

### Understanding Honey Bee (*Apis mellifera*) Colony Losses: A Multifactorial Perspective

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#### **ABSTRACT**

Bees play an essential role as pollinators of crops and wild plants thereby contributing to biodiversity. Beekeeping is an important economic and agricultural activity. However, beekeepers are faced with numerous external factors, including climate change, intensive agricultural production, the extensive use of pesticides and the high incidence of honey bee diseases. In researches' work to date, numerous potential factors have been identified that contribute to colony population decline, reduced colony health, and colony losses. This review summarizes the most important factors affecting honey bee colonies and their health. The main causes of colony loss include pests and pathogens, environmental and beekeeping stressors, apiculture practices and pesticide residues. Reducing honey bee colony losses requires an integrated approach that recognises the multifactorial nature of the problem. Coordinated strategies should consider both direct biological threats, such as parasites and pathogens, and indirect influences, such as environmental changes, pesticide exposure and forage quality.

Keywords: Apis mellifera, pesticides, pathogens, climate change, beekeeping management

#### **INTRODUCTION**

Animal-based pollination contributes to 30% of global food production, and bee-pollinated crops, contribute to approximately one-third of the total human dietary supply. Bees are significant pollinators, because of their effectiveness and widespread occurrence. They contribute to the biodiversity of plants in nature (Khalifa et al., 2021). Honey bee (*Apis mellifera* Linnaeus, 1758) is the most frequent floral visitor in natural habitats worldwide. By pollinating crops and wild plants, bees play an essential role in food production and the conservation of biodiversity in ecosystems (Hung et al., 2018; Beaurepaire et al., 2020; Marín-García et al., 2022).

One of the branches of agriculture is beekeeping, which involves the breeding of honey bees (*A. mellifera*) that produce and utilise various products of the hive, such as

royal jelly, wax, propolis, pollen, and venom, the most important of which is honey. Beekeeping is significant economic or agricultural activity, as it enables the production of numerous crops that are important for the production of food for animals and humans (Marín-García et al., 2022; Kady et al., 2021). A total of 11 honey bee species have been identified. The most widely managed and globally distributed species is the Western honey bee, *A. mellifera* (Mduda et al., 2025). The annual loss of bee colonies is primarily due to the spread of non-native pathogens and pests, as well as problems with hive management. Scientists should focus primarily on the impact of pests, diseases, and management-related issues (Smith et al., 2013; Tlak Gajger et al., 2019).

Honey bee colony mortality occurs in all countries with a developed beekeeping industry, e.g. in Poland, where colony mortality is above the acceptable level, which is reported as 10% (Topolska et al., 2018; Mazur et al., 2022). The death of bee colonies has been reported in many western countries. Very high-profile reports of the death of colonies come from the USA (vanEngelsdorp et al., 2009). Winter mortality of honey bee colonies in Latin America averages 20.6%, which is between the levels reported in Europe, where losses are generally lower (12.5%), and the United States, where higher levels are reported (40.4%) (Van Der Zee et al., 2012; Goulson et al., 2015a; Requier et al., 2024). In Slovenia, the death of bee colonies is also being observed. In some areas, beekeepers have detected the death of colonies in early autumn, and later. We do not yet know the final state of wintering success in recent years. However, the consequences of the death of colonies will certainly be noticeable in the natural environment. There are well-known observations from fruit growers and nature observers, when during the flowering period of fruit trees in some areas there were no bees to pollinate the flowers. Indicators that contribute substantially to managed honey bee colonies are reduced floral resources (Clermont et al., 2015; Kuchling et al., 2018), inadequate beekeeping management (Gray et al., 2019) and climate changes (Switanek et al., 2017; Overturf et al., 2022).

This manuscript provides an overview of the major drivers of honey bee colony losses, with a focus on pests and pathogens, environmental stressors, apiculture practices and pesticide use.

## MULTIPLE STRESSORS THAT AFFECT HONEY BEES

In the activities of researchers so far, numerous possible causes have been "recorded" that contribute to the reduction of colonies population, reduced colonies health and the death of the colony (Christen, 2023).

One of the main concerns in modern agriculture is a use of pesticides and their degradation products (Gradish et al., 2019). Bees can be exposed to a combination of pesticide compounds in agricultural fields. Thirty-two different pesticides belonging to nine chemical classes have been found in beehives (Mullin et al., 2010). Synergistic combinations of pesticides, neonicotinoids and fungicides have also been identified (Almasri et al., 2020; Rondeau & Raine, 2022).

Another factor affecting honey bee colonies is the direct and indirect damage caused by varroa mites. The negative effects of varroa parasitism on the brood or adult bees are well known. Parasitism causes reduced bee weight, lower protein content, and other consequences, and indirectly causes reduced bee resistance to various pathogens. Secondary infections, including viruses, play a very important role. Viruses are the hidden enemies of honey bees, as compared to other pathogens, because most of the viral infections pass without clinical manifestation of

characteristic disease signs. It was also demonstrated that viral infections in honey bee colonies are one of the main source of septicity and considered as a key risk for their health (Ullah et al., 2021). In the United States, colony losses are reported with varroa mites playing a major role in the loss (Traynor et al., 2020; Insolia et al., 2022). Emerging diseases and pests are also a problem; e.g. Nosema ceranae and the small hive beetle. The increasing spread of exotic pathogens could be a factor in the worldwide occurrence of diseases, and it is due to the greater connection and globalisation (Crowl et al., 2008).

Reducing the diversity of plant species in nature, especially in agricultural areas, is another critical component of honey bee health. In most agricultural activities, the intensity of processing and production is increasing, and biodiversity in the natural environment is also reportedly decreasing (Brittain et al., 2013).

Honey bee colony mortality is associated with increase in pests and diseases, as well as anthropogenic activities such as the use of agrochemicals, habitat loss, and climate change (Potts et al., 2010; Paudel et al., 2015). Researchers therefore assume that several factors are usually required for colony death. To date, numerous studies have been carried out on the effect of various factors on an individual bee or on the entire bee colony (Mullin et al., 2010; Brittain et al., 2013; Gradish et al., 2019; Jacques et al., 2017). Below, several factors that are widely discussed in both research and beekeeping communities are outlined.

#### PESTS AND PATHOGENS

#### Varroa

The ectoparasitic mite *Varroa destructor* is on the global level, the major factor contributing to the loss of honey bee colonies. Therefore, the fight against *V. destructor* should be a priority in current honey bee health research. Varroa mites are causing disease called varroosis. Mites reproduce in the brood cells of developing bees (Korená Hillayová et al., 2022; Tlak Gaiger et al., 2019).

Early detection and accurate assessment of infection levels are key to effective varroa control (Gregorc & Sampson, 2019). Counting the mites that fall naturally onto the bottom board is a common approach, as it correlates well with the overall mite burden in a colony (Ritter, 1981). 'Sugar shake' is another simple, non-destructive technique for sampling varroa mites. In this method, icing sugar is sprinkled on the bodies of the living bees. The icing sugar causes the varroa mites to lose their adhesion and detach themselves permanently from the host bees (Fakhimzadeh et al., 2011). Another method can be used with water or alcohol (70%) (Toufailia et al., 2014). Depending on how many varroa mites are detected using the above methods, the use of miticides

or other treatments may be necessary. Regular assessment of the mite infestation in the colonies will determine the right time for control. Organic acids and essential oils have become widely used due to their effectiveness and low impact on hive products. Thymol, a compound found in thyme, is used in products such as Apiguard® and Thymovar®, which have proven to be very effective in reducing mite populations without affecting the quality of the honey (Fassbinder et al., 2002).

Formic acid is another widely used organic treatment, its performance can be influenced by environmental conditions such as temperature (Fries, 1991). Oxalic acid proves to be particularly effective in colonies without brood, where its application can lead to an almost complete elimination of mites (Gregorc & Planinc, 2001). HopGuard®, which is based on beta acids from hops, has shown a rapid effect and is effective at killing varroa mites in colonies with open and sealed brood (Degrandi-Hoffman et al., 2012; Rademacher et al., 2015). Grooming and hygienic behaviour within bee colonies are also important factors in containing the infestation of varroa mites (Macedo et al., 2002; Spivak & Gilliam, 1998). In Austria, the management of V. destructor remains a major challenge for beekeepers, and further research is recommended to better understand management practices and improve treatment effectiveness (Oberreiter and Brodschneider, 2020).

The efficacy of five authorised veterinary medicinal products in the control of *V. destructor* mite infestations was evaluated in honey bee colonies in five experimental apiaries in different regions of Croatia, during the 2014 beekeeping season. The examined treatments were CheckMite+ (with coumaphos), VMP-Bayvarol (flumethrin), Apiguard and Thymovar (both thymol-based), and ApiLife Var, (combination of thymol, eucalyptus oil, levomenthol, and camphor). The results of their study showed that CheckMite+ was consistently the most effective treatment. In contrast, thymol-based products were less reliable, particularly in different climatic conditions. Overall treatment efficacy differed between locations, influenced by environmental factors, colony strength, and timing of treatment (Tlak Gajger et al., 2019).

Additionally, biotechnical methods such as drone brood removal — where drone combs are introduced, then removed and destroyed before the mites can emerge — and interrupting summer brood by caging the queen or splitting the colony have been shown to be effective in reducing varroa pressure. These methods interrupt the mite's reproductive cycle and help to reduce the need for chemical treatments. However, they are intensive, require careful timing, and can reduce short-term productivity, which can make them less practical for large-scale or less experienced beekeepers (Gregorc and Sampson, 2019). Integrated pest management strategies, which combine multiple mite control techniques such as monitoring, organic treatments,

and biotechnical interventions, are recommended as a sustainable approach to maintain colony health (Jack and Ellis, 2021).

#### Viruses

Clinically visible symptoms of honey bee virus diseases are mostly associated with another infectious agent, like the presence of microsporidia *N. apis* and strong infestations with *V. destructor* mites (Evans & Spivak, 2010). There have been at least more than 20 viruses identified to infect honey bees worldwide, generally from *Dicistroviridae* as well as *Iflaviridae* families, like ABPV (Acute Bee Paralysis Virus), BQCV (Black Queen Cell Virus), KBV (Kashmir Bee Virus), SBV (Sacbrood Virus), CBPV (Chronic bee paralysis virus), SBPV (Slow Bee Paralysis Virus) along with IAPV (Israeli acute paralysis virus), DWV (Deformed Wing Virus) (Ullah et al., 2020).

The *V. destructor* mite acts as a vector for harmful viruses (Gisder et al., 2009), most notably the DWV, which causes visible symptoms associated with colony losses. In addition, three other viruses ABPV, IAPV, KBV, become more virulent when spread by *V. destructor* and are all known to contribute to colony decline in areas where *V. destructor* is present (Smith et al., 2013).

#### Vespa

Another predator that threatens honey bee colonies is *Vespa velutina*. This species is considered native to northern India, southern China, and the western part of Indonesia (Lima et al., 2022). It is spreading in Europe, as it does not have many predators or competitors. Its spread is favoured by the high reproduction rate, regional temperatures and precipitation levels. It also benefits from the abundant availability of food, and its life cycle supports its successful introduction and spread into new habitats (Flores et al., 2019).

V. velutina has been included on the European Union list of Invasive Alien Species of Union concern, because it causes significant damage to the environment and beekeeping activities (Kishi & Goka, 2017). Hornets typically attack honey bees as they return to the hive, catching them mid-air at the hive entrance. They kill the bees using their jaws, remove the protein-rich thorax, and carry it back to their nest as food for their larvae. The intense predation by *V. velutina* can weaken honey bee colonies and lead to their collapse, as foraging is disrupted and returns to the hive become increasingly rare (Requier et al., 2019). This not only results in economic losses in beekeeping, but also negatively affects pollination services in the ecosystem (Laurino et al., 2019). Strategies for controlling *V. velutina* are focussed on detecting and destroying nests. However, locating nests is a challenge, especially if they are hidden in foliage or built in high, inaccessible places. Once nests are found, they must be

completely eradicated, to ensure that the queen, brood, and most workers are eliminated to prevent recolonization (Feás Sánchez & Charles, 2019).

Leza et al. (2019) discovered that the presence of *V. velutina* in honey bee colonies has negative impact on honey bee health and on the activation of their antioxidant system to protect themselves against this biotic stressor. Their study showed increased levels of mRNA expression of oxidative stress-related genes (od2, tpx3, trxR1, gtpx1, gstS1, coxI, cytC and if2mt), increased enzymatic catalase activity and an increase in lipid peroxidation in *V. velutina* positive samples.

#### Nosema

A different problem that is affecting honey bee colonies is nosemosis. It is caused by two species of microsporidian parasites, Nosema apis and N. ceranae. More common and more lethal is N. ceranae. Nosema infection can have serious negative effects on honey bee colonies, including reduced foraging activity, decreased colony growth and productivity. It can lead to physiological and behavioural changes, weaken their immune system and decrease bee longevity. N. ceranae has also been directly linked to the loss of honey bee colonies. The antibiotic fumagillin was previously the only authorised antibiotic used to treat nosemosis. Research aimed at finding alternatives to fumagillin has shown that probiotics, propolis, various plant extracts, royal jelly, essential plant oils and similar substances, can reduce the production of nosema spores in honey bees and improve the survival of infected bees (Chaimanee et al., 2021; Kim et al.,

In a study that was held at University of Belgrade, Faculty of Veterinary Medicine, they were testing Agaricus bisporus mushroom extract. The extracts were tested on bees in a laboratory experiment. The extract from A. bisporus was found to have both anti-nosema and immunoprotective effect (Glavinic et al., 2021). In a study, Kim et al. (2023) selected the most effective entomopathogenic mushroom culture extracts to combat nosemosis. They showed that all 342 fungal culture extracts had an inhibitory effect, with the exception of 2 genera and 1 fungal species. The high inhibitory activity of about 80% or more was also demonstrated in fungal isolates of 15 species and 10 genera. The treatments with culture extracts of Paecilomyces marquandii 364 and Pochonia bulbillosa 60 were effective in reducing the mortality of honey bees caused by nosemosis. The extracts of these two fungal isolates also increased the survival of honey bees.

#### **ENVIRONMENTAL STRESSORS**

Honey bee colony losses are caused by a complex interaction of biotic and abiotic stress factors. Throughout history, bees experienced multiple extinction events and unexplained population declines. One of the earliest known cases occurred during the transition from the Cretaceous to the Palaeogene, about 65.5 million years ago (Schulte et al., 2010). This mass extinction, geologically characterised by the K-Pg boundary, had a significant impact on the Xylocopinae bee lineage. Genetic analyses (COI, Cytb, EF-1 $\alpha$  genes) suggest that the collapse of plant-insect relationships during this time - likely due to catastrophic climate change negatively impacted bee survival (Rehan et al., 2013). Plant diversity, flower shapes, abundance, and the quality and quantity of nectar and pollen (Venjakob et al., 2022) have an impact on honey bee foraging behaviour, colony health, overall honey production, and its quality (Somme et al., 2015; Nicolson et al., 2022). There are differences in the foraging behaviour of bees. They visit nectar-rich flowers and collect a greater amount of nectar and produce higher honey yields (Nicolson et al., 2022).

In medieval Europe, historical records also document repeated episodes of bee mortality. The Annals of Ulster mention bee mortality in Ireland in 951 and again in 992, at a time characterised by extreme weather conditions, fungal crop diseases (ergot), and famine (Fleming, 1871). In 1035, Bavaria experienced a severe decline in bee populations following an unusually cold summer that destroyed flowering crops. These cases illustrate a recurring pattern: unfavourable weather and reduced floral resources lead to the collapse of bee colonies. The bee mortality continued in modern times. The first bees introduced to North America in 1621 initially thrived, but after 1670, managed colonies began to decline, likely due to disease (Pellett, 1938). In 1903, Utah lost 2,000 colonies to a mysterious "disappearing disease" (Critchlow, 1904), and in the 1990s, over half of Pennsylvania's colonies vanished without clear cause (Finley et al., 1996).

A particularly devastating case occurred on the Isle of Wight between 1905 and 1919, where three epidemics wiped out 90% of the bees on the island. The crisis was eventually attributed to a combination of the tracheal mite (*Acarapis woodi*), *N. apis*, and the CBPV, exacerbated by poor climate conditions that limited the availability of flowers (Bailey, 1964). These historical examples show that bee losses due to environmental stressors are not a new phenomenon, but a recurring pattern.

Habitat change is another critical stress factor. While habitat loss typically has a negative impact, studies from Indonesia have shown that forested environments rich in honeydew can improve colony performance (Buchori et al., 2019).

Two new stress factors — climate change and invasive plant species—present further challenges. In addition, there are subspecies-specific sensitivities and certain beekeeping practises, such as queen replacement strategies, that can unintentionally increase overwintering mortality (Switanek et al., 2017; Suchail, et al., 2000).

The issue is further complicated by the use of processed sugar products in beekeeping, which may contain harmful compounds such as hydroxymethylfurfural (HMF) (Bailey, 1964). A laboratory study examined the toxicity of hydroxymethylfurfural (HMF), a compound formed in overheated or poorly stored sugar syrups, on caged worker honey bees. Bees fed with HMF-contaminated syrup showed increased mortality and behavioural impairments such as reduced feeding and uncoordinated movement. The results indicate that even moderate HMF levels found in commercial bee feed can be harmful, underscoring the need for careful feed management and quality control in beekeeping (Gregorc and Sampson, 2019; LeBlanc et al., 2009).

Environmental factors further compound the problem. Data from Austria, for example, indicate that bee colonies foraging in maize-dominated landscapes experienced significantly higher winter losses — likely due to exposure to pesticide-contaminated pollen and guttation water, as well as poor nutritional value (Höcherl et al., 2012; Urbanowicz et al., 2019). Similarly, feed dominated by melezitose-producing honeydew has been associated with digestive disorders and higher loss rates (Gregorc, 2020).

In Austria, the loss rate of bee colonies in the winter of 2018/2019 was average compared to previous years (Brodschneider et al., 2019). Most beekeepers (69.7%) recorded no or low losses (under 20%), which indicates that many were able to replenish the lost colonies independently in the following summer season (Brodschneider et al., 2010). Long-term analyses show that winters with high losses are typically followed by an increase in new colonies, while winters with low losses result in fewer new colonies in the following season (Brodschneider et al., 2019).

Regional differences in the loss rates of bee colonies exist not only in Austria, but also in countries such as the Czech Republic (Brodschneider et al., 2019). These differences are often attributed to landscape and climate differences, both of which influence honey bee colony development and winter mortality (Kuchling et al., 2018; Van Esch et al., 2020). It is also known that weather effects influence the survival of bee colonies (Switanek et al., 2017). Altitude also appears to play a role: bee colonies that overwintered at an altitude of over 600 meters showed lower loss rates, possibly due to colder temperatures, different landscapes, or lower

transmission of pathogens at higher altitudes (Switanek et al., 2017; Kuchling et al., 2018; Forfert et al., 2016; Dynes et al., 2019).

#### APICULTURE PRACTICES

Queen problems, a critical factor for colony losses, accounted for 3.6-4.4% of total losses, which is comparable to the results from other COLOSS countries (Gray et al., 2019; Brodschneider et al., 2019). Only a minority of beekeepers (7.8%) reported more queen issues than usual during the foraging season. A higher incidence of queen problems was linked with greater winter losses, which is consistent with findings from the USA, where colonies with signs of emergency or supersedure queen cells had lower survival rates (Tarpy et al., 2013). Factors contributing to queen problems may include exposure to neonicotinoids (Williams et al., 2015) and the logistics of queen transport (Withrow et al., 2019). The age of the queen bee going into winter also has a significant impact older queens reduce the likelihood of colony survival (Genersch et al., 2010; Morawetz et al., 2019; Zee et al., 2014; Giacobino et al., 2016). Beekeepers who replaced more than 25% of their queens with younger ones reported significantly fewer losses (Genersch et al., 2010; Zee et al., 2014; Amiri et al., 2017; Ricigliano et al., 2018), highlighting the benefit of maintaining young, well-mated queens to build strong overwintering colonies (Amiri et al., 2017; Ricigliano et al., 2018). The annual replacement of gueens appears to be a practical strategy to reduce colony loss rates (Genersch et al., 2010; Giacobino et al., 2016).

Beekeeping practises have a direct impact on survival rates and are largely under the control of the individual beekeeper (Jacques et al., 2017). Migratory operations experienced lower losses than stationary ones, possibly because migratory beekeepers are more experienced or their colonies have access to better forage sources (Zee et al., 2014). Smaller-scale beekeepers tended to have higher losses, which could be due to a lack of training or experience compared to those managing more colonies (Morawetz et al., 2019; Brodschneider et al., 2010; Gray et al., 2019; Castilhos et al., 2019).

The purchase of wax from external sources correlated with higher loss rates, likely because pesticides and pathogens can accumulate in beeswax over time (Calatayud-Vernich et al., 2019; Harriet et al., 2017; Calatayud-Vernich et al., 2018). However, some studies show that contaminated wax does not directly affect the survival of bee colonies (Payne et al., 2019).

Colony collapse disorder (CCD) is a pathological condition marked by the sudden disappearance of adult worker bees from a hive, while the queen and brood (eggs, larvae, prepupae, and pupae) remain alive. A characteristic feature is that the bees leave the hive and do not return, with no corpses found inside or nearby—excluding acute poisoning

as a likely cause (vanEngelsdorp et al., 2009; vanEngelsdrop et al. 2010; Goulson et al., 2015b). There is also no evidence of hive invasion by predators such as wasps or hornets. Visual inspections typically show an infestation with the ectoparasitic mite *V. destructor* (Le Conte et al., 2010), and intestinal parasites such as *N. ceranae* are frequently present (Fries et al., 2006). Other non-specific signs include a shortened nurse phase (from 25 to 5 days), delayed pest attacks, and colonies dominated by young adult bees. The syndrome occurs in its classic form mainly in the United States and, to a lesser extent, in Europe (Ellis et al., 2010).

#### PESTICIDE USE

Bees can be exposed to pesticides through both direct contact and oral ingestion. Numerous chemical substances are used that can have different levels of toxicity to bees. Nurse bees are exposed to pesticides inside the hive when they consume contaminated food sources, and forager bees come into contact with pesticides during their foraging activity. Nurse bees generally have limited direct exposure to pesticides outside the hive, but they can be exposed to these chemicals through ingestion of contaminated food sources, direct contact with contaminated wax, and miticides (Gradish et al., 2019). They cause acute mortalities or sublethal effects in individual bees, including queens (Tlak Gajger et al., 2017), and also a noticeable, moderate or severe death of honey bee colonies. In addition, it is also possible to detect subclinical changes in bees that leave consequences at the level of organs, tissues and cells (Gregorc et al., 2004, 2012, 2016, 2018a; Silva-Zacarin et al., 2006; Malagoli & Gregorc, 2010). The connections between these consequences and the longevity of bees are not yet known.

The most well-known pesticides are imidacloprid and fipronil, which act systemically in the plant (Gill et al., 2012; Nicodemo et al., 2014). Reduced insect biodiversity and their health in agriculture settings can be result of the increased use of pesticides and fertilisers (Hallmann et al., 2017). Insects, including managed honey bees, provide important services to natural and agricultural ecosystems. Sulfoxaflor and flupyradifurone as new generation insecticides can also have harmful effects on beneficial insects (Siviter & Muth, 2020). Neonicotinoids as neurotoxins with high toxicity to most arthropods can affect the health of honey bees (Dively et al., 2015; Gregorc et al., 2018a). In plants, they act systemically by penetrating the plant tissue and protecting all parts of the crop, and are widely applied as seed dressings. They are used as a pest control in arable farming and horticulture (Goulson, 2013).

A variety of acaricides are used by beekeepers to control the ectoparasitic mite V. destructor (Tihelka, 2018). The accumulation of acaricides used to control varroa and of degradation products, that accumulate in the plants (wax)

can also be a problem. Throughout the entire period of varroa control, numerous chemical substances are used, the effect of which on the bee organism and its organ systems is not fully understood (Gregorc & Bowen, 1998, 1999, 2000; Silva-Zacarin et al., 2006; Gregorc et al., 2012, 2018b, 2018a). Amitraz, a chemosynthetic agent, is one of the most commonly used acaricides in Europe (Brodschneider et al., 2023).

There are other conventional pesticides and their degradation products that affect the health of honey bees. Conventional pesticides are products, often synthetic in nature, that farmers use to control pests, weeds, and diseases that can harm crops (Abdollahdokht et al., 2022). Degradation products are new chemicals that form when a substance breaks down or decomposes due to factors like time, light, temperature, or other environmental conditions (Guo et al., 2024).

In tropical and temperate agricultural landscapes alike, the intensive use of pesticides, particularly neonicotinoids and other systemic insecticides, has caused alarm among scientists and beekeepers (Mitchell et al., 2017). A 2019 study in tropical agricultural landscapes in Thailand examined how insecticide exposure affects honey bee colonies. The researchers monitored colonies placed in areas with varying farming intensities and found multiple insecticides, most often chlorpyrifos and imidacloprid, in hive samples. Colonies near intensive monocultures showed reduced brood area and adult bee population growth, indicating that even sub-lethal pesticide exposure can hinder colony development. The study underscores the need for stricter pesticide regulation and long-term monitoring to safeguard bee health (Buchori et al., 2019).

A 2019 field study in Romania assessed honey bee exposure to neonicotinoids in maize-growing areas. Residue analysis and colony monitoring showed that bees near treated fields accumulated clothianidin and thiamethoxam, especially during maize flowering. Although no acute mortality occurred, exposed colonies exhibited reduced foraging and brood development, indicating sublethal effects. These findings confirm that field-level exposure to neonicotinoids can impair colony performance and support the EU's restrictive pesticide policies (Căuia, 2020). Honey bee populations are increasingly threatened by various environmental stressors, most notably exposure to pesticides (Gil-Lebrero et al., 2017). A nationwide monitoring program in Italy (2015-2019) investigated pesticide residues linked to honey bee mortality. Over 1,000 samples of dead bees and hive materials were analysed, revealing frequent detections of insecticides-especially neonicotinoids and pyrethroids—often in combination with other substances. Mortality peaks were associated with spring and earlysummer pesticide use, while acaricide residues indicated chronic contamination within hives. The study concludes that bee deaths often result from combined pesticide exposure and highlights the need for coordinated monitoring and regulation of both agricultural and beekeeping chemicals (Martinello et al., 2020).

The intensification of agriculture, including the use of pesticides and fertilisers, poses an additional threat to bee health. Sublethal exposure to pesticides — via water, nectar, pollen, or contaminated hive materials — can reduce longevity, immunity, and reproductive function (Jacques et al., 2017; Papaefthimiou et al., 2002).

#### INTERACTIONS AMONG STRESSORS

Tesovnik et al. (2020) report in their study that the simultaneous action of multiple stressors, both natural and chemical results in a greater effect than any single stressor alone. Adult honey bees were exposed to thiamethoxam during the larval and/or adult stage and infected with N ceranae. There was a synergistic interaction of thiamethoxam and nosema, which reflected in lowest honey bee survival.

The synergistic effects of *N. ceranae* and *V. destructor* led to severe alternations in midgut histomorphology, particularly damaging the midgut epithelium. Simultaneous exposure to acetamiprid and *V. destructor* inhibited the immune response and energy metabolism of honey bees. The combination of all three stressors caused the most dramatic damage, disrupting midgut structures as well as aromatic amino acids and lipid metabolism (Kang et al., 2025). Honey bees were exposed to N. ceranae, the herbicide glyphosate and the fungicide difenoconazole. The combination of nosema and pesticides had a strong impact on physiological markers of the nervous system, detoxification, antioxidant defences and social immunity of honey bees (Almasri et al., 2021). Exposure to DWV alone, had a minimal impact. Conversely, when bees were simultaneously treated with acaricides, their mortality rate increased (Lin et al., 2025). Two pesticides (sulfoxaflor and coumaphos) enhanced parasite reproduction, thereby contributing to host colony losses (Frizzera et al., 2025).

#### CONCLUSION

In conclusion, reducing honey bee colony losses requires an integrated approach that recognises the multifactorial nature of the problem. Beekeeping is becoming a very demanding agricultural activity, the results of which depend on many factors. Inappropriate work and behaviour of the beekeeper is only one of the factors. Other possible negative factors affecting beekeeping are agricultural technologies, the behaviour of people in the wider environment and environmental influences. Many beekeepers realise too late that they are responding inadequately to the needs of the bee colony. Coordinated action must address both direct biological threats, such as parasites and pathogens, and

indirect influences such as environmental changes, pesticide exposure, and forage quality. Many studies have demonstrated that these stressors can interact to generate synergistic effects on honey bee health. Taken together, these findings emphasise the urgent need for softer pollinator practises, stricter regulations, and ongoing monitoring to protect bee populations and the ecosystems they support.

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# Razumevanje izgub družin medonosne čebele (*Apis mellifera*): večfaktorska perspektiva

#### IZVLEČEK

Čebele imajo ključno vlogo kot opraševalke kulturnih in divjih rastlin ter tako pomembno prispevajo k biotski raznovrstnosti. Čebelarstvo predstavlja pomembno gospodarsko in kmetijsko dejavnost. Čebelarji se soočajo s številnimi zunanjimi dejavniki, med katerimi so podnebne spremembe, intenzivna kmetijska pridelava, raba pesticidov ter visoka pojavnost bolezni medonosne čebele. Doslej izvedene raziskave so opredelile številne potencialne dejavnike, ki prispevajo k zmanjševanju populacije v družinah, zmanjšani vitalnosti ter k izgubam čebeljih družin. Ta pregledni članek povzema najpomembnejše dejavnike, ki vplivajo na medonosne čebele in njihovo zdravje. Med glavnimi vzroki izgube družin so škodljivci in povzročitelji bolezni, okoljski dejavniki, čebelarske prakse ter ostanki pesticidov. Zmanjševanje izgub družin medonosne čebele zahteva celosten pristop, ki upošteva večfaktorsko naravo problema. Usklajene strategije morajo upoštevati neposredne biološke grožnje, kot so paraziti in patogeni, kakor tudi posredne vplive, med katere sodijo okoljske spremembe, izpostavljenost pesticidom ter kakovost pašnih virov.

Ključne besede: Apis mellifera, pesticidi, patogeni, klimatske spremembe, čebelarsko upravljanje