IoT) with genetic, ecological, and socio-economic factors. Efforts should also focus on improving technology transfer to beekeepers in diverse regions, particularly regarding cost-effective mite control solutions. Collaboration between researchers, beekeepers, and policymakers needs to be strengthened to ensure that scientific findings are effectively translated into practical applications.

Conclusion

Honey bees provide essential ecosystem services, particularly through pollination, which supports biodiversity and global food production. However, the worldwide infestation of Varroa mites continues to pose a serious threat to honey bees' health and productivity. Current control strategies heavily rely on chemical treatments, leading to the development of resistance and a gradual decline in their effectiveness. This resistance is driven by a combination of complex genetic, biochemical, and management-related factors. A thorough understanding of resistance mechanisms, such as target-site mutations and enhanced metabolic detoxification, therefore requires large-scale, longitudinal studies to devise strategies for the sustainable control of *Varroa* mites.

Continuous monitoring of insecticide resistance, rotation of acaricides, and increased use of non-chemical methods are essential cornerstones of *Varroa* management. Integration of conventional methods with emerging technologies is necessary to achieve sustainable solutions within the framework of integrated pest management.

Advances such as RNA interference (RNAi), gene-editing tools like CRISPR-Cas9, and smart monitoring systems based on the Internet of Things (IoT) offer promising directions for future control strategies. These technologies are crucial for ensuring the health and productivity of honey bee colonies.

AI Usage Statement: No artificial intelligence (AI) tools or technologies were used in the preparation, writing, or analysis of this review paper. The authors are fully responsible for all content.

Authors' contributions: SA prepared the initial draft, M.A.A., S.M., and MS reviewed and edited. All authors have read and approved the manuscript.

Conflict of Interest: The authors declare that they have no conflicts of interest.

Data availability: The data used and analyzed during this project are available from the corresponding author on reasonable request.

Ethics Statement: not applicable

Funding: No funding information is available.

REFERENCES

Abbott KL. Spatial dynamics of supercolonies of the invasive yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island, Indian Ocean. Diversity and Distributions 2006;(1):101-10.https://doi.org/10.1111/j.13669516.2006.00193.x

Almecija G, Schimmerling M, Del Cont A, Poirot B, Duquesne V. *Varroa destructor* resistance to tau-fluvalinate: relationship between in vitro phenotypic test and VGSC L925V mutation. Pest Management Science 2022;78(12):5097-105. https://doi.org/10.1002/ps.7126

Anderson KE, Sheehan TH, Mott BM, Maes P, Snyder L, Schwan MR, Walton A, Jones BM, Corby-Harris V. Microbial ecology of the hive and pollination landscape: bacterial associates from floral nectar, the alimentary tract and stored food of honey bees (*Apis mellifera*). PloS one 2013;8(12):e83125. https://doi.org/10.1371/journal.pone.0083125

Bahreini R, González-Cabrera J, Hernández-Rodríguez CS, Moreno-Martí S, Muirhead S, Labuschagne RB, Rueppell O. Arising amitraz and pyrethroids resistance mutations in the ectoparasitic *Varroa destructor* mite in Canada. Scientific Reports 2025;15(1):1587. https://doi.org/10.1038/s41598-025-85279-6

Bąk BE, Wilde J, Siuda MA. Characteristics of north-eastern population of *Varroa destructor* resistant to synthetic pyrethroids. Medycyna Weterynaryjna 2012;68(10):603-606.

Bakar MA, Aqueel MA, Raza AB, Arshad M, Mahmood R, Qadir ZA. Comparative efficacy of five commercial synthetic acaricides against *Varroa destructor* (Anderson and Trueman) in *Apis mellifera* L. colonies. Pakistan Journal of Zoology 2018;50(3) http://dx.doi.org/10.17582/journal.pjz/2018.50.3.857.861 .

Balabanidou V, Grigoraki L, Vontas J. Insect cuticle: a critical determinant of insecticide resistance. Current Opinion in Insect Science 2018;27:68-74. https://doi.org/10.1016/j.cois.2018. 03.001.

Beaurepaire AL, Krieger KJ, Moritz RF. Seasonal cycle of inbreeding and recombination of the parasitic mite *Varroa destructor* in honeybee colonies and its implications for the selection of acaricide resistance. Infection, Genetics and Evolution 2017;50:49-54. https://doi.org/10.1016/j.meegid.2017.02.011

Boi M, Serra G, Colombo R, Lodesani M, Massi S, Costa C. A 10 year survey of acaricide residues in beeswax analysed in Italy. Pest Management Science 2016;72(7):1366-72. https://doi.org/10.1002/ps.4161

Campbell EM, Budge GE, Bowman AS. Gene-knockdown in the honey bee mite *Varroa destructor* by a non-invasive approach: studies on a glutathione S-transferase. Parasites & Vectors 2010;3(1):73. https://doi.org/10.1186/1756-3305-3-73

Campbell EM, Pert CC, Bowman AS. RNA-interference methods for gene-knockdown in the sea louse, *Lepeophtheirus salmonis*: studies on a putative prostaglandin E synthase. Parasitology 2009;136(8):867-74. https://doi.org/10.1017/S0031182009990357

Cao CW, Zhang J, Gao XW, Liang P, Guo HL. Overexpression of carboxylesterase gene associated with organophosphorous insecticide resistance in cotton aphids, *Aphis gossypii* (Glover). Pesticide biochemistry and physiology 2008;90(3):175-80. https://doi.org/10.1016/j.pestbp.2007.11.004

Casida JE, Durkin KA. Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. Annual Review of Entomology 2013;58:99-117. https://doi.org/10.1146/annurev-ento-120811-153645

Castonguay E, Angers B. The key role of epigenetics in the persistence of asexual lineages. Genetics Research International 2012;2012(1):534289. https://doi.org/10.1155/ 2012/534289

Celikkol E, Dogac E. The status of pyrethroid resistance mutation frequencies in *Varroa destructor* populations in the most important beekeeping areas of Türkiye. Experimental and Applied Acarology 2025;94(2):32. https://doi.org/10.1007/s10493-025-01002-0

Cheng X, Umina PA, Lee SF, Hoffmann AA. Pyrethroid resistance in the pest mite, *Halotydeus destructor*: dominance patterns and a new method for resistance screening. Pesticide Biochemistry and Physiology 2019; 159:9-16. https://doi.org/10.1016/j.pestbp.2019.04.010

Corley SW, Jonsson NN, Piper EK, Cutullé C, Stear MJ, Seddon JM. Mutation in the RmβAOR gene is associated with amitraz resistance in the cattle tick *Rhipicephalus microplus*. Proceedings of the National Academy of Sciences 2013;110(42):16772-7. https://doi.org/10.1073/pnas.1309072110

Dang K, Doggett SL, Veera Singham G, Lee CY. Insecticide resistance and resistance

mechanisms in bed bugs, Cimex spp.(Hemiptera: Cimicidae). Parasites & vectors 2017;10(1):318. https://doi.org/10.1186/s13071-017-2232-3

de Mattos IM, Soares AE, Tarpy DR. Effects of synthetic acaricides on honey bee grooming behavior against the parasitic *Varroa destructor* mite. Apidologie 2017;48(4):483-94. https://doi.org/10.1007/s13592-017-0491-9

De Rouck S, İnak E, Dermauw W, Van Leeuwen T. A review of the molecular mechanisms of acaricide resistance in mites and ticks. Insect Biochemistry and Molecular Biology 2023;159:103981. https://doi.org/10.1016/j.ibmb.2023.103981

Dmitryjuk M, Żółtowska K, Frączek R, Lipiński Z. Esterases of *Varroa destructor* (Acari: Varroidae), parasitic mite of the honeybee. Experimental and Applied Acarology 2014;62:499-510. https://doi.org/10.1007/s10493-013-9754-y

Dong K, Du Y, Rinkevich F, Nomura Y, Xu P, Wang L. Molecular biology of insect sodium channels and pyrethroid resistance. Insect Biochemistry and Molecular Biology 2014;50:1-17. https://doi.org/10.1016/j.ibmb.2014.03.012

Dujin T, Jovanović V, Šuvakov D, Milković Z. Effects of extended use of amitraz-based products on the formation of resistant strains of *Varroa jacobsoni*. Veterinarski Glasnik 1991;(45):851-855.

Ellis JD, Evans JD, Pettis J. Colony losses, managed colony population decline, and Colony Collapse Disorder in the United States. Journal of Apicultural Research 2019;49(1):134-6. https://doi.org/10.3896/IBRA.1.49.1.30

Elzaki MEA, Miah MA, Peng Y, Zhang H, Jiang L, Wu M, et al. Deltamethrin is metabolized by CYP6FU1, a cytochrome P450 associated with pyrethroid resistance, in *Laodelphax striatellus*. Pest Management Science 2018;74(6):1265-71. https://doi.org/10.1002/ps.4808; (45)

Elzen PJ, Baxter JR, Spivak M, Wilson WT. Amitraz resistance in varroa: new discovery in North America. American Bee Journal 1999;139:362.

Elzen PJ, Baxter JR, Spivak M, Wilson WT. Control of *Varroa jacobsoni* Oud. resistant to fluvalinate and amitraz using coumaphos. Apidologie. 2000;31(3):437-41. https://doi.org/10.1051/apido:2000134

Erban T, Harant K, Chalupnikova J, Kocourek F, Stara J. Beyond the survival and death of the deltamethrin-threatened pollen beetle *Meligethes aeneus*: an in-depth proteomic study employing a transcriptome database. Journal of Proteomics 2017;150:281-9. https://doi.org/10.1016/j.jprot.2016.09.016

European Commission. Commission Implementing Regulation (EU) No 485/2013 of 24 May 2013 amending Implementing Regulation (EU) No 540/2011 as regards the conditions of approval of the active substances clothianidin, thiamethoxam, and imidacloprid, and prohibiting the use and sale of seeds treated with plant protection products containing those active substances. Official Journal of the European Union. 2013.

Evans JD, Cook SC. Genetics and physiology of *Varroa* mites. Current Opinion in Insect Science 2018;26:130-5. https://doi.org/10.1016/j.cois.2018.02.005

FAO. Guidelines for the management of honey bee diseases and pests. Rome: Food and Agriculture Organization of the United Nations 2014.https://openknowledge.fao.org/server/api/core/bitstreams/3cef7eea-d03-4a7e-0a4-e5c 90f76af10/content (Accessed on 18.09.2026)

Farjamfar M, Saboori A, González-Cabrera J, Hernández Rodríguez CS. Genetic variability and pyrethroid susceptibility of the parasitic honey bee mite *Varroa destructor* (Acari: Varroidae) in Iran. Experimental and Applied Acarology 2018;76:139-48. https://doi.org/10.1007/s10493-018-0296-1

Fernandez N, Omar G. New indications of decrease in the efficiency of the active ingredient fluvalinate. Boletín del Colmenar 1997;4:10-11.

Ferrari S, Silva M, Guarino M, Berckmans D. Monitoring of swarming sounds in bee hives for early detection of the swarming period. Computers and Electronics in Agriculture 2007;64(2):72-7. https://doi.org/10.1016/j.compag.2008.05.010

French-Constant RH, Pittendrigh B, Vaughan A, Anthony N. Why are there so few resistance-associated mutations in insecticide target genes? Philosophical Transactions of the Royal Society B: Biological Sciences 1998;353(1376):1685-93. https://doi.org/10.1098/rstb.1998.0319

Flores KB, Wolschin F, Amdam GV. The role of methylation of DNA in environmental adaptation. Integrative & Comparative Biology 2013; *53*, 359–372. https://doi.org/10.1093/icb/ict019

Gallai N, Salles JM, Settele J, Vaissière BE. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecological Economics

2009;68(3):810-21. https://doi.org/10.1093/icb/ict019

Garbian Y, Maori E, Kalev H, Shafir S, Sela I. Bidirectional transfer of RNAi between honey bee and *Varroa destructor*: *Varroa* gene silencing reduces *Varroa* population. PLoS Pathogens 2012;8(12):e1003035. https://doi.org/10.1371/journal.ppat.1003035

Genath A, Sharbati S, Buer B, Nauen R, Einspanier R. Comparative transcriptomics indicates endogenous differences in detoxification capacity after formic acid treatment between honey bees and varroa mites. Scientific reports 2020;10(1):21943. https://doi.org/10.1038/s41598-020-79057-9

Genersch E. Honey bee pathology: current threats to honey bees and beekeeping. Applied Microbiology and Biotechnology. 2010; 87:87-97. https://doi.org/10.1007/s00253-010-2573-8

Georghiou GP, Taylor CE. Genetic and biological influences in the evolution of insecticide resistance. Journal of Economic Entomology 1977;70(3):319-23. https://doi.org/10.1093/jee/70.3.319

Gerson U, Mozes-Koch R, Cohen E. Enzyme levels used to monitor pesticide resistance in *Varroa jacobsoni*. Journal of Apicultural Research 1991;30(1):17-20. https://doi.org/10.1080/00218839.1991.11101229

Giacobino A, Pacini A, Molineri A, Rodriguez G, Crisanti P, Cagnolo NB, et al. Potential associations between the mite *Varroa destructor* and other stressors in honeybee colonies (*Apis mellifera* L.) in temperate and subtropical climate from Argentina. Preventive Veterinary Medicine 2018;159:143-52. https://doi.org/10.1016/j.prevetmed.2018.09.011

González-Cabrera J, Bumann H, Rodríguez-Vargas S, Kennedy PJ, Krieger K, Altreuther G, et al. A single mutation is driving resistance to pyrethroids in European populations of the parasitic mite, *Varroa destructor*. Journal of Pest Science 2018;91:1137-44. https://doi.org/10.1007/s10340-018-0968-y

Gonzalez-Cabrera J, Davies TE, Field LM, Kennedy PJ, Williamson MS. An amino acid substitution (L925V) associated with resistance to pyrethroids in *Varroa destructor*. PLoS One 2013;8(12):e82941. https://doi.org/10.1371/journal.pone.0082941

González-Cabrera J, Rodríguez-Vargas S, Davies TE, Field LM, Schmehl D, Ellis JD, et al. Novel mutations in the voltage-gated sodium channel of pyrethroid-resistant *Varroa destructor* populations from the Southeastern USA. PLoS One 2016;11(5):e0155332. https://doi.org/10.1371/journal.pone.0155332

Goodwin RM, Taylor MA, McBrydie HM, Cox HM. Base levels of resistance to common control compounds by a New Zealand population of *Varroa destructor*. New Zealand Journal of Crop and Horticultural Science 2005;33(4):347-52. https://doi.org/10.1080/01140671.2005.9514369

Gracia MJ, Moreno C, Ferrer M, Sanz A, Peribáñez MÁ, Estrada R. Field efficacy of acaricides against *Varroa destructor*. PLoS One 2017;12(2):e0171633. https://doi.org/10.1371/journal.pone.0171633

Grbić M, Van Leeuwen T, Clark RM, Rombauts S, Rouzé P, Grbić V, Osborne EJ, Dermauw W, Thi Ngoc PC, Ortego F, Hernández-Crespo P. The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. Nature 2011;479(7374):487-92. https://doi.org/10.1038/nature10640

Han B, Wu J, Wei Q, Liu F, Cui L, Rueppell O, et al. Life-history stage determines the diet of ectoparasitic mites on their honey bee hosts. Nature Communications 2024;15(1):725. https://doi.org/10.1038/s41467-024-44915-x

Hernández-Rodríguez CS, Marín Ó, Calatayud F, Mahiques MJ, Mompó A, Segura I, et al. Large-scale monitoring of resistance to coumaphos, amitraz, and pyrethroids in *Varroa destructor*. Insects 2021;12:27. https://doi.org/10.3390/insects12010027

Hernández-Rodríguez CS, Moreno-Martí S, Almecija G, Christmon K, Johnson JD, Ventelon M, et al. Resistance to amitraz in the parasitic honey bee mite *Varroa destructor* is associated with mutations in the β-adrenergic-like octopamine receptor. Journal of Pest Science 2022;1-17. https://doi.org/10.1007/s10340-021-01471-3

Hernández-Rodríguez CS, Moreno-Martí S, Emilova-Kirilova K, González-Cabrera J. A new mutation in the octopamine receptor associated with amitraz resistance in *Varroa destructor*. Pest Management Science 2025;81(1):308-15. https://doi.org/10.1002/ps.8434

Higes M, Martín-Hernández R, Hernández-Rodríguez CS, González-Cabrera J. Assessing the resistance to acaricides in *Varroa destructor* from several Spanish locations. Parasitology Research 2020;119(11):3595-601. https://doi.org/10.1007/s00436-020-06879-x

Hollingworth RM. Chemistry, biological activity, and uses of formamidine pesticides. Environmental Health Perspectives

1976;14:57-69. https://doi.org/10.1289/ehp.761457

Hubert J, Erban T, Kamler M, Kopecky J, Nesvorna M, Hejdankova S, et al. Bacteria detected in the honeybee parasitic mite *Varroa destructor* collected from beehive winter debris. Journal of Applied Microbiology 2015;119(3):640-54. https://doi.org/10.1111/jam.12899

Hubert J, Nesvorna M, Kamler M, Kopecky J, Tyl J, Titera D, et al. Point mutations in the sodium channel gene conferring tau-fluvalinate resistance in *Varroa destructor*. Pest Management Science 2014;70(6):889-94. https://doi.org/10.1002/ps.3679

Inak E, De Rouck S, Koc-Inak N, Erdem E, Rüstemoğlu M, Dermauw W, Van Leeuwen T. Identification and CRISPR-Cas9 validation of a novel β-adrenergic-like octopamine receptor mutation associated with amitraz resistance in *Varroa destructor*. Pesticide Biochemistry and Physiology 2024;204:106080. https://doi.org/10.1016/j.pestbp.2024.106080

Johnson RM, Dahlgren L, Siegfried BD, Ellis MD. Acaricide, fungicide and drug interactions in honey bees (*Apis mellifera*). PloS One 2013;29;8(1):e54092. https://doi.org/10.1371/journal.pone.0054092

Johnson RM, Dahlgren L, Siegfried BD, Ellis MD. Acaricide, fungicide and drug interactions in honey bees (*Apis mellifera*). PLoS One 2013;8(1):e54092. https://doi.org/10.1371/journal.pone.0054092

Johnson RM, Ellis MD, Mullin CA, Frazier M. Pesticides and honey bee toxicity – USA. Apidologie 2013;41(3):312-31. https://doi.org/10.1051/apido/2010018

Johnson RM, Huang ZY, Berenbaum MR. Role of detoxification in Varroa destructor (Acari: Varroidae) tolerance of the miticide tau-fluvalinate. International Journal of Acarology 2010;36(1):1-6. https://doi.org/10.1080/01647950903468273

Kamler M, Nesvorna M, Stara J, Erban T, Hubert J. Comparison of tau-fluvalinate, acrinathrin, and amitraz effects on susceptible and resistant populations of *Varroa destructor* in a vial test. Experimental and Applied Acarology 2016;69:1-9. https://doi.org/10.1007/s10493-016-0023-8

Kanga LH, Marshall K, Legaspi JC. Mechanisms of insecticide resistance in field populations of the Varroa mite (Acari: Mesostigmata: Varroidae) in Florida. Florida Entomologist 2016;99(2):324-6. https://doi.org/10.1653/024.099.0231

Kim DH, Rossi JJ. RNAi mechanisms and applications. Biotechniques 2008;44(5):613-6. https://doi.org/10.2144/000112792

Kim S, Yoon KA, Cho S, Lee J, Lim Y, Lee SH. Molecular and kinetic properties of three acetylcholinesterases in the Varroa mite, *Varroa destructor*. Pesticide Biochemistry and Physiology 2022;188:105277. https://doi.org/10.1016/j.pestbp.2022.105277

Le Conte Y, Huang ZY, Roux M, Zeng ZJ, Christidès JP, Bagnères AG. *Varroa destructor* changes its cuticular hydrocarbons to mimic new hosts. Biology Letters 2015;11(6):20150233. https://doi.org/10.1098/rsbl.2015.0233

Ledón-Rettig CC, Richards CL, Martin LB. Epigenetics for behavioral ecologists. Behavioral Ecology 2013;24(2):311-24. https://doi.org/10.1093/beheco/ars145

Leza MM, Llado G, Miranda-Chueca MA. Comparison of the efficacy of Apiguard (thymol) and Apivar (amitraz) in the control of *Varroa destructor* (Acari: Varroidae). Spanish Journal of Agricultural Research 2015;13(3):e05SC01.

Lin H, Chuan-hua X, Jin-jun W, Ming L, Wen-cai L, Zhi-mo Z. Resistance selection and biochemical mechanism of resistance to two Acaricides in *Tetranychus cinnabarinus* (Boiduval). Pesticide Biochemistry and Physiology 2009;93(1):47-52. https://doi.org/10.1016/j.pestbp.2008.11.001

Lin Z, Su X, Wang S, Ji T, Hu FL, Zheng HQ. Fumigant toxicity of eleven Chinese herbal essential oils against an ectoparasitic mite (*Varroa destructor*) of the honey bee (*Apis mellifera*). Journal of Apicultural Research 2020;59(2):204-10. https://doi.org/10.1080/00218839.2019.1688493

Liu N. Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. Annual Review of Entomology 2015;60(1):537-59. https://doi.org/10.1146/annurev-ento-10814-020828

Lodesani M, Colombo M, Spreafico M. Ineffectiveness of Apistan® treatment against the mite *Varroa jacobsoni* Oud in several districts of Lombardy (Italy). Apidologie 1995;26(1):67-72. https://doi.org/10.1051/apido:19950109

Lu K, Song Y, Zeng R. The role of cytochrome P450-mediated detoxification in insect adaptation to xenobiotics. Current Opinion in Insect Science 2021;43:103-7. https://doi.org/10.1016/j.cois.2020.11.004

Maggi MD, Ruffinengo SR, Damiani N, Sardella NH, Eguaras MJ. First detection of *Varroa destructor* resistance to coumaphos in Argentina. Experimental and Applied Acarology 2009;47:317-20. https://doi.org/10.1007/s10493-008-9216-0

Maggi MD, Ruffinengo SR, Mendoza Y, Ojeda P, Ramallo G, Floris I, et al. Susceptibility of *Varroa destructor* (Acari: Varroidae) to synthetic acaricides in Uruguay: Varroa mites' potential to develop acaricide resistance. Parasitology Research 2011;108:815-21. https://doi.org/10.1007/s00436-010-2122-5

Maggi MD, Ruffinengo SR, Negri P, Eguaras MJ. Resistance phenomena to amitraz from populations of the ectoparasitic mite *Varroa destructor* of Argentina. Parasitology Research 2010;107:1189-92. https://doi.org/10.1007/s00436-010-1986-8

Marsky U, Rognon B, Douablin A, Viry A, Rodríguez Ramos MA, Hammaidi A. Amitraz Resistance in French *Varroa* Mite Populations—More Complex Than a Single-Nucleotide Polymorphism. Insects 2024;15(6):390. https://doi.org/10.3390/insects15060390

Martin SJ. Acaricide (pyrethroid) resistance in *Varroa destructor*. Bee World 2004;85(4):67-9.https://doi.org/10.1080/0005772X.2004.11099632

Mathieu L, Faucon JP. Changes in the response time for *Varroa jacobsoni* exposed to amitraz. Journal of Apicultural Research 2000;39(3-4):155-8. https://doi.org/10.1080/00218839.2000.11101036

Mendes EC, Mendes MC, Sato ME. Diagnosis of amitraz resistance in Brazilian populations of *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) with larval immersion test. Experimental and Applied Acarology 2013;61:357-69. https://doi.org/10.1007/s10493-013-9697-3

Millán-Leiva A, Hernández-Rodríguez CS, González-Cabrera J. New PCR–RFLP diagnostics methodology for detecting *Varroa destructor* resistant to synthetic pyrethroids. Journal of Pest Science 2018;91:937-41.

Millán-Leiva A, Marín Ó, Christmon K, vanEngelsdorp D, González-Cabrera J. Mutations associated with pyrethroid resistance in *Varroa* mite, a parasite of honey bees, are widespread across the United States. Pest Management Science 2021;77(7):3241-9. https://doi.org/10.1007/s10340-018-0964-2

Minakuchi C, Namiki T, Shinoda T. Krüppel homolog 1, an early juvenile hormone-response gene downstream of Methoprene-tolerant, mediates its anti-metamorphic action in the red flour beetle *Tribolium castaneum*. Developmental Biology 2009;325:341-50. https://doi.org/10.1002/ps.6366

Mitton GA, Quintana S, Gimenez Martinez P, Mendoza Y, Ramallo G, Brasesco C, et al. First record of resistance to flumethrin in a varroa population from Uruguay. Journal of Apicultural Research 2016;55(5):422-7. https://doi.org/10.1080/00218839.2016.1257238

Morawetz L, Köglberger H, Griesbacher A, Derakhshifar I, Crailsheim K, Brodschneider R, et al. Health status of honey bee colonies (*Apis mellifera*) and disease-related risk factors for colony losses in Austria. PLoS One 2019;14(7):e0219293. https://doi.org/10.1371/journal.pone.0219293

Mozes-Koch R, Slabezki Y, Efrat H, Kalev H, Kamer Y, Yakobson BA, et al. First detection in Israel of fluvalinate resistance in the varroa mite using bioassay and biochemical methods. Experimental and Applied Acarology 2000;24:35-43. https://doi.org/10.1023/A:1006379114942

Muthusamy R, Ramkumar G, Karthi S, Shivakumar MS. Biochemical mechanisms of insecticide resistance in field population of Dengue vector *Aedes aegypti* (Diptera: Culicidae). International Journal of Mosquito Research 2014;1(2):1-4.

Namountougou M, Simard F, Baldet T, Diabaté A, Ouédraogo JB, Martin T, et al. Multiple insecticide resistance in *Anopheles gambiae* sl populations from Burkina Faso, West Africa. PLoS One 2012;7(11):e48412. https://doi.org/10.1371/journal.pone.0048412

Nazzi F, Le Conte Y. Ecology of *Varroa destructor*, the major ectoparasite of the western honey bee, *Apis mellifera*. Annual Review of Entomology 2016;61:417-32. https://doi.org/10.1146/annurev-ento-10715-023731

Obaid MK, Islam N, Alouffi A, Khan AZ, da Silva Vaz Jr I, Tanaka T, et al. Acaricides resistance in ticks: selection, diagnosis, mechanisms, and mitigation. Frontiers in Cellular and Infection Microbiology 2022;12:941831. https://doi.org/10.3389/fcimb.2022.941831

Papežíková I, Palíková M, Kremserová S, Zachová A, Peterová H, Babák V, et al. Effect of oxalic acid on the mite *Varroa destructor* and its host the honey bee *Apis mellifera*. Journal of Apicultural Research 2017;56(4):400-8. https://doi.org/10.1080/00218839.2017.1327937

Pettis JS, Delaplane KS. Coordinated responses to honey bee decline in the USA. Apidologie 2010;41(3):256-63. https://doi.org/10.1051/apido/2010013

Pietropaoli M, Formato G. Liquid formic acid 60% to control varroa mites (*Varroa destructor*) in honey bee colonies (*Apis mellifera*): protocol evaluation. Journal of Apicultural Research 2018;57(2):300-7. https://doi.org/10.1080/00218839.2017.1376767

Pohorecka K, Kiljanek T, Antczak M, Skubida P, Semkiw P, Posyniak A. Amitraz marker residues in honey from honeybee colonies treated with Apiwarol. Journal of Veterinary Research 2018;62(3):297-301. https://doi.org/10.2478/jvetres-2018-0043

Prullage JB, Tran HV, Timmons P, Harriman J, Chester ST, Powell K. The combined mode of action of fipronil and amitraz on the motility of *Rhipicephalus sanguineus*. Veterinary Parasitology 2011;179(4):302-10. https://doi.org/10.1016/j.vetpar.2011.03.041

Puranik SI, Akbar A, Ghagane SC. Economic benefits of honey and honey products. In: Honey: Composition and health benefits. 2023. John Wiley & Sons, Ltd.: Hoboken, NJ, USA 2023. pp. 330-339, https://doi.org/10.1002/9781119113324.ch24

Qin G, Jia M, Liu T, Xuan T, Yan Zhu K, Guo Y, et al. Identification and characterisation of ten glutathione S-transferase genes from oriental migratory locust, *Locusta migratoria* (Meyen). Pest Management Science 2011;67(6):697-704. https://doi.org/10.1002/ps.2110

Ramsey SD, Ochoa R, Bauchan G, Gulbronson C, Mowery JD, Cohen A, et al. *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. Proceedings of the National Academy of Sciences 2019;116(5):1792-801. https://doi.org/10.1073/pnas.181837111

Rangel J, Ward L. Evaluation of the predatory mite *Stratiolaelaps scimitus* for the biological control of the honey bee ectoparasitic mite *Varroa destructor*. Journal of Apicultural Research 2018;57(3):425-32. https://doi.org/10.1080/00218839.2018.1457864

Reinbacher L, Ferrari MF, Angeli S, Schausberger P. Effects of *Metarhizium anisopliae* on host choice of the bee-parasitic mite *Varroa destructor*. Acarologia 2018;58(2):287-95. https://doi.org/10.24349/acarologia/20184241

Rinkevich FD. Detection of amitraz resistance and reduced treatment efficacy in the Varroa Mite, *Varroa destructor*, within commercial beekeeping operations. PLoS One 2020;15(1):e0227264. https://doi.org/10.1371/journal.pone.0227264

Rivero A, Vezilier J, Weill M, Read AF, Gandon S. Insecticide control of insect vectors: where are we going? PLoS Pathogens 2010;6(8):e1001000. https://doi.org/10.1371/journal.ppat.1001000

Rodríguez-Dehaibes SR, Otero-Colina G, Sedas VP, Jiménez JAV. Resistance to amitraz and flumethrin in *Varroa destructor* populations from Veracruz, Mexico. Journal of Apicultural Research 2005;44(3):124-5. https://doi.org/10.1080/00218839.2005.11101162

Rodriguez-Vivas RI, Jonsson NN, Bhushan C. Strategies for the control of *Rhipicephalus microplus* ticks in a world of conventional acaricide and macrocyclic lactone resistance. Parasitology Research 2018;117:3-29. https://doi.org/10.1007/s00436-017-5677-6

Rosenkranz P, Aumeier P, Ziegelmann B. Biology and control of *Varroa destructor*. Journal of Invertebrate Pathology 2010;103:S96-S119. https://doi.org/10.1016/j.jip.2009.07.016

Roth MA, Wilson JM, Gross AD. Assessing *Varroa destructor* acaricide resistance in *Apis mellifera* colonies of Virginia. Apidologie 2021;1-13. https://doi.org/10.1007/s13592-021-00901-6

Sammataro D, Untalan P, Guerrero F, Finley J. The resistance of varroa mites (Acari: Varroidae) to acaricides and the presence of esterase. International Journal of Acarology 2005;31(1):67-74. https://doi.org/10.1080/01647950508684419

Sanford MT. Introduction, spread and economic impact of *Varroa* mites in North America. In: Mites of the honey bee. Hamilton: Dadant and Sons; 2001. p. 149-62.

Santiago GP, Otero-Colina G, Sánchez DM, Guzmán MER, Vandame R. Comparing effects of three acaricides on *Varroa jacobsoni* (Acari: Varroidae) and *Apis mellifera* (Hymenoptera: Apidae) using two application techniques. Florida Entomologist 2000;468-76. https://doi.org/10.2307/3496722

Shakesby AJ, Wallace IS, Isaacs HV, Pritchard J, Roberts DM, Douglas AE. A water-specific aquaporin involved in aphid osmoregulation. Insect Biochemistry and Molecular Biology 2009;39:1-10. https://doi.org/10.1016/j.ibmb.2008.08.008

Shehzad M, Bodlah I, Siddiqui JA, Bodlah MA, Fareen AGE, Islam W. Recent insights into pesticide resistance mechanisms in *Plutella xylostella* and possible management strategies. Environmental Science and Pollution Research 2023;30(42):95296-311. https://doi.org/10.1007/s11356-023-29271-5

Siddiqui JA, Fan R, Naz H, Bamisile BS, Hafeez M, Ghani MI, et al. Insights into insecticide-resistance mechanisms in invasive species: Challenges and control strategies. Frontiers in Physiology 2023;13:1112278. https://doi.org/10.3389/fphys.2022.1112278

Sparks TC, Storer N, Porter A, Slater R, Nauen R, McGaughey W. Insecticide resistance management and industry: The origins and evolution of the Insecticide Resistance Action

Committee (IRAC) and the mode of action classification scheme. Pest Management Science 2021;77(6):2609-19. https://doi.org/10.1002/ps.6254

Spreafico M, Eördegh FR, Bernardinelli I, Colombo M. First detection of strains of *Varroa destructor* resistant to coumaphos. Results of laboratory tests and field trials. Apidologie 2001;32(1):49-55. https://doi.org/10.1051/apido:2001110

Stahlmann-Brown P, Hall RJ, Pragert H, Robertson T. *Varroa* appears to drive persistent increases in New Zealand colony losses. Insects 2022;13(7):589. https://doi.org/10.3390/insects13070589

Stara J, Pekar S, Nesvorna M, Erban T, Vinsova H, Kopecky J, Doskocil I, Kamler M, Hubert J. Detection of tau-fluvalinate resistance in the mite *Varroa destructor* based on the comparison of vial test and PCR–RFLP of kdr mutation in sodium channel gene. Experimental and Applied Acarology 2019; 77(2):161-71. https://doi.org/10.1007/s10493-019-00353-9

Steenberg T, Kryger P, Holst N. A scientific note on the fungus Beauveria bassiana infecting *Varroa destructor* in worker brood cells in honey bee hives. Apidologie 2010;41(1):127-8. https://doi.org/10.1051/apido/2009057

Strachecka A, Borsuk G, Olszewski K, Paleolog J, Lipiński Z. Proteolysis on the body surface of pyrethroid-sensitive and resistant *Varroa destructor*. Acta Parasitologica 2013;58(1):64-9. https://doi.org/10.2478/s11686-013-0109-y

Strachecka A, Borsuk G, Olszewski K, Paleolog J. A new detection method for a newly revealed mechanism of pyrethroid resistance development in *Varroa destructor*. Parasitology Research 2015;114(11):3999-4004. https://doi.org/10.1007/s00436-015-4627-4

Thompson HM, Brown MA, Ball RF, Bew MH. First report of Varroa destructor resistance to pyrethroids in the UK. Apidologie 2002;33(4):357-66. https://doi.org/10.1051/apido:2002027

Tlak Gajger I, Svečnjak L, Bubalo D, Žorat T. Control of Varroa destructor mite infestations at experimental apiaries situated in Croatia. Diversity 2019;12(1):12. https://doi.org/10.3390/d12010012

Traynor KS, Mondet F, de Miranda JR, Techer M, Kowallik V, Oddie MA, Chantawannakul P, McAfee A. Varroa destructor: A complex parasite, crippling honey bees worldwide. Trends in parasitology 2020; 36(7):592-606. https://doi.org/ 10.1016/j.pt.2020.04.004

Trueman JW, Anderson DL. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Experimental & Applied Acarology 2000 Mar;24(3):165-89. https://doi.org/10.1023/A:1006456720416

Valentine A, Martin SJ. A survey of UK beekeeper's Varroa treatment habits. Plos One 2023; 18(2):e0281130. https://doi.org/10.1371/journal.pone.0281130

Vandervalk LP, Nasr ME, Dosdall LM. New miticides for integrated pest management of *Varroa destructor* (Acari: Varroidae) in honey bee colonies on the Canadian prairies. Journal of Economic Entomology 2014;107(6):2030-6. https://doi.org/10.1603/EC14048

Van-Engelsdorp D, Caron D, Hayes J, Underwood R, Henson M, Rennich K, Spleen A, Andree M, Snyder R, Lee K, Roccasecca K. A national survey of managed honey bee 2010–11 winter colony losses in the USA: results from the Bee Informed Partnership. Journal of Apicultural Research 2012;51(1):115-24. https://doi.org/10.3896/IBRA.1.51.1.14

Vlogiannitis S, Mavridis K, Dermauw W, Snoeck S, Katsavou E, Morou E, Harizanis P, Swevers L, Hemingway J, Feyereisen R, Van Leeuwen T. Reduced proinsecticide activation by cytochrome P450 confers coumaphos resistance in the major bee parasite Varroa destructor. Proceedings of the National Academy of Sciences 2021;118(6): e2020380118. https://doi.org/10.1073/pnas.2020380118

Vu PD, Rault LC, Jenson LJ, Bloomquist JR, Anderson TD. Voltage-gated chloride channel blocker DIDS as an acaricide for Varroa mites. Pesticide Biochemistry and Physiology 2020;167:104603. https://doi.org/10.1016/j.pestbp.2020.104603

Vu PD. Toxicological analysis of acaricides for Varroa mite management Doctoral dissertation, Virginia Tech. Blacksburg, VA, USA, 2016, (Access date: February 19, 2016).

Walshe DP, Lehane SM, Lehane MJ, Haines LR: Prolonged gene knockdown in the tsetse fly Glossina by feeding double-stranded RNA. Insect Molecular Biology 2009;18:11-19. https://doi.org/10.1111/j.1365-2583.2008.00839.x

Wang R, Huang ZY, Dong KE. Molecular characterization of an arachnid sodium channel gene from the varroa mite (*Varroa destructor*). Insect Biochemistry and Molecular Biology 2003;33(7):733-9. https://doi.org/10.1016/S0965-1748(03)00068-7

Wang R, Liu Z, Dong K, Elzen PJ, Pettis J, Huang Z. Association of novel mutations in a sodium

channel gene with fluvalinate resistance in the mite, *Varroa destructor*. Journal of Apicultural Research 2002;41(1-2):17-25. https://doi.org/10.1080/00218839.2002.11101064

Wilfert L, Long G, Leggett HC, Schmid-Hempel P, Butlin R, Martin SJ, Boots M. Deformed wing virus is a recent global epidemic in honeybees driven by Varroa mites. Science 2016;351(6273):594-7. https://doi.org/10.1126/science.aac99

Xia J, Han L, Zhao Z. Investigating the relationship of DNA methylation with mutation rate and allele frequency in the human genome. BMC Genomics 2012;13(Supplement 8):S7. https://doi.org/10.1186/1471-2164-13-S8-S7

Zhan Y, Fan S, Zhang M, Zalom F. Modelling the effect of pyrethroid use intensity on mite population density for walnuts. Pest Management Science 2015;71(1):159-64. https://doi.org/10.1002/ps.3799