BEE AND BEEKEEPING

III

EDITORS

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 Institution of Economic Development and Social Researches Publications®
(The Licence Number of Publicator: 2014/31220)
TÜRKİYE TR: +90 342 606 06 75
USA: +1 631 685 0 853
E mail: iksadyayinevi@gmail.com
www.iksadyayinevi.com

ISBN: 978-625-378-539-0
Cover Design: İbrahim KAYA
December / 2025
Ankara / Türkiye
Size: 16x24cm

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Preface

Beekeeping is not merely an agricultural activity encompassing the production of honey and other bee products; it is also a multidisciplinary field of strategic importance in terms of ecosystem sustainability, agricultural productivity, biodiversity, and food security. In recent years, increasing environmental pressures, climate change, pesticide use, pathogen load, and global trade dynamics have made scientific studies on the health of honeybees and the safety of bee products more critical than ever. In this context, beekeeping goes beyond the classical production perspective and strongly intersects with many advanced scientific fields such as molecular biology, biosensor technologies, microbiota analysis, ecotoxicology, economics, and behavioral biology.

This book aims to bring together innovative, current, and interdisciplinary approaches developed for monitoring the health status of honeybees, ensuring the quality and origin of bee products, and better understanding bee-plant interactions. The chapters in the book address the current state and future potential of molecular and biosensor-based diagnostic approaches in the diagnosis of bee diseases; This book evaluates the monitoring and traceability of chemical residue limits in honey and other bee products from the perspective of advanced analytical and biosensor technologies. In addition, the response of honey production to environmental and economic shocks is examined within the framework of unit root tests, offering a scientific perspective on the economic vulnerability of beekeeping. The effects of pesticides, a significant factor directly affecting bee health, on the microbiota of honeybees are discussed in light of current findings; and the long-term consequences of these effects on colony health and the immune system are evaluated. The book also addresses new generation honey authenticity analysis methods developed to combat imitation and adulteration of honey and other bee products, within the framework of evolving analytical techniques and omics approaches. The lesser-known but extremely important aspect of bee-plant interactions, the electrostatic field perception of bees and the effects of the electrical signatures of flowers on pollination efficiency, are presented from an innovative ecological and behavioral perspective.

Finally, attention is drawn to colony sustainability through the analysis of environmental, biological, and managerial factors affecting the reproductive health of male bees (drones). The effects of pollen and nectar characteristics of different fruit types on bee preferences are evaluated in terms of pollination ecology and agricultural productivity. In this respect, the book aims to both provide a deep understanding of bee biology and to interpret agriculture-ecosystem relationships on a scientific basis.

This work aims to be a current reference source for academics, graduate students, researchers, experts working in the beekeeping sector, and policymakers, as well as to inspire scientific and technological approaches that will shape the future of beekeeping. With an interdisciplinary perspective, it is hoped that this book will contribute to the protection of honey bees, the safety of bee products, and the development of sustainable beekeeping practices…

Aralık 2025 / Bayburt/TÜRKİYE
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CHAPTER I

THE IMPACT OF POLLEN AND NECTAR CHARACTERISTICS OF FRUIT SPECIES ON BEE PREFERENCES

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DOI: https://www.doi.org/10.5281/zenodo.18138021

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1. INTRODUCTION

Approximately 80% of flowering plants are entomophilous. Bees (*Hymenoptera: Apidae*) constitute the majority of pollinators (Klein et al., 2007)In fruit production, the effectiveness of pollination directly affects quality criteria such as fruit set, size, shape integrity, and seed number Honeybees (*Apis mellifera*) are the most widespread pollinator species worldwide and play an indispensable role in the pollination of both wild and cultivated plant species (Potts et al., 2016).

Bees visit flowers because they are a source of pollen and nectar. Pollen is the primary source of protein, amino acids, lipids, vitamins, and minerals for bee colonies; It plays a critical role in brood development, bee physiology, and immune system regulation (Di Pasquale et al., 2013). Pollen quality is generally assessed by its protein content (10–35%) and amino acid diversity. In particular, the proportion of essential amino acids such as leucine, isoleucine, valine, and tryptophan can determine bees' pollen preference (Roulston, Cane, & Buchmann, 2000). Nectar is the primary carbohydrate source and meets the colony's energy needs. Bees prefer flowers based on nectar volume and sugar concentration. Nectars with a sucrose/fructose/glucose ratio between 35–65% are generally identified as the most attractive sources (Nicolson & Thornburg, 2007). Nectar varies not only in its sugar content but also in volatile organic compounds (VOCs), pH, mineral content, and secondary metabolites (e.g., alkaloids, phenolics) (Simcock, Gray, & Wright, 2014). A study conducted by Leponiemi et al. (2023) examined the factors that determine honeybees' nectar and pollen collection preferences using DNA metabarcoding. In the study, honey and pollen samples collected from six different apiaries were compared with surrounding flowering plants to determine which plants the bees preferred. The findings indicate that bees do not choose nectar and pollen sources randomly; they prefer nutritious sources such as high protein pollen and nectar containing high sugar content. Furthermore, it was determined that pollen source selection is greater than nectar, and that season and flower morphology influence bee preferences. The study reveals that bees prefer certain plant species for both efficient pollination and a balanced diet, emphasizing the importance of diversity and suitable plant species in agricultural practices.

The decline in pollinators in agricultural ecosystems not only leads to reduced productivity but also to a decrease in biodiversity and disrupted ecological balance. Therefore, understanding the factors that determine bees' flower preferences is a strategic priority from both ecological and economic perspectives.

2. THE EFFECT OF POLLEN CHARACTERİSTİCS ON BEE PREFERENCE

2.1. The Role of Pollen in Bee Nutrition

Honeybees (*Apis mellifera*) collect pollen from various plant species for the healthy development of the colony. Pollen is an essential protein source for larval development, the maturation of worker bee glands, particularly the hypopharyngeal glands, and immune system support (Di Pasquale et al., 2013). The nutritional value of pollen is determined by its crude protein, essential amino acids, lipids, sterols, and vitamin content.

Roulston et al. (2000) reported the average protein content of pollen to range from 10–35%, but emphasized that there are significant differences among plant sources. Pollen from the legume *(Fabaceae)* and fruit tree *(Rosaceae)* families, in particular, are among the high protein and preferred sources for bees. However, pollen from some fruit species has been reported to have low digestibility and be less preferred by bees (Su et al., 2022).

The impact of pollen quality on bee health is not limited to its nutritional content. Some pollens contain phytochemical compounds that directly affect bee development. For example, flavonoids, phenolic acids, and carotenoids play a role in reducing oxidative stress and strengthening bee immunity (Alaux, Ducloz, Crauser, & Le Conte, 2010). Therefore, pollen diversity and composition are critical parameters for the sustainability of colony performance.

2.2. Comparison of Pollen Characteristics of Fruit Species

a) Apple (*Malus domestica* L.): Apple flower pollen contains high protein (25–30%) and essential amino acids (Neff 2013, 2012). Bees exhibit intense pollen collection in apple orchards; pollen grains are medium-sized (25–30 µm) and smooth-surfaced. These morphological characteristics allow bees to easily adhere to body hairs, increasing both collectability and pollination efficiency (IPBES 2016). Increasing bee density in apple orchards increases fruit set rates by 20–40% (Garibaldi et al., 2013).

b) Pear (*Pyrus communis* L.): Pear flower pollen generally has a low protein content (10–12%) and a low sugar content. Additionally, pear blossoms are known for their lower volatile emission compared to other fruit species, leading to limited bee interest in these flowers (Su et al., 2022). It has been reported that honeybees visit apple blossoms in the same orchard 3–4 times more frequently than pear blossoms (Benedek, Nyeki, et al., 2000). A comparative study conducted in France and Italy determined that only 35% of pear blossoms were visited by bees, while this rate was 85% for apple blossoms (Vicens & Bosch, 2000). This low visitation rate, combined with environmental factors such as low temperature and wind, especially in early spring, reduces pollination success. Wild bee species such as *Osmia cornuta* have been shown to be more effective than honeybees in almond pollination (Bosch & Blas, 1994). Therefore, it is recommended to prioritize the protection of not only honeybees but also other pollinator species in pear orchards.

c) Apricot (*Prunus armeniaca* L.): Apricot trees are an important source of pollen and nectar for honeybees (*Apis mellifera* L.) due to their intense flowering, especially in spring. A study examining the effect of cross-pollination on fruit set through visits by honeybees to "Sundrop" apricot flowers showed that pollinator bees worked faster on the flowers than nectar foragers (5.3 vs. 2.7 flowers per minute). Foraging bees visited for up to 6 hours per day under good weather conditions, reaching 9 bees per tree. Neither nectar volume nor composition appeared likely to reduce foraging activity (Austin, Hewett, Noiton, & Plummer, 1996). In a laboratory study by Lan, Ding, Ma, Jiang, and Huang (2021), A. *mellifera* colonies were fed only apricot or pear pollen; hypopharyngeal gland development and lifespan were significantly higher in apricot pollen-fed bees. Additionally, bees consuming apricot pollen have been reported to exhibit a tendency to recognize and re-select the same pollen source in olfactory preference tests. A study conducted in Central Asia determined that bee visitation density in apricot orchards reached 6.8 visits/flower/hour, and post-pollination fruit set rates reached up to 90%. Similarly, in Turkey, bee activity on apricot flowers was reported to be highest in the morning. Furthermore, apricot flowers have been reported to strongly appeal to bees' visual perception due to their UV-reflective petals

d) Cherry (*Prunus avium* L.) and Plum (*Prunus domestica* L.): Cherry and plum pollen are rich in lipids and phenolic compounds, making them an attractive source for bees. However, because these species have a short flowering period, they are only heavily used by bees for short periods (Abrol, 2012). In a study conducted in Germany, the visitation rate of *Apis mellifera* to cherry blossoms was measured as 4.6 visits/flower/hour, while Bombus terrestris was reported to be active at a rate of 1.5 visits/flower/hour in the same orchards. The presence of both species increased fruit set in cherry by 15% (Holzschuh, Dudenhöffer, & Tscharntke, 2012).

e) Strawberry (*Fragaria* × *ananassa* Duch.): Although strawberries are self-pollinating, bee visits play an important role in fruit shape and size. As the number of visits by honeybees to strawberry flowers increases, the rate of smooth, large fruit also increases (Chagnon, Gingras, & DeOliveira, 1993). A study conducted in Canada found that honeybees visited strawberry flowers at an average rate of 2.5 visits/flower/hour, while bumblebees were more active at 3.1 visits/flower/hour. As bee density increased, the rate of deformed fruit in strawberries decreased from 25% to 8% (Garibaldi et al., 2013). Strawberry pollen is moderate in protein (18–20%), but the flower morphology facilitates pollen collection by bees. Furthermore, the continuous flowering of strawberry plants provides a long-term pollen source for bees (Garibaldi et al., 2013).

f). Citrus (*Citrus* spp.): Citrus species provide an important source of nectar and pollen for honeybees (*Apis mellifera* L.) during peak bloom periods. A study examining the relationship between morphological characteristics of citrus flowers and honeybee preferences showed that bees visited larger-flowered cultivars (e.g., "Orlando" and "Minneola" tangelo), while bee visitation rates were significantly lower on smaller-flowered hybrids. This was suggested to be due to morphological and biochemical factors such as flower size, petal aperture, nectar availability, and total nectar/pollen reward. It was emphasized that not only genetic compatibility but also floral structure and bee attractiveness should be considered for efficient pollination and fruit set among citrus cultivars. Research shows that honeybee visitation intensity in citrus orchards increases in the morning, decreases midday, and persists until the end of the flowering period (Albrigo, Russ, Rouseff, & Bazemore, 2012). It has been suggested that insect pollination (especially bees) in citrus orchards increases fruit set by 2.4-fold, and that approximately 60% of total yield may be due to pollination (Monasterolo et al., 2024). Furthermore, it has been determined that flowers left for bee pollination in three-leafed oranges exhibited positive effects in terms of fruit weight, acidity, and yield compared to flowers whose pollination was prevented (Malerbo-Souza, Nogueira-Couto, & Couto, 2004). Similar results were reported in observations made in the Mediterranean region of Turkey; honeybees were observed to frequent citrus flowers between 8:00 and 10:00 in the morning, and activity decreased at noon as nectar decreased (Baydar & Gürel, 1998).

g). Quince (*Cydonia oblonga* Mill.): Regular visits by honeybees to quince orchards increase the effectiveness of natural pollination and support fruit yield and quality. A study observing bee visits and feeding behavior on the flowers of six different quince cultivars over three years found an average of seven visits per flower per day under good weather conditions. Approximately 51.6% of the bees collected pollen, 19.9% collected only nectar, and 28.5% collected both pollen and nectar (Benedek, Szabó, & Nyéki, 2000).

h). Almond (*Prunus dulcis* L.): A study comparing the feeding behavior and pollination efficiency of honeybees and Osmia cornuta on almond flowers found that the stigma contact rate of O. cornuta was very high at 98.7% during visits per flower. This rate was found to decrease to 39.5% in nectar-gathering *A. mellifera* individuals and to 76.3% in pollen-nectar-gathering individuals. Furthermore, O. cornuta visited more flowers per unit of time; as a result, the rate of fruit set with a single visit ranged from 21.8–38.1% in O. cornuta, compared to 16.7–26% for *A. mellifera* (pollen-nectar-gathering) and 9.1–0% for *A. mellifera* (nectar-only collecting). These results suggest that O. cornuta may be a more effective pollinator than *A. mellifera* in fruit trees such as almonds (Bosch & Blas, 1994).

2.3. Pollen Morphology and Bee Foraging Behavior

Bees are affected by both flower morphology and the structural characteristics of pollen grains during pollen collection. The surface structure (exine thickness, ornamentation), shape, and size of pollen determine its ability to adhere to bee body hairs (Eşerler, Vardarlı, Savaş, & Mutlu, 2023). In most fruit species, pollen grains are 20–40 µm in diameter, an optimum size for bees to easily transport.

A study conducted in Turkey examined 46 different pollen types collected by honeybees in the Antalya flora. Pollens from the Rosaceae family were determined to be the most preferred group in terms of both protein content and morphological suitability (Baydar & Gürel, 1998). This result demonstrates that fruit species are an ecologically important food source for bees.

2.4. Pollen Diversity, Colony Health, and Pollination Success

Feeding colonies only a single type of pollen (monofloral diet) weakens the bee immune system and leads to imbalances in larval development (Di Pasquale et al., 2013). In contrast, a mixture of pollen collected from fruit species and wild plants increases colony health and pollination efficiency. Various studies have reported that hives with high pollen diversity experience faster larval development, longer worker bee lifespan, and increased disease resistance (Alaux et al., 2010).

Therefore, the presence of multiple flowering plants in orchards, rather than a single species, is recommended for both bee health and fruit yield. Bees' pollen preferences are therefore not only a feeding behavior but also a factor that directly affects agricultural ecosystem productivity.

3. THE EFFECT OF NECTAR CHARACTERİSTİCS ON BEE PREFERENCE

3.1. The Importance of Nectar in Bee Nutrition

Nectar is the primary carbohydrate source for bees and is vital for the maintenance of colony metabolism. Honey bees (*Apis mellifera*) select flowers based on parameters such as the type of sugar in the nectar, its density, pH, volatile compound profile, and even temperature (Nicolson & Thornburg, 2007). Honey bees generally prefer nectars with a sucrose-equivalent sugar concentration between 30–50% (Pyke, 2016). Nectars with low sugar content increase collection costs, while overly concentrated nectars hinder absorption and are therefore not preferred by bees (Cnaani, Thomson, & Papaj, 2006).

The primary components of nectar are sucrose, fructose, and glucose. However, bees' preferences are determined not only by these ratios, but also by the amines, amino acids, organic acids, phenolic compounds, and volatile terpenes found in nectar. For example, aromatic amino acids such as phenylalanine create a positive conditioning effect on bees' floral memory; therefore, species containing this compound in their nectar are visited more frequently by bees (Simcock et al., 2014).

Bees evaluate floral scents not only through olfaction but also through learning and memory mechanisms. Experimentally, it has been shown that certain volatile compounds (e.g., benzaldehyde, linalool, geraniol) guide bees' flower selection and increase the likelihood of these scents being learned again (Wright et al., 2013)

Fruit flowers generally produce terpenoids, phenylpropanoids, and benzene-derived compounds. While benzaldehyde and linalool predominate in apple blossoms, β-ocimene and limonene are prominent in apricot flowers, while geraniol and nerol are prominent in citrus (Knudsen, Eriksson, Gershenzon, & Ståhl, 2006). These chemical differences influence bees' flower recognition and revisiting of the same species. The interaction of nectar volatiles with bee memory is important for consistent pollination. The bees' tendency to revisit the same flowers ensures that pollen is transferred between the correct species, which directly increases fruit set rates (Chittka, Thomson, & Waser, 1999).

The pH of nectar generally ranges from 4.0 to 7.0. Honey bees prefer nectars with a neutral or slightly acidic pH. Overly acidic nectars can negatively affect the bee's digestive system (Nicolson & Thornburg, 2007). Some fruit species contain low concentrations of alkaloids (e.g., caffeine) or phenolic compounds in their nectar. Research by Wright et al. (2013) showed that caffeine-containing nectars strengthen bee memory and increase the revisit rate of the same flowers. Therefore, secondary metabolites may serve as cognitive signals that guide bee behavior.

Nectar quantity is also directly proportional to visitation density. Bee density per unit of time increases significantly in flowers with high nectar volume. However, in the presence of very dense colonies, competition for nectar can occur on the same flowers, leading bees to seek alternative sources (Seeley, 2009).

3.2. The Relationship Between Nectar Characteristics and Pollination Success

Bees' selection based on specific nectar characteristics directly affects the efficiency of fruit pollination. While pollination rates are generally between 70–90% in species producing high-sugar nectar, such as apples and cherries, this rate can drop to as low as 40–60% in pear orchards (Benedek, Nyeki, et al., 2000). This demonstrates the extent to which nectar rewards guide bee behavior.

Nectar attractiveness influences not only individual bee behavior but also the division of labor at the colony level. More forager bees are directed to nectar sources that provide high energy yields, increasing the homogeneity of pollination within the orchard. Furthermore, bee flower preferences are shaped not only by individual but also by learning and guidance mechanisms at the colony level. When forager bees find productive resources, they transmit information to other bees through dance communication, resulting in a "collective orientation" toward a particular flower type within the colony (Seeley, 2009).

3.3. Comparison of Nectar Characteristics of Fruit Species

a) Apple (*Malus communis* L.): Apple blossoms are one of the most frequently visited fruit species by bees. The nectar volume of apple blossoms is generally 0.5–1.2 µL/flower, with an average sugar concentration of 35–45% (Delaplane & Mayer, 2000). The sucrose-dominant nature of apple nectar (approximately 55% sucrose, 25% fructose, and 20% glucose) allows honeybees to obtain high energy yields. Additionally, apple blossoms secrete volatile compounds such as linalool, benzaldehyde, and geraniol; these compounds support bees' homing behavior (Knudsen et al., 2006). A study investigating the relationship between bee visitation density per flower, nectar production, nectar characteristics, and the foraging behaviors of both honeybees and wild bees in 18 different apple cultivars over three years found that nectar production in apple flowers was highly variable among cultivars, with cultivars with higher nectar production leading to increased overall bee visitation density (Benedek & Finta, 2006). In a study by Benedek and Nyéki (1995), the average bee visitation frequency on apple flowers was 5.4 visits/flower/hour. This rate was recorded as 1.2 visits/flower/hour on pear flowers in the same region. It has been reported that honeybees concentrate on apple flowers in the morning hours, with activity decreasing at noon due to increased temperature.

b) Pear (*Pyrus communis* L.): Pear flowers are generally visited to a limited extent by honeybees due to their low nectar volume and low sugar concentration. However, this deficiency is partially compensated by bees' tendency to turn to pear flowers when no other sources are available during the flowering period. The nectar volume of pear flowers is quite low (0.1–0.3 μL/flower), and the average sugar concentration is around 20–25%. This leads to bees' lower interest in pear flowers. A study by Su et al. (2022) reported that the concentrations of volatile compounds (hexyl acetate, benzyl alcohol, phenylethyl alcohol) detected in pear flowers were low, while apricot and apple flowers had a stronger aromatic profile. This suggests that bees generally use pear flowers as transit points for short visits.

c) Apricot (*Prunus armeniaca* L.): Apricot flowers are one of the most preferred fruit species by bees because they secrete high-sugar (40–48%) and abundant nectar (Lan et al., 2021). Apricot nectar also contains phenylalanine and linalool, compounds that stimulate bees' taste receptors. These volatiles enhance both olfactory memory and learning behavior (Simcock et al., 2014).

d) Cherry (*Prunus avium* L.): Cherry trees are a critical source of pollen and nectar for honeybees, especially during peak bloom periods. Cherry blossoms secrete abundant nectar in the morning, which is generally rich in sucrose (40–50%). The red-pigmented petals of cherry blossoms also contrast with the bees' UV vision system, increasing visual appeal (Neff 2013, 2012).

e) Citrus (*Citrus* spp.): Flowers of citrus species such as lemon (*Citrus limon*), lime (*C. aurantiifolia*), orange (*C. sinensis*), and grapefruit (*C. paradisi*) are highly attractive to honeybees (*Apis mellifera*) and provide efficient honey production (Malerbo-Souza et al., 2004; Monasterolo et al., 2024). These species and their hybrids, due to their rich nectar content, provide rewarding resources for bees and play an important role in pollination services. The white color and intense aromatic compounds of citrus flowers facilitate bee detection, increasing flower visits. The contrasting leaf background of the flowers also supports visual orientation. However, bees' attraction to citrus flowers is mostly for nectar collection.

4. CONCLUSIONS AND RECOMMENDATIONS

The pollen and nectar characteristics of fruit species are directly linked to bees' ecological preferences and colony health. Through this interaction, bees are indispensable actors in agricultural production. The nectar volume offered by flowers and the nutritional value of pollen are key factors shaping bee visitation preferences. Nectar volume and flower density significantly increase bee visit frequency. Furthermore, nutritional qualities such as pollen protein-lipid ratio or pollen viability also play a role in bee preference behavior. Not only the availability of floral resources but also their quality influences bees' decisions. In some fruit species, floral tube length, corolla structure, and flower accessibility facilitate or hinder bees' nectar-gathering behavior. This influence depends not only on nectar or pollen quantity but also on morphological compatibility. Therefore, when selecting suitable varieties and species for orchards, it is necessary to consider not only nectar and pollen abundance but also morphological criteria such as flower structure and accessibility.

Orchard arrangements that take into account bees' natural preferences can increase pollination efficiency. This, in turn, can increase both fruit yield and quality. They are also critical for supporting biodiversity and implementing sustainable agricultural policies. Therefore, it is recommended that fruit producers and agricultural planners consider bee behavior when planning orchards. Protecting pollination services is crucial not only for beekeeping but also for global food security.

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CHAPTER II

ANALYSIS OF FACTORS AFFECTING THE REPRODUCTIVE HEALTH OF HONEYBEE (*APIS MELLIFERA*) DRONES

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DOI: https://www.doi.org/10.5281/zenodo.18138074

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1. INTRODUCTION

Honey bees are social insects that live in communities called bee families or colonies. (Richards vd., 2023). When examined closely, three different individuals are detected within a bee colony in terms of morphological, physiological and behavioral characteristics. Queen bee, worker bee and drones (Halvaci et al., 2023). Queen bees and worker bees are female individuals and develop from fertile eggs, while drones emerge from infertile eggs through parthenogenesis (Khan and Ghramh, 2024).

As male bees, drones fulfill the essential role of mating with queens to ensure the continuation of their species (Halvaci et al., 2023). However, the reproductive health of honey bee drones is not solely determined by their innate biology but is influenced by a myriad of factors, both natural and anthropogenic.

One of the primary biological factors impacting drone reproductive health is genetic diversity (Shultz et al., 2024). Genetic diversity within honey bee colonies is crucial for resilience against diseases, environmental stressors, and adaptation to changing conditions. Inbreeding depression, a consequence of reduced genetic diversity, can lead to decreased drone fertility and colony fitness. For instance, studies conducted by Gençer and Firatli (2005) and Zaitoun et al. (2009) have demonstrated that inbred honey bee drones exhibit lower sperm viability and increased susceptibility to diseases, highlighting the importance of genetic diversity in maintaining healthy drone populations.

Environmental factors also play a significant role in shaping drone reproductive health. Climate and weather conditions, such as temperature, humidity, and wind speed, can profoundly impact drone mating behavior and reproductive success. Adverse weather conditions, including extreme temperatures or heavy rainfall, can disrupt drone mating flights, leading to decreased genetic diversity within colonies. Research by Koeniger and Koeniger (2007) and Neves et al. (2011) has shown that cold temperatures and high humidity levels can inhibit drone flight activity and impair mating success rates, underscoring the vulnerability of drones to environmental fluctuations.

Furthermore, human activities and management practices within beekeeping operations can exert considerable pressure on drone reproductive health. Intensive queen rearing programs, aimed at selecting for specific traits in honey bee queens, may inadvertently lead to a reduction in drone genetic diversity and compromise overall colony health. Likewise, the transportation of honey bee colonies for pollination services exposes drones to stressors such as vibration, temperature fluctuations, and limited food, which can negatively impact their reproductive fitness.

As we delve deeper into the factors influencing the reproductive health of honey bee drones, it becomes evident that a multifaceted approach is required to address these challenges effectively. By integrating scientific research, conservation efforts, and sustainable beekeeping practices, we can strive to preserve the health and vitality of honey bee populations, safeguarding their crucial role as pollinators and ensuring the sustainability of agricultural ecosystems.

2. BIOLOGICAL FACTORS

The reproductive health of male honeybees (drones) is influenced by many biological factors, including genetic makeup, nutritional status, environmental conditions, physiological development, pathogen presence, and pesticide exposure. Genetic makeup and racial differences play a decisive role in testicular development, sperm production capacity, and sperm quality. While inadequate or unbalanced nutrition during the larval stage negatively impacts testicular development, a diet rich in protein and vitamins promotes higher sperm viability and motility.

Temperature and humidity are important environmental factors, with high temperatures, in particular, negatively impacting spermatogenesis. Drone age and physiological maturity also determine reproductive success. Parasites such as Varroa destructor and viruses such as DWV cause degeneration of testicular tissue and decreased sperm production. Furthermore, neonicotinoid pesticides and other chemical residues reduce sperm count and viability, impairing drone fertility. A strong antioxidant defense system and a balanced gut microbiota support reproductive health by mitigating the effects of oxidative stress.

Biological factors are fundamental determinants of honey bee drone reproductive health, encompassing genetics, physiology, and age. Understanding these factors is crucial for maintaining robust drone populations within honey bee colonies.

2.1. Genetics

Genetic diversity is crucial for the health and resilience of honey bee colonies, including drone populations. Inbreeding depression, resulting from reduced genetic diversity, can lead to decreased drone fertility, increased susceptibility to diseases, and compromised colony fitness.

Inbreeding Depression: Inbreeding depression occurs when closely related individuals mate, leading to the expression of harmful recessive alleles and reduced fitness in offspring. Several studies have demonstrated the negative effects of inbreeding depression on honey bee drones. For example, a study (Stürup et al., 2013) investigated the effects of inbreeding on honey bee drone health. The study found that inbred drones exhibited lower sperm viability and increased mortality rates compared to outbred drones, highlighting the reproductive consequences of reduced genetic diversity within colonies.

2.1.1.Genetic Diversity and Disease Resistance

Genetic diversity within honey bee colonies is essential for resistance against pathogens and parasites (Desai & Currie, 2015). Studies have shown that genetically diverse colonies are better equipped to resist diseases and environmental stressors. For instance, research by Rangel et al. (2018) demonstrated that genetically diverse colonies exhibited greater resistance to diseases such as Varroa mites and Nosema spp. compared to genetically homogeneous colonies. Similarly in a study conducted (Rangel et al., 2020), found that colonies with higher genetic diversity had lower incidences of disease and higher survival rates, underscoring the importance of genetic diversity in maintaining healthy honey bee populations.

2.1.2. Selective Breeding for Genetic Diversity

Beekeepers and researchers often employ selective breeding programs to enhance genetic diversity and resilience in honey bee populations. These programs aim to selectively breed queens and drones from genetically diverse stocks to improve overall colony health and productivity. For example, research conducted by Kovačić et al. (2020) investigated the effects of selective breeding on honey bee genetic diversity. The study found that selective breeding programs led to diversity loss, which can translate into the loss of local adaptations. Overall, genetic diversity plays a critical role in shaping the reproductive health and resilience of honey bee drones. Conservation efforts focused on promoting genetic diversity, minimizing inbreeding, and preserving diverse honey bee stocks are essential for ensuring the long-term viability of honey bee populations.

2.2. Physiology

The reproductive physiology of honey bee drones is finely tuned to ensure successful mating and colony reproduction. Understanding the physiological mechanisms underlying drone fertility is essential for maintaining healthy honey bee populations.

2.2.1. Spermatogenesis and Sperm Viability

Honey bee drones undergo spermatogenesis in their reproductive organs, culminating in the production of viable sperm. The size and quality of drone spermatozoa directly influence mating success and colony fitness. In a study conducted to investigate the relationship between seminal vesicle size and sperm production in drones. The study found that drones with larger seminal vesicles produced greater quantities of sperm, resulting in higher mating success rates (Hayashi and Satoh, 2019). Additionally, a study by Czekońska et al. (2013) demonstrated that environmental stressors such as heat stress could impair sperm viability and reduce drone fertility, highlighting the importance of environmental conditions in maintaining optimal sperm quality.

2.2.2. Physiological Regulation of Mating Behavior

Honey bee mating behavior is regulated by complex physiological mechanisms, including hormonal signaling and sensory perception. For example, drones produce pheromones to attract and court queens during mating flights. Research by Villar et al. (2019) investigated the role of pheromones in honey bee mating behavior. The study found that drones produce specific pheromones that signal their reproductive status and attract queens for mating, highlighting the importance of chemical communication in drone reproduction.

2.2.3. Flight Physiology and Mating Behavior

Drone mating behavior is closely linked to their flight physiology and energy metabolism. Drones must expend significant energy to engage in mating flights and pursue queens. Research by Koeniger et al. (2005) investigated the metabolic demands of honey bee mating flights. The study found that drones undergo rapid metabolic changes during mating flights, requiring efficient energy utilization and flight performance. Additionally, a study by Mattila and Seeley (2007) examined the relationship between drone age and mating behavior. The research found that younger drones exhibited higher mating success rates and outcompeted older drones for mating opportunities, highlighting the importance of age-related physiological factors in drone reproduction.

Overall, understanding the physiological mechanisms underlying honey bee drone fertility is essential for maintaining healthy honey bee populations and ensuring successful colony reproduction. Conservation efforts focused on optimizing environmental conditions, promoting optimal sperm quality, and understanding the hormonal regulation of mating behavior are crucial for safeguarding honey bee reproductive health.

2.3. Age

Drone age plays a significant role in determining reproductive performance and mating success within honey bee colonies. Understanding the age-related dynamics of drone reproductive health is essential for effective colony management and conservation efforts.

2.3.1. Mating Success and Competition

Honey bee drones undergo physiological changes as they age, which can impact their ability to compete for mating opportunities. Mattila and Seeley (2007), investigated the relationship between drone age and mating success. The study found that younger drones exhibited higher mating success rates compared to older drones, suggesting that age-related factors influence drone competitiveness during mating flights. Additionally, in a study examined the mating behavior of drones from different age cohorts (Metz and Tarpy, 2022). The research revealed that younger drones were more likely to mate with queens, indicating age-related differences in mating behavior and success rates.

2.3.2. Sperm Viability and Quality

The age of drones also influences the quality and viability of spermatozoa produced during spermatogenesis. Studies have shown that older drones may experience declines in sperm quality and viability, which can impact colony reproductive success. In a study by Czekońska et al.(2013), investigated the effects of drone age on sperm production and viability. The study found that older drones had lower sperm counts and higher levels of sperm abnormalities compared to younger drones, highlighting the age-related decline in sperm quality. Additionally, a study by Rousseau et al. (2016) examined the relationship between drone age and sperm viability. The research revealed that sperm viability declined with increasing drone age, underscoring the importance of age-related factors in maintaining optimal reproductive health.

2.3.3. Longevity and Colony Fitness

The lifespan of honey bee drones is relatively short compared to other castes within the colony. As drones age, they may experience declines in vigor and overall fitness, which can impact colony productivity and survival. Hayashi et al., (2017), investigated the longevity of honey bee drones and its implications for colony fitness. The study found that older drones had reduced flight performance and mating success rates, leading to decreased colony reproductive output. Additionally, a study by Metz and Tarpy. (2019) examined the effects of drone lifespan on colony productivity. The research revealed that colonies with longer-lived drones exhibited higher reproductive output and queen mating frequency, highlighting the importance of drone age in colony fitness and reproductive success.

Overall, understanding the age-related dynamics of honey bee drone reproductive health is essential for effective colony management and conservation efforts. By considering age-related factors such as mating success, sperm viability, and longevity, beekeepers can optimize colony productivity and ensure the sustainability of honey bee populations.

3. ENVIRONMENTAL FACTORS

The reproductive health of male honeybees (drones) is highly sensitive to environmental factors, and these factors play a decisive role in sperm production, viability, motility, and mating success. Temperature, humidity, light duration, and seasonal changes affect all processes from drone development to maturation. High temperatures (above 35°C), particularly during the pupal stage, cause degeneration of testicular tissue, decreased sperm production, and structural deterioration of sperm cells, while low temperatures prolong developmental time and delay maturation. Adequate humidity (55–70%) is crucial for sperm viability; excessive dryness leads to dehydration of the seminal plasma, disrupting the integrity of the sperm membrane. Light duration and seasonal variations also affect drone production and reproductive performance; drones developing in spring and summer generally have higher sperm quality, while autumn drones have lower reproductive capacity. Furthermore, environmental stressors such as pesticide residues, air pollution, and plant-derived toxins increase oxidative damage, negatively impacting sperm motility and viability. Therefore, the balance of environmental conditions is critical for the healthy development and successful reproduction of drones.

3.1. Climate and Weather

Climate and weather conditions play a crucial role in shaping the mating behavior and reproductive success of honey bee drones. Temperature, humidity, wind speed, and other environmental factors influence drone flight activity and mating flights, ultimately impacting colony genetic diversity and productivity.

3.1.1. Temperature

Temperature extremes can significantly affect drone mating behavior and reproductive success. Cold temperatures can inhibit drone flight activity, reducing opportunities for mating and leading to decreased genetic diversity within colonies. Conversely, excessive heat can stress drones, impairing sperm viability and fertility. For example, a study investigated the effects of temperature on drone mating flights (Stürup et al., 2013). The researchers found that both cold and hot temperatures negatively impacted drone flight activity, highlighting the vulnerability of drones to temperature extremes.

3.1.2. Humidity

Humidity levels also play a role in drone mating behavior and reproductive success. High humidity can impede drone navigation and flight efficiency, leading to decreased mating success rates. Conversely, low humidity levels may increase water loss and stress levels in drones, affecting their overall fitness and reproductive performance.

Research by Neves et al. (2011) examined the impact of humidity on drone flight activity and mating success. The study revealed that high humidity levels reduced drone flight activity and mating success rates, underscoring the importance of humidity regulation for successful drone mating.

3.1.3. Wind Speed

Wind speed can influence drone flight behavior and mating flights. Strong winds may deter drones from flying, limiting mating opportunities and reducing genetic diversity within colonies. Conversely, calm winds facilitate drone flight activity and increase the likelihood of successful mating.

A study conducted by Reyes et al. (2019). investigated the effects of wind speed on honey bee foraging behavior and flight activity. The research demonstrated that windy conditions reduced drone flight activity and mating success rates, highlighting the significance of wind speed in shaping drone reproductive behavior.

Overall, climate and weather conditions exert a profound influence on honey bee drone mating behavior and reproductive success. Beekeepers must consider these factors when managing colonies and scheduling mating flights to optimize genetic diversity and colony productivity.

3.2. Pesticides and Chemicals

Exposure to pesticides and other chemicals poses a significant threat to the reproductive health and overall well-being of honey bee drones. These toxic substances can impair drone fertility, weaken immune defenses, and increase susceptibility to diseases, ultimately jeopardizing colony survival.

3.2.1. Neonicotinoid Pesticides

Neonicotinoids are a class of systemic insecticides commonly used in agriculture to control pests. However, their widespread use has raised concerns about their impact on pollinators, including honey bees. Numerous studies have demonstrated the detrimental effects of neonicotinoids on honey bee health. For example, a study conducted by Ciereszko et al., (2017) investigated the effects of imidacloprid, a neonicotinoid insecticide, on honey bee colony health. The research found that exposure to imidacloprid significantly reduced drone sperm viability and increased mortality rates, highlighting the reproductive toxicity of neonicotinoids on honey bee drones.

3.2.2. Organophosphate and Pyrethroid Insecticides

Organophosphate and pyrethroid insecticides are commonly used in agricultural and urban settings to control insect pests. However, these chemicals can have adverse effects on honey bee health, including reduced fertility and increased susceptibility to diseases.

Research by Fisher and Rangel (2018) investigated the effects of sublethal doses of organophosphate and pyrethroid insecticides on honey bee drone health. The study revealed that exposure to these chemicals significantly reduced drone sperm viability and longevity, highlighting the reproductive toxicity of organophosphate and pyrethroid insecticides.

3.2.3. Pesticides and Herbicides

In addition to insecticides, fungicides and herbicides can also impact honey bee drone health. These chemicals may have indirect effects on drones by altering floral resources or disrupting colony foraging behavior. Research by Fisherand Rangel, (2018) investigated the effects of pesticides on honey bee drone sperm viability. The research found that exposure to pesticides reduced sperm viability in drones, indicating the potential reproductive toxicity of these chemicals on honey bee drones.

Overall, the widespread use of pesticides and chemicals poses a significant threat to honey bee drone reproductive health. Beekeepers and policymakers must implement measures to mitigate pesticide exposure and promote sustainable pest management practices to safeguard honey bee populations and ensure their long-term viability.

3.3. Habitat Loss

Habitat loss and fragmentation are significant threats to honey bee populations, including drones. The conversion of natural habitats for agriculture, urbanization, and other human activities can disrupt honey bee foraging behavior, nesting sites, and mating opportunities, ultimately impacting colony health and reproductive success.

3.3.1. Conversion of Natural Habitats

The conversion of natural habitats, such as grasslands, forests, and meadows, for agricultural purposes is a major driver of habitat loss for honey bees. Agricultural intensification and expansion lead to the loss of floral resources and nesting sites essential for honey bee foraging and reproduction.
A study (Naug, 2002) investigated the effects of habitat loss on honey bee foraging behavior. The research found that honey bee foraging activity significantly declined in landscapes with reduced floral diversity and increased agricultural land use, highlighting the negative impact of habitat loss on honey bee populations.

3.3.2. Urbanization and Land-Use Changes

Urbanization and land-use changes also contribute to habitat loss and fragmentation for honey bees. The expansion of urban areas and infrastructure reduces available foraging resources and nesting sites, limiting drone mobility and mating opportunities. Research by Samuelson (2019) investigated the effects of urbanization on honey bee reproductive success. The study found that urban areas with limited floral diversity and green spaces resulted in reduced drone mating success rates, highlighting the negative impact of urbanization on honey bee populations.

3.3.3. Deforestation and Fragmentation

Deforestation and habitat fragmentation further exacerbate habitat loss for honey bees. Fragmented habitats create barriers to drone dispersal and mating, leading to decreased genetic diversity within colonies. A study by Williams et al.(2009) examined the effects of habitat fragmentation on honey bee genetic diversity. The research found that fragmented landscapes reduced drone dispersal and gene flow, resulting in genetic isolation and increased inbreeding within colonies.

Overall, habitat loss and fragmentation pose significant threats to honey bee populations, including drones. Conservation efforts focused on preserving natural habitats, enhancing floral diversity, and minimizing land-use changes are essential for maintaining healthy honey bee populations and ensuring their long-term viability.

4. MANAGEMENT PRACTICES AND HUMAN ACTIVITIES

The reproductive health of male honeybees (drones) is significantly affected not only by biological factors but also by management strategies and human activities implemented in beekeeping.

Colony management errors, particularly inadequate nutrition, frequent colony splitting, and frequent queen replacements, can negatively impact drone development and sperm quality. Intensive colony inspections and frame arrangements disrupt the temperature and humidity balance in the brood area, impairing the development of larval drones. Failure to consider the genetic diversity of drones in artificial rearing and queen production programs can lead to reduced intra-colony genetic variation and long-term reductions in male fertility.

Furthermore, agricultural activities in the surrounding area, particularly the use of pesticides and fungicides, contaminate drones' food sources, reducing sperm quality and viability. Excessive sugar syrup consumption by beekeepers or imbalanced colony nutrition in the event of pollen deficiency negatively impacts drones' energy metabolism and mating performance. Transported beekeeping and stress-inducing practices can also impair drones' flight muscle strength and mating behavior. Therefore, balanced nutrition, reduction of chemical exposure, preservation of genetic diversity and adoption of environmentally friendly beekeeping practices are of great importance to protect the reproductive health of drones.

4.1. Beekeeping Practices

Beekeeping practices have a significant impact on honey bee drone reproductive health and colony sustainability. Effective management strategies aimed at optimizing drone production, genetic diversity, and environmental conditions are essential for maintaining healthy honey bee populations.

4.1.1. Queen Rearing and Drone Production

Queen rearing practices play a crucial role in determining the genetic diversity and reproductive potential of honey bee colonies. Beekeepers often selectively breed queens from genetically diverse stocks to improve colony productivity and resilience. However, intensive queen rearing programs may inadvertently reduce drone genetic diversity and compromise colony health. Research by Tarpy et al. (2015) investigated the effects of queen mating frequency on colony genetic diversity. The study found that colonies with higher queen mating frequencies exhibited greater genetic diversity and resistance to diseases, highlighting the importance of maintaining diverse mating stocks for optimal colony health.

4.1.2. Transportation Stress

The transportation of honey bee colonies for pollination services can expose drones to stressors such as vibration, temperature fluctuations, and limited foraging opportunities. These stressors can impact drone reproductive health and genetic diversity, ultimately affecting colony productivity. A study by Melicher et al.(2019). investigated the effects of transportation stress on honey bee colony health. The research found that transported colonies experienced increased mortality rates and reduced brood production, indicating the detrimental effects of transportation stress on colony fitness and reproductive success.

4.1.3. Hive Management Practices

Effective hive management practices are essential for promoting honey bee health and productivity. Beekeepers must monitor hive conditions, disease prevalence, and environmental factors to optimize colony performance. Research by Guzman-Novoa et al. (2020) investigated the effects of hive management practices on honey bee colony health. The study found that colonies managed with integrated pest management techniques exhibited lower rates of disease and higher survival rates compared to conventionally managed colonies, highlighting the importance of proactive management strategies in maintaining healthy honey bee populations.

Overall, beekeeping practices play a critical role in shaping honey bee drone reproductive health and colony sustainability. By implementing proactive management strategies, beekeepers can optimize colony productivity, genetic diversity, and environmental conditions, ultimately contributing to the long-term viability of honey bee populations.

4.2. Transportation

The transportation of honey bee colonies for pollination services is a common practice in modern beekeeping. However, this process can expose drones to stressors such as vibration, temperature fluctuations, and limited foraging opportunities, which can impact their reproductive health and genetic diversity.

4.2.1. Impact on Drone Reproductive Health

Transportation stress can also impact the reproductive health of honey bee drones. Research by Zhao et al., (2021) investigated the effects of transportation stress on drone sperm viability. The study found that drones from transported colonies exhibited lower sperm viability and increased levels of sperm abnormalities compared to drones from non-transported colonies. These findings suggest that transportation stress can impair drone reproductive health and potentially reduce colony genetic diversity.

4.2.2. Management Strategies to Mitigate Transportation Stress

Beekeepers can implement management strategies to mitigate the effects of transportation stress on honey bee colonies. Research by Simone-Finstrom et al., (2016) investigated the effectiveness of pre-transportation hive ventilation in reducing stress levels in honey bee colonies. The study found that colonies provided with adequate ventilation prior to transportation exhibited lower stress responses and improved survival rates during transit. These findings suggest that proactive management practices, such as optimizing hive ventilation, can help minimize the impact of transportation stress on honey bee colonies.

4.2.3. Long-Term Implications for Colony Health and Genetic Diversity:

The long-term implications of transportation stress on honey bee colony health and genetic diversity are significant. Prolonged exposure to transportation stressors can weaken colony immunity, increase susceptibility to diseases, and reduce overall fitness. A study by El-Seedi. (2022) investigated the effects of transportation stress on honey bee colony genetic diversity. The study found that colonies subjected to frequent transportation events exhibited lower levels of genetic diversity and increased rates of inbreeding, highlighting the potential long-term consequences of transportation stress on honey bee population dynamics.

Overall, transportation stress poses a significant threat to honey bee colony health and genetic diversity. Beekeepers must implement proactive management strategies to minimize stress levels during transportation and safeguard the reproductive health and viability of honey bee populations.

5. CONCLUSION

In conclusion, the reproductive health of honey bee drones is influenced by a complex interplay of biological, environmental, and anthropogenic factors. Throughout this compilation, we have explored the various elements that impact drone fertility and survival, ranging from genetic diversity and physiological factors to environmental conditions and human activities.

Furthermore, it is evident that maintaining genetic diversity within honey bee colonies is paramount for mitigating the negative impacts of inbreeding and enhancing colony resilience. By prioritizing genetic diversity in queen rearing programs and implementing sustainable beekeeping practices, beekeepers can contribute to the long-term health and viability of honey bee populations.

Moreover, environmental stressors such as climate change, habitat loss, and pesticide exposure pose significant challenges to honey bee health and reproductive success. Addressing these challenges requires collaborative efforts from beekeepers, researchers, policymakers, and the broader community to promote pollinator-friendly practices and protect honey bee habitats.

Addressing the challenges facing honey bee drones requires a multifaceted approach that integrates scientific research, conservation efforts, and sustainable beekeeping practices. By promoting genetic diversity, preserving natural habitats, and implementing responsible management practices, we can strive to safeguard the reproductive health of honey bee drones and ensure the resilience of honey bee populations.

In essence, the well-being of honey bee drones is intricately linked to the health of their colonies and the broader ecosystem. As stewards of these vital pollinators, it is our collective responsibility to prioritize their conservation and protection. By working together to address the underlying factors affecting drone reproductive health, we can secure a sustainable future for honey bees and the ecosystems they support.

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CHAPTER III

BEES' ELECTROSTATIC FIELD PERCEPTION AND FLOWER ELECTRICAL SIGNATURES: EFFECTS ON POLLINATION EFFICIENCY

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DOI: https://www.doi.org/10.5281/zenodo.18138109

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1. INTRODUCTION

The mutually beneficial relationship between flowering plants and pollinators is a fundamental biological process critical to both the continuity of natural ecosystems and the efficiency of global agricultural production. According to current ecological analyses, approximately 75% of flowering plants and at least one-third of human food depend on animal pollination, with honeybees (*Apis mellifera*) and other Apoidea species providing a significant portion of this service (Klein et al., 2006). Bees' broad ecological tolerance, colony organization, and high foraging capacity make them primary pollinators in many ecosystems (Seeley, 2009).

For many years, pollinator-plant interactions have been primarily addressed within the framework of classical sensory categories such as visual signals (color, UV patterns), chemical cues (volatile organic compounds, nectar profile), and floral morphology (Clarke, Whitney, Sutton, & Robert, 2013). However, the rapid development of biophysical and neuroethological studies in the last decade has revealed a new sensory communication channel that significantly expands this framework: the electrostatic properties of flowers. While bees acquire a positive electric charge by ionizing air molecules during flight through wingbeats, flower surfaces are generally negatively charged due to grounding, epidermal structure, and atmospheric ions. This opposing polarity creates a natural electrostatic attraction and not only facilitates pollen transfer but also serves as a sensory cue that helps bees identify flowers (Sutton, Clarke, Morley, & Robert, 2016). Experiments conducted on Bombus terrestris have shown that bees can detect the electric field patterns of flowers, quickly learn these patterns, associate them with rewards, and actively use them in their choice behaviors (Clarke et al., 2013). Furthermore, the temporary change in surface charge caused by a bee landing on a flower serves as a "visit trace" for new arrivals, thus ensuring energy efficiency in resource use (Greggers et al., 2013).

These findings clearly demonstrate that flower-bee interactions cannot be explained solely by the classical model based on visual and chemical signals; floral electrostatic signals constitute a third sensory channel in pollination ecology. Thus, pollination processes are being redefined not as a one-dimensional communication system but as a complex sensory network integrating visual, chemical, and electrical cues.

Modern ecosystems, however, are increasingly surrounded by anthropogenic electromagnetic fields originating from mobile communication systems, Wi-Fi networks, high-voltage power lines, and other radio-frequency devices. Whether this artificial electrical noise alters the natural electrostatic signatures of flowers and its potential effects on bee behavior is a growing topic of ecological and agricultural research (Molina-Montenegro et al., 2023; Shepherd et al., 2018).

This book chapter examines the biophysical basis of flower electrostatic properties, bees' electrostatic field detection mechanisms, the behavioral outcomes of these signals, and the potential disruptive effects of anthropogenic electromagnetic fields on this sensory channel from a holistic ecological perspective.

2. ELECTROSTATIC PROPERTIES OF FLOWERS

Communication between plants and pollinators has long been explained by classical sensory cues such as color, scent, and morphological structure. However, recent biophysical research has revealed that the electrical charges accumulated on flower surfaces are an integral component of this communication network.

The surface potential of most flowers is slightly negative; this is related to the plant's grounded root system, the electrical properties of the petal epidermis, and its constant contact with the atmospheric electric field (Madariaga et al., 2024; Volkov, 2006). In contrast, bees lose electrons and gain a positive charge by rubbing against air molecules during flight. This creates a natural potential difference between the bee and the flower, creating both a physical attraction that facilitates pollen transfer and a complementary sensory cue that can be detected from a distance for bees (Clarke et al., 2013; Sutton et al., 2016). Figure 1 summarizes the main sources of electrostatic potential on flower surfaces and their interaction with bees.

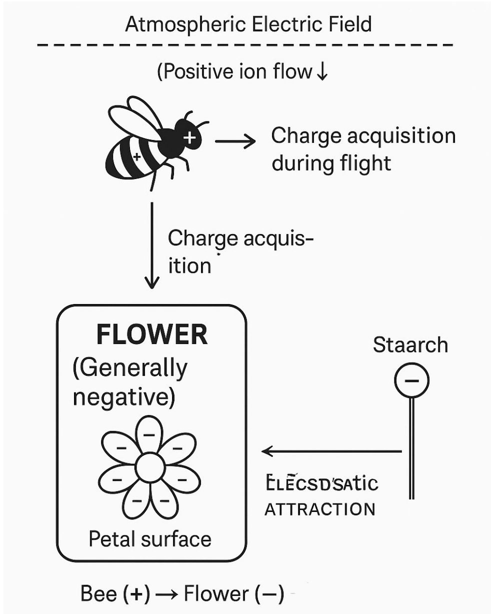


Figure 1. Electrostatic Flower–Bee Interaction

Flower surface potential is dynamic and sensitive to environmental conditions. The atmospheric electric field changes continuously throughout the day depending on factors such as insolation, cloud cover, air ionization, wind speed, and relative humidity (Rycroft, Israelsson, & Price, 2000)Under dry conditions, the electrical resistance of the petal epidermis increases, making the surface charge more stable, while under humid conditions, surface conductance increases, causing charge dissipation to accelerate. Therefore, the morphology and microclimate of each plant species create a unique "electrical signature." Sutton et al. (2016) showed that surface potentials in Petunia and Lavandula species can range from a few hundred millivolts to a few volts, and this variation creates distinctive species-specific electric field patterns (Figure 2).

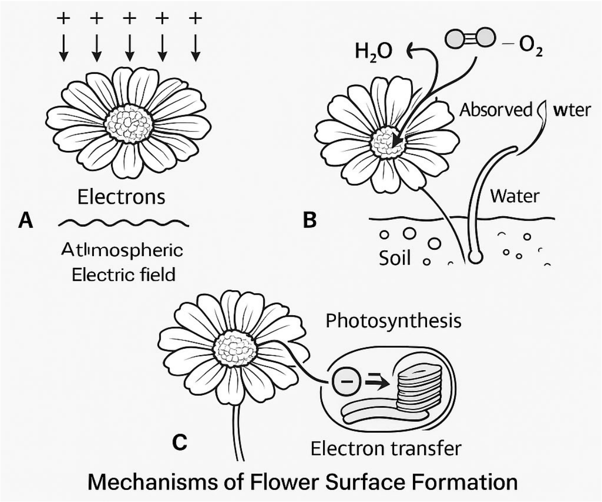


Figure 2. Mechanisms of Flower Surface Charge Formation

One of the most obvious consequences of electrostatic charge differences is their effect on pollen grain behavior. Pollen grains are generally neutral or slightly negatively charged; when a positively charged bee approaches a flower, the pollen grains are drawn to the bee hairs by electrostatic attraction without mechanical contact. Clarke et al. (2013) experimentally demonstrated that a positively charged artificial bee model attracts approximately three times more pollen than a neutral model. This process reduces pollen loss, particularly in species with thin pollen, thus improving reproductive success. This mechanism is illustrated in Figure 3.

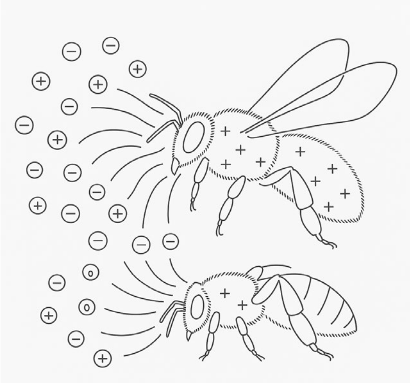


Figure 3. Electrostatic Attraction of Pollen to Positively Charged Bees

When a bee lands on a flower, some of its positive charge transfers to the floral surface, creating a transient change in the flower's electrical signature. Greggers et al. (2013) showed that this change remains detectable for 1–3 minutes and serves as a "visiting signal" to newly arriving individuals. This information prevents bees from unnecessarily visiting flowers that have depleted nectar, allowing them to optimize their energy (Figure 4).

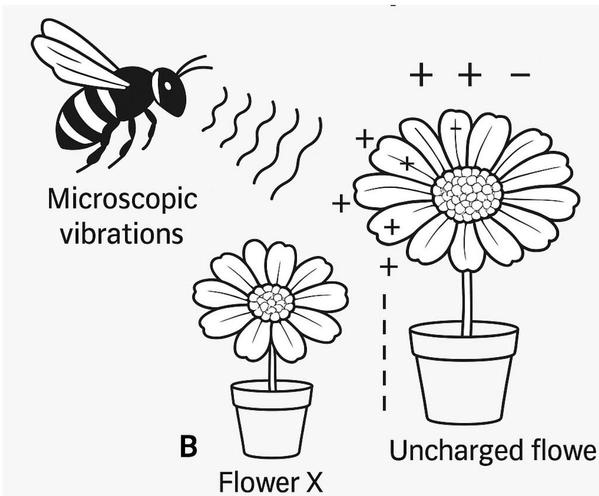


Figure 4. Floral Electrostatic Field Detection by Bees

Flower morphology is also a critical factor determining the three-dimensional structure of the electrostatic field. Petal shape, tube length, epidermal cell architecture, and surface hair growth alter the intensity distribution of the electric field surrounding the flower. Sutton et al. (2016) mapped these electric field patterns and showed that each species produces a unique electrostatic morphology. Bees can detect these fields from a distance of approximately 10 cm, a significant advantage that allows bees to accurately identify flowers, especially in low-light or windy conditions.

Electric field strengths measured across different species vary widely. Species such as Petunia integrifolia and Lavandula angustifolia produce higher field gradients, while species such as Helianthus annuus have more moderate field strengths (Volkov, 2006). This diversity suggests that plants have invested not only in classical sensory signals but also in electrostatic signatures as an evolutionary tool for attracting pollinators. Consequently, the electrostatic properties of flowers are a critical component in pollination ecology, both for pollen transfer and for guiding bee behavior. These electrical signals, working in conjunction with visual and chemical cues, ensure that plant-bee interactions are orchestrated by a holistic sensory network.

3. BEE ELECTROSTATIC FIELD DETECTION MECHANISM

Bees' ability to perceive floral electric field patterns reveals a new layer of communication in pollination ecology that goes beyond classical sensory systems (vision, smell, taste). Neuroethological and biophysical studies on Bombus terrestris and *Apis mellifera* have shown that electric field perception relies on both the charging processes acquired during flight and the highly sensitive responses of mechanosensory structures (Clarke et al., 2013; Sutton et al., 2016) (Figure 5).

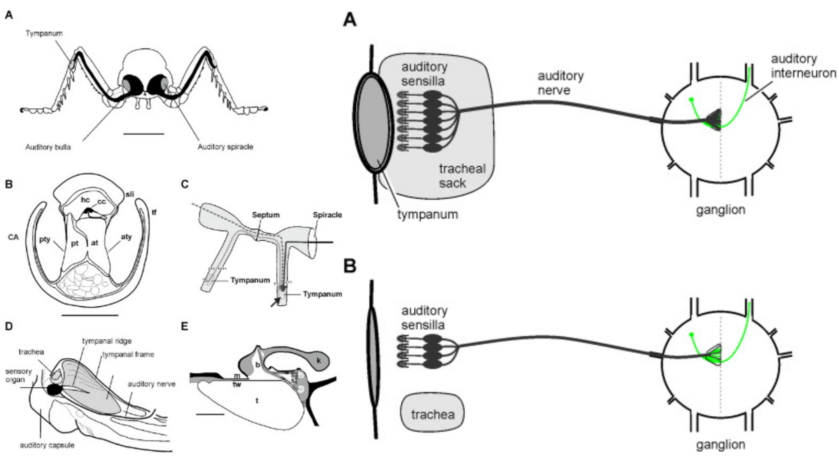


Figure 5. Mechanosensory Hair Structure and Electric-Field Detection Pathway

3.1. Charge Acquired by Bees During Flight

Bees ionize air molecules during flight by flapping their wings, and as a result of the loss of electrons, they acquire a positive charge. When bees fly, they acquire a positive (+) charge. This charge is weak but sufficient to attract pollen/particles (Clarke et al., 2013).

This charge strengthens the electrostatic gradient between the flower and the bee, increasing the efficiency of pollen transfer and making mechanosensory structures more sensitive to changes in the electric field. The image below (Figure 6) summarizes this process.

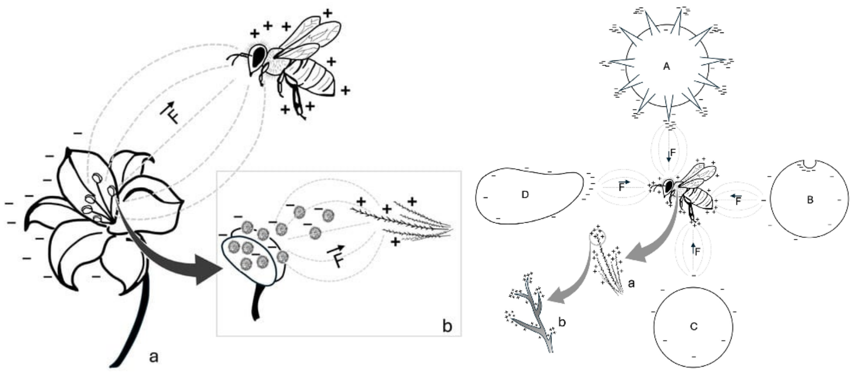


Figure 6. Bee Electrostatic Charge Acquisition During Flight

3.2. The Role of Mechanosensory Hairs in Electrostatic Sensation

The bee body is covered with fine, flexible mechanosensory hairs located on the thorax, abdomen, legs, and antenna segments. These hairs are highly sensitive biomechanical receptors that can detect even the smallest changes in external electric fields. A minimal change in electric field intensity causes a bending of the hairs at the nanometer scale. This mechanical deflection is converted into an electrical signal by stimulating voltage-sensitive ion channels in the hair root.

Using a Laser Doppler Vibrometer (LDV), Sutton et al. (2016) recorded nanometer-level vibrations at the hair tips, even at electric fields as low as 100 V/m. This finding is one of the strongest evidences that bee hairs are sensitive enough to detect weak electric fields. This process is conceptually illustrated in Figure 7.

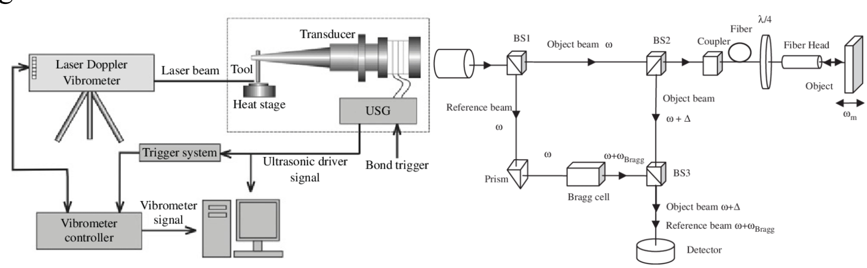


Figure 7. Electric-Field–Induced Vibration of Mechanosensory Hairs (LDV Concept)

3.3. The Role of the Antenna and Johnston's Organ

The bee antenna is a complex sensory platform capable of simultaneously processing chemical, mechanical, and electrical information. Although the Johnston's organ, located on the second segment of the antenna, has traditionally been described as a structure that detects air vibrations, Greggers et al. (2013) have shown that this organ is also sensitive to changes in electric fields.

Antenna segments acquire a positive charge during flight, and even the slightest change in the electric field direction triggers neural activation in the sensilla. Therefore, the antenna serves as a receptive center that provides continuous information about the direction and intensity of the electric field.

3.4. Transmission of Signals to the Central Nervous System

Signals from the mechanosensory hairs and antennal sensilla are transmitted via peripheral neurons to the protocerebrum and, in particular, to the mushroom bodies, a region responsible for learning and memory processes. Clarke et al. (2013) reported that bees learned different electric field patterns after approximately 30–40 trials, and that this learning rate was comparable to olfactory learning.

This result demonstrates that electrostatic signals are not merely perceived environmental information but also an active component of bee cognition.

3.5. Differences in Sensitivity Among Species

Bee species exhibit varying levels of sensitivity to electrostatic fields:

- Bombus terrestris exhibits greater mechanical sensitivity to electric field changes due to its longer and stiffer hair structure.
- *Apis mellifera*, despite having finer hair structure, can behaviorally distinguish differences in electric field direction and intensity.

These differences are closely related to the species' ecological niches and foraging strategies.

3.6. The Role of Electrostatic Information in Recognizing Visited Flowers

When a bee lands on a flower, some of its positive charge is transferred to the flower, temporarily altering the flower's electrostatic signature. Greggers et al. (2013) demonstrated that this change remains detectable for 60–180 seconds and serves as a "visit signature" for newly arriving bees. The logic of this mechanism is illustrated in Figure 8.

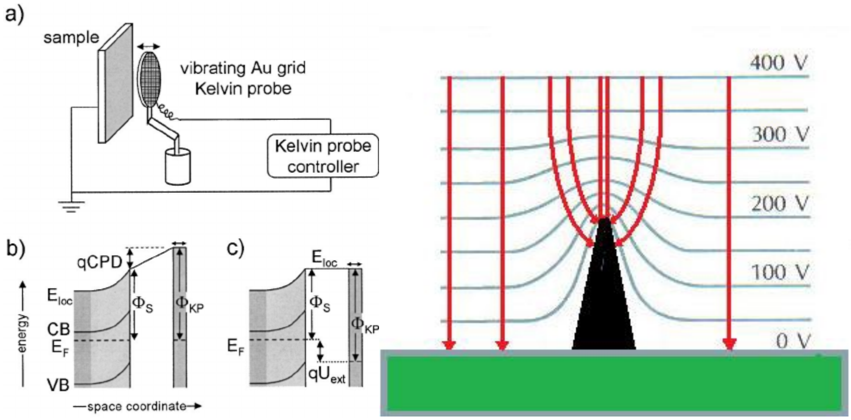


Figure 8. Floral Electric Field Mapping (Kelvin Probe / Field Mill Concept)

This mechanism prevents bees from revisiting flowers with depleted nectar, optimizes flight paths, and increases the colony's overall energy efficiency.

4. EFFECTS OF ELECTROSTATİC SİGNALS ON BEE BEHAVİOR

Bees' interaction with flowers is not limited to visual (color, UV patterns) and chemical (volatile compounds, nectar odor) signals. Weak electric fields generated around flowers also constitute an important sensory component that shapes bees' flower detection, approach, selection, and nectar search strategies. Studies such as Clarke et al. (2013), Sutton et al. (2016), and Zakon (2016) clearly demonstrate that electrostatic signals serve as an independent information channel in bee decision-making.

4.1. Flower Detection and Approach Behavior

Bees not only utilize visual contrast and UV patterns when detecting flowers; they also perceive electric field gradients around the flower. Sutton et al. (2016) showed that Bombus terrestris individuals can distinguish weak electric field changes from approximately 10 cm away from flowers. These electrical signals form a complementary guidance mechanism that allows bees to detect flower location from a distance, acquire preliminary information about petal morphology, and optimize landing angles.

4.2. Flower Choice and Resource Evaluation

Experiments conducted by Clarke et al. (2013) demonstrated that bees can choose the correct flower based solely on electric field patterns. Even when color and odor cues were completely removed from the experimental setting, bees continued to make the correct choice by associating electric field patterns with reward. This result demonstrates that electrostatic patterns are a type of information that can be learned, remembered, and associated with reward for bees. Therefore, bees recognize the electrical signatures of flowers with high nectar content over time and tailor their foraging strategies based on this sensory information. The laboratory setups for this process are shown in the image below (Figure 9).

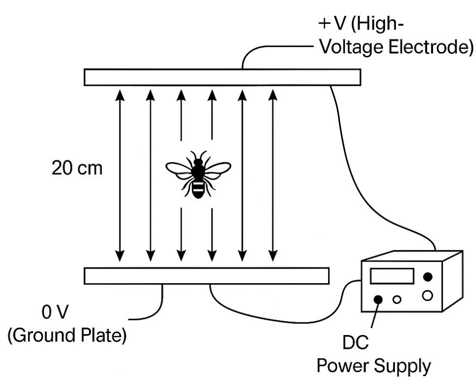


Figure 9. Parallel Plate Electric Field Chamber (Controlled E-Field Setup)

4.3. Recognizing Visited Flowers

When a bee lands on a flower, some of its positive charge is transferred to the flower surface, and this interaction causes a short-lived change in the flower's electrical signature. Greggers et al. (2013) found that this change remains detectable for 60–180 seconds and is interpreted by newly arriving bees as a "visit signature." This information allows bees to bypass flowers that have recently depleted nectar, reduce unnecessary visits, and adjust their flight path more efficiently. Therefore, electrostatic signals contribute significantly to more economical flower-to-flower migrations and increased energy conservation at the colony level.

4.4. The Role of Electrostatic Signals in Nectar Foraging Strategies

The time bees spend on a flower, their nectar collection speed, and their contact point selection are largely influenced by the electrical signals generated by the flower. Morley and Robert (2018) showed that electric fields shorten bees' landing time, facilitate the determination of the correct contact point, and accelerate orientation to nectar-rich areas. These advantages make electrostatic information an even more valuable guidance mechanism for bees, especially in low-light conditions, environments where odor signals are dispersed by wind, and habitats with high flower density.

4.5. Learning, Memory, and Decision-Making Processes

Bees not only perceive electric fields; they actively use these signals in their learning processes. Clarke et al. (2013) demonstrated that bees can learn electric field patterns within 30–40 trials, and this learning rate is similar to olfactory learning. These findings suggest that electric fields are integrated into bee cognitive processing, processed by mushroom bodies, and play a key role in guiding reward-driven behaviors.

4.6. Interflower Migration and Energy Optimization

Thanks to electrostatic signals, bees can establish shorter and more efficient flight paths, reducing time and energy costs by avoiding unnecessary flower visits, and thus increasing the colony's total nectar and pollen yield. The importance of this sensory advantage is clearly evident in bees with impaired electrostatic perception. Indeed, Migdał et al. (2025) reported that under conditions where electrostatic perception is impaired, bees select incorrect flowers more frequently, foraging time is significantly prolonged, and total energy consumption increases significantly, demonstrating that electrostatic signals play a critical role in colony-level foraging efficiency.

4.7. Behavioral Differences Between Species

There are significant differences in electrostatic sensitivity between Bombus terrestris and *Apis mellifera*. Bombus terrestris exhibits greater mechanical sensitivity to changes in electric fields due to its stiffer and longer hair structure, while *Apis mellifera*, despite its thinner hairs, is quite capable of distinguishing the direction and intensity of electric fields at a behavioral level. These sensory differences are thought to be related to the ecological niches, feeding strategies, and foraging behaviors of both species.

4.8. Integration of Electrostatic, Visual, and Chemical Cues

When assessing the suitability of a flower, bees utilize multiple sensory categories, such as UV patterns, volatile organic compounds (VOCs), nectar sugar content, and the flower's electrostatic signature. This integrated assessment allows bees to accurately determine both reward potential and the flower's visitability. Especially in low light conditions, high plant density, or habitats with increased competition, electrostatic signals become a discriminatory and orientation guide for bees, compensating for the weakening of other sensory cues.

5. EFFECTS OF ANTHROPOGENIC ELECTROMAGNETIC FIELDS (EMF) ON BEE BEHAVIOR AND ELECTROSTATIC PERCEPTION

The natural electrostatic communication system between plants and bees relies on the bees' highly sensitive detection of weak electric fields generated by charges on the flower surface. However, the modern environment is surrounded by intense electromagnetic noise generated by mobile communication networks, Wi-Fi systems, radio base stations, high-voltage power lines, and other electronic infrastructure. There is growing scientific evidence that these artificial electromagnetic fields (EMFs) can disrupt both the natural electrical signatures of flowers and the ability of bees to perceive these signals (Erdoğan & Cengiz, 2019).

Natural floral electric fields perceived by bees are generally in the range of 50–200 V/m; mechanosensory hairs can detect even small changes in these weak fields through nanometer-scale vibrations. In contrast, high-frequency electrical noise generated by anthropogenic EMF sources can disrupt the ability of flowers to perceive these signals (Figure 10).

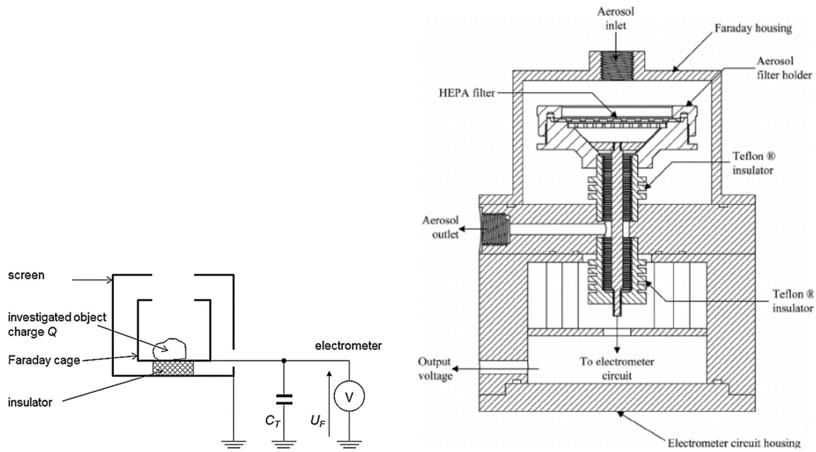


Figure 10. Faraday Cage & Electrometer Setup (Charge Measurement in Bees).

EMF exposure not only weakens electrostatic perception; it can also affect bees' flight stability and navigation behavior (Erdoğan & Cengiz, 2019). Harst, Kuhn, and Stever (2006) reported that bees exposed to 900 MHz GSM signals took significantly longer to return to the hive, and individuals exhibited deviations in their flight paths. Favre (2011) showed that stress behavior called "piping" significantly increased in colonies under GSM signal exposure. These findings suggest that EMF affects the neurophysiological processes involved in bee navigation.

The effects of EMF exposure on cognitive processes are also noteworthy. Bees integrate multiple categories of information, such as odor, visual patterns, and electrostatic signals, in the mushroom body region during learning and memory processes. Thielens, Greco, Verloock, Martens, and Joseph (2020) showed that RF signals alter neuronal response dynamics in antennal sensilla, resulting in reduced learning success in PER (Proboscis Extension Reflex) conditioning tests. Bees exposed to EMF require more trials to learn signals associated with reward, and their long-term memory performance is impaired.

At the colony level, EMF exposure can affect social organization. Increased "piping" behavior, as demonstrated by Fèvre and Dearden (2024) is one of the early indicators of acute stress responses in the colony. Some controlled experiments have reported that EMF exposure disrupts the task distribution of worker bees, causes delays in brood care, and negatively impacts hive entry and exit traffic. Although some of these results are based on a limited number of studies, they suggest that intra-colony organization may be sensitive to electromagnetic noise.

The weakening of electrostatic perception by EMF can increase the error rate in bees' behavioral processes, such as discriminating between flowers, recognizing recently visited flowers, and determining correct landing spots. This can lead to longer flight times, increased energy consumption, and decreased foraging efficiency at the colony level. Migdal et al. (2023) reported that the accuracy of bees discriminating flower patterns under EMF exposure was significantly reduced. However, there is no full consensus in the literature. Some studies suggest that low-intensity environmental EMF has minimal effects, while others have reported significant behavioral and physiological effects. These discrepancies suggest that the effects of EMF may depend on variables such as type, duration of exposure, field strength, frequency, and environmental context.

Increased environmental electromagnetic noise can impair bees' ability to detect natural electrostatic signals from flowers, potentially leading to knock-on ecological consequences for pollination processes. Therefore, EMF is considered a new stress factor that threatens the sensory basis of bee-plant interactions in modern ecosystems.

6. ELECTROSTATIC MEASUREMENT TECHNIQUES AND EXPERIMENTAL APPROACHES

Understanding electrostatic signals in bee-flower interactions requires high-precision measurements of both the electrical charges on the flower surface and the electrostatic charges acquired by bees during flight. Modern research; It utilizes a broad methodological framework, ranging from non-contact surface potential measurements to the analysis of mechanosensory hair responses using Laser Doppler Vibrometry (LDV), from quantifying bee loading in Faraday cages to controlled electric field experiments (Clarke et al., 2013; Sutton et al., 2016).

6.1. Measuring Electrostatic Charges on Flower Surfaces

The electrical potential of the flower surface is most often determined with non-contact electrostatic meters. Portable electrostatic field meters such as the Extech 480846, Simco FMX-004, and Prostat PRS-812 provide high sensitivity in the field. In laboratory settings, the Trek 347 or Monroe Electronics models offer higher resolution.

In the standard application protocol, the sensor is held approximately 10 mm from the flower surface, and the average surface potential is calculated by taking measurements at different points from the petal tip to the base (Sutton et al., 2016) .This method allows for the reproducible determination of species-specific “electrostatic signatures.”

6.2. Measuring the Charge Acquired by Bees During Flight

The most reliable method for measuring the electrostatic charge acquired by bees after flight is a Faraday cage-electrometer combination. High-sensitivity electrometers such as the Keithley 6514 or 6517B can measure the charge carried by bees at the picoculum (pC) level.

After a short period of free flight, bees are guided into the Faraday cage, and the net charge of the individual is recorded by the electrometer upon entry. Sutton et al. (2016) have shown that bees typically carry a positive charge between +50 and +200 pC based on these measurements. This setup is shown in Figure 10.

6.3. Measuring the Response of Mechanosensory Hairs to Electric Fields (LDV)

Nanometer-scale vibrations of bee hairs to electric fields are one of the strongest direct evidence of electrostatic sensing capacity. Laser Doppler Vibrometer (LDV) systems (Polytec PDV-100, OFV-5000, etc.) used for this purpose record micro vibrations occurring at the feather tips with high resolution.

In the experimental protocol, the bee is lightly anesthetized, the LDV laser is focused on the designated feather, and a controlled electric field is applied. As the electric field changes, the amplitude, resonance, and frequency response of the feather are recorded (Sutton et al., 2016). LDV-based measurement setups are shown in Figure 11.

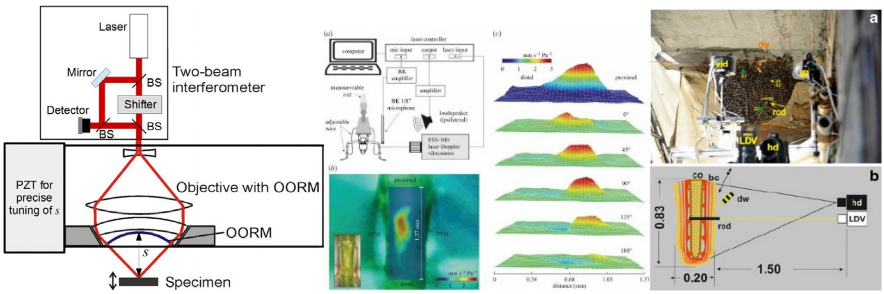


Figure 11. Laser Doppler Vibrometry (LDV) Setup for Measuring Bee Hair Deflection

6.4. Mapping Floral Electric Field Patterns

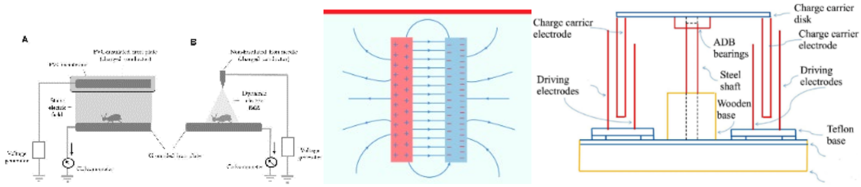
Three-dimensional electric field patterns of flowers can be mapped using Kelvin probe devices (e.g., KP Technology SKP5050) or rotating electrode-based Field Mill systems (e.g., Monroe Electronics models). These technologies measure potential gradients without contacting the flower surface and quantitatively reveal the effect of floral morphology on the electric field.

These techniques allow the species-specific electrical signatures of flowers to be reconstructed as three-dimensional models.

6.5. Controlled Electrostatic Field Experiments

Parallel-plate electric field chambers are used to study the electric field sensitivity of bees. The field strength generated in these chambers is calculated by the voltage applied across the plate gap, and stable electric fields are typically obtained in the range of 10–150 V/m.

The typical setup used by Sutton et al. (2016) has a 20 cm plate spacing and a voltage capacity of 0–300 V DC. These environments offer a wide range of research opportunities, from feather vibration experiments to directional sensitivity studies. This setup is shown in Figure 12.



Figuge 12. Parallel-Plate Electrostatic Field Chamber Used in Bee Electroreception Experiments

6.6. Experimental Arenas for Assessing Bee Behavior

Y-mazes, dual-choice arenas, and circular flight arenas are used to examine how bees use electrostatic signals at a behavioral level. Clarke et al. (2013) developed an "electrostatically stimulated feeder" system that evaluates bee choice behavior with electrostatically charged artificial flowers. These arenas allow for quantitative measurement of bee decision-making time, landing site selection, and error rates (Figure 13).

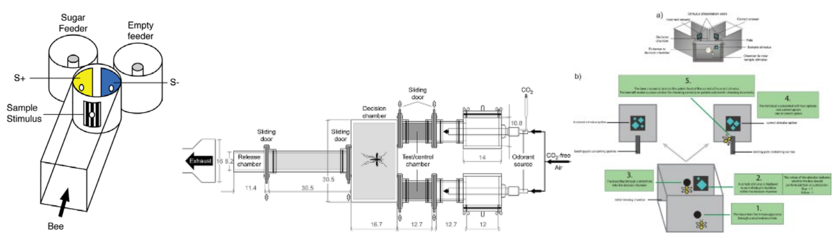


Figure 13. Electrostatic Choice Arena (Y-Maze / Dual-Choice Behavioral Setup)

6.7. EMF Exposure Experiments

RF signal generators (e.g., Rohde & Schwarz SMB100A), power amplifiers, and EMF meters (e.g., TES 593) are used to study the effects of anthropogenic electromagnetic fields on bees. Spectrum analyzers such as the Narda SRM-3006 verify the accuracy of signal parameters. These devices offer the opportunity to study the effects of RF fields of specific frequencies and intensities on bee behavior in a controlled manner.

6.8. Data Processing and Analysis Software

Polytec software is used for processing LDV data; MATLAB-based simulation modules are used for electric field models; and ArenaTracker, ImageJ, and specialized motion tracking software are used for behavioral analysis. These software enables sophisticated analyses such as frequency analysis, signal filtration, three-dimensional field reconstruction, and behavioral classification.

7. THE IMPORTANCE OF ELECTROSTATIC SIGNALS FOR ECOSYSTEMS, PLANT REPRODUCTION, AND AGRICULTURAL POLLINATION

The electrostatic dimension of bee-flower interactions is not merely a sensory element that guides individual behaviors; it is a fundamental biophysical mechanism that directly impacts plant reproductive success, pollination efficiency, and the sustainability of ecosystem functions. The electrical signatures of flowers and the electrostatic sensing ability of bees create an invisible communication network that increases the efficiency of the pollination process in both natural ecosystems and agricultural production systems.

Plant reproductive success depends largely on the efficient transport of pollen to the stigma surface. The electrostatic attraction created when a positively charged bee approaches the flower surface enhances pollen transfer not only through mechanical contact but also through electrical forces. Clarke et al. (2013) demonstrated the effectiveness of this mechanism in pollen transport by demonstrating that a positively charged artificial bee model attracted approximately three times more pollen than a neutral model. This effect reduces pollen loss, particularly in species with thin pollen and growing in wind-exposed habitats, increasing the plant's seed-setting rate.

For bees, electrostatic signals contain critical information such as the flower's freshness, nectar quantity, and recent visitation. Greggers et al. (2013) showed that the electrical signature change caused by a bee landing on a flower transferring its positive charge to the surface remains detectable for 1–3 minutes, and that newly arriving bees use this information as a "visited signal" that saves energy. This mechanism allows bees to avoid unnecessary flower visits and establish more efficient flight paths, creating a net energy gain at the colony level.

At the ecosystem level, electrostatic signals play a crucial role in regulating interactions between plant communities. Different plant species produce unique electrostatic signatures; this diversity facilitates bees' orientation to the right species, even in habitats with high plant density. This prevents excessive pressure on densely visited flowers while also providing less frequently visited species with the opportunity to pollinate. This balancing mechanism enhances the stability of pollination networks, particularly in arid areas, high-altitude ecosystems, and regions with a high concentration of endemic species. The importance of electrostatic signals in agricultural ecosystems is becoming increasingly evident. Pollinator-dependent plants species such as apple (Malus domestica), pear, strawberry (Fragaria × ananassa), blueberry, rapeseed (Brassica napus), and cucurbits indirectly benefit from bee electrostatic perception. Morley and Robert (2018) reported that electrostatic interactions shorten bee-flower contact time, increase pollen exchange, and enable bees to position themselves more accurately on the flower. Consequently, they have a positive impact on crop set, fruit quality, and seed yield.

The importance of this mechanism becomes even more evident when considering the global economic value of pollination services. Economic analyses from the European Union estimate the annual economic value of bee pollination to exceed €15 billion (Klein et al., 2007). Electrostatic signals can be considered one of the invisible yet functionally critical components of this vast ecological service.

The increasing presence of anthropogenic electromagnetic noise (EMF) in the modern environment poses the risk of masking flowers' natural electrostatic signatures and diminishing bee sensory sensitivity. Shepherd et al. (2018) and Panagopoulos, Johansson, and Carlo (2015) have shown that RF-EMF exposure can reduce pollination efficiency by impairing bee electric field perception and behavioral accuracy. These findings demonstrate that electrostatic signals are critical not only for behavioral but also for environmental stress ecology.

In conclusion, electrostatic interactions are a versatile mechanism that forms the biophysical basis of plant-pollinator interactions and play an essential role in ecosystem resilience and agricultural production security. The integrity of this sensory channel is a fundamental element that must be preserved for both the functional sustainability of natural ecosystems and agricultural productivity.

8. CONCLUSION

This section has presented a comprehensive review of the rapidly expanding literature on the role of electrostatic signals in bee-flower interactions in recent years. The traditional approach has primarily explained pollination processes through visual (color, UV patterns) and chemical (volatile compounds, nectar odor) cues. However, pioneering studies such as Clarke et al. (2013); (Greggers et al., 2013; Sutton et al., 2016) have clearly demonstrated that the electrical signatures of flowers are also an integral part of this communication network. The positive charge bees acquire during flight and the slightly negative potential of flower surfaces create a natural electrostatic gradient that supports pollen transfer and flower selection processes.

The presence of electrostatic signals provides significant ecological advantages for both plants and bees. The electrostatic attraction generated by a positively charged bee approaching a flower in plants enhances reproductive success by extending pollen transfer beyond mechanical contact. For bees, the electrical signature of a flower: It functions as a sensory guide, encoding critical information such as freshness, nectar content, and recent visitation. The "visitation trail" mechanism, specifically described by Greggers et al. (2013) allows bees to avoid unnecessary visits and conserve energy at the colony level.

At the ecosystem scale, electrostatic communication is a complementary process that strengthens the stability of pollination networks. The unique electrical signatures produced by different plant species contribute to bees' ability to distinguish the correct flower species even in complex and dense plant communities. This mechanism supports the maintenance of ecosystem functions by regulating pollinator behavior, particularly in habitats dominated by challenging environmental conditions. In agricultural production systems, electrostatic interactions shorten bee-flower contact time, increasing pollen exchange, and consequently having a positive impact on fruit set rate and crop yield.

However, the increasing presence of anthropogenic electromagnetic fields (EMFs) in the modern environment appears to pose a risk of masking the natural electrostatic signatures of flowers and weakening bees' sensory sensitivity. Shepherd et al. (2018) and Panagopoulos et al. (2015) reported that RF-EMF exposure can negatively impact both electrostatic perception and behavioral processes such as orientation, learning, and decision-making. This suggests that the electrostatic sensory channel is critical not only in the context of pollination biology but also in modern environmental stresses.

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CHAPTER IV

NEW GENERATION APPROACHES TO HONEY AUTHENTICITY ANALYSIS

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DOI: https://www.doi.org/10.5281/zenodo.18138159

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1. INTRODUCTION

Honey is a bee product with high nutritional value and biological activity due to its enzymes, phenolic compounds, organic acids, and rich carbohydrate structure (Alvarez-Suarez, Tulipani, Romandini, Bertoli, & Battino, 2010; Bogdanov, Jurendic, Sieber, & Gallmann, 2008). This biochemical richness makes honey not only a traditional food but also a functional product with high commercial value. Increasing global demand has made economically motivated counterfeiting a more visible problem. The 2020 report of the European Union Food Fraud Network indicates that a significant portion of honey circulating in international markets exhibits findings of questionable authenticity (European Commission, 2020; Moore, Spink, & Lipp, 2012).

Today, honey fraud has gone far beyond simple sugar additions. The carbohydrate profiles of corn, rice, or other starch-based syrups are increasingly approaching those of natural honey, making detection difficult with conventional physicochemical tests. Furthermore, practices such as removing pollen structure through ultrafiltration, blending honey from different geographical regions, heat treatments, or aroma and color manipulations also obscure the true botanical and geographical identity of the product (Kaškonienė & Venskutonis, 2010; Moore et al., 2012). Inappropriate heat treatments lead to significant deterioration in key quality indicators such as diastase activity, HMF levels, and volatile compound profiles.

Therefore, modern honey authentication studies require a multi-component approach rather than relying on a single analysis. Nuclear Magnetic Resonance (NMR), Isotope Ratio Mass Spectrometry (IRMS), LC-MS/MS, DNA barcoding, FTIR/Raman spectroscopy, and chemometric classification methods have gained significant ground in identifying current types of fraud (Hansen, Kunert, Raezke, & Seifert, 2024; Martinello, Stella, Baggio, Biancotto, & Mutinelli, 2022; Elisabetta Schievano, Stocchero, Morelato, Facchin, & Mammi, 2011). In particular, the ability of NMR-based metabolic fingerprint analyses to assess both syrup adulteration and geographical/botanical origin differences within the same measurement is a key advantage that distinguishes the method.

Fraud is not limited to chemical manipulation; it is also associated with labeling errors, missing records, and attempts to conceal origin throughout the supply chain. The length of the global trade chain, inconsistencies in auditing standards, and information asymmetry between producer and consumer further complicate this process (Almiani, Mirza, Zufferey, Alyammahi, & Lamghari, 2025). Therefore, it is increasingly important to supplement laboratory analyses with digital solutions particularly blockchain-based traceability systems that ensure data integrity throughout the supply chain (Galvez, Mejuto, & Simal-Gandara, 2018).

This review examines the primary methods used in honey fraud, the strengths and limitations of current analytical verification techniques, and the potential contributions of digital traceability models that increase supply chain transparency to the industry. It has been demonstrated that a holistic approach that evaluates chemical, biological and administrative dimensions together may be the most effective solution in combating honey fraud.

2. THE SCALE OF GLOBAL HONEY FRAUD AND ITS ECONOMİC IMPACTS

The global honey trade has grown significantly over the past two decades, creating a multibillion-dollar market with an annual production of approximately 2.1 million tons (FAO, 2023). However, this economic growth has made honey one of the food products most vulnerable to counterfeiting. The 2020 report of the European Union Food Fraud Network reported that approximately one-third (approximately 32%) of honey on international markets was suspected of non-conformity in terms of authenticity (European Commission, 2020). This rate makes honey one of the most counterfeited food categories, along with olive oil and dairy products.

Multiple factors contribute to the prevalence of honey counterfeiting. Chief among these are the product's high commercial value, its relatively vulnerable chemical composition to manipulation, the multi-actor and fragmented nature of the supply chain, and the specialized nature of laboratory verification processes (Bose & Padmavati, 2024). Countries such as China, India, Argentina, Turkey, and Ukraine account for the majority of global production. Price differences and export pressures in these countries have occasionally led to the introduction of lower-quality products in syrup or blended form. Indeed, Chinese honey exports have been one of the most frequent areas of international trade investigations over the last 10-15 years (Elisabetta Schievano, Morelato, Facchin, & Mammi, 2013).

Market controls conducted in the US also yield significant findings. A widely reported journalistic investigation conducted by Food Safety News in 2011 found no pollen in some samples taken from large chain supermarkets. While not a scientific study, this study generated significant public debate about the potential use of ultrafiltration to conceal origin information. The detection of high amounts of syrup-laced honey during operations conducted by the US Customs and Border Protection Agency between 2013 and 2022 during the same period highlights the economic dimension of the problem.

In the European Union, a multi-country analysis published in 2022 found that approximately 46% of imported honey contained suspected syrup adulteration (European Parliament, 2023). This situation both increases price pressure in producing countries and puts domestic producers within the EU at a serious competitive disadvantage. Counterfeiting of origin in high-value-added products with geographical indications such as Manuka, Anzer, or Sidr honey leads to direct economic losses (Yildiz et al., 2022).

Similar problems are also reported in Turkey. Various studies have detected traces of C4 sugars or rice/corn-derived syrups in some commercial honeys (Guler et al., 2014; Kivrak, Kivrak, & Karababa, 2016; Tosun, 2013). The Ministry of Agriculture and Forestry's official inspection lists also indicate that the number of honey recalled for "imitation and adulteration" has increased in recent years (Ministry of Agriculture and Forestry of Türkiye, 2021). These findings demonstrate the criticality not only of laboratory practices but also of supply chain transparency and regulatory oversight.

Overall, the economic impacts of honey fraud are not limited to producer revenue losses. Artificially depressing market prices disadvantages small and medium-sized beekeepers, while diminished consumer confidence and resulting discrepancies in international trade threaten the long-term sustainability of the sector. Therefore, reducing economic losses requires both strengthening scientific verification methods and establishing effective traceability mechanisms throughout the supply chain.

3. TYPES OF HONEY ADULTERATION

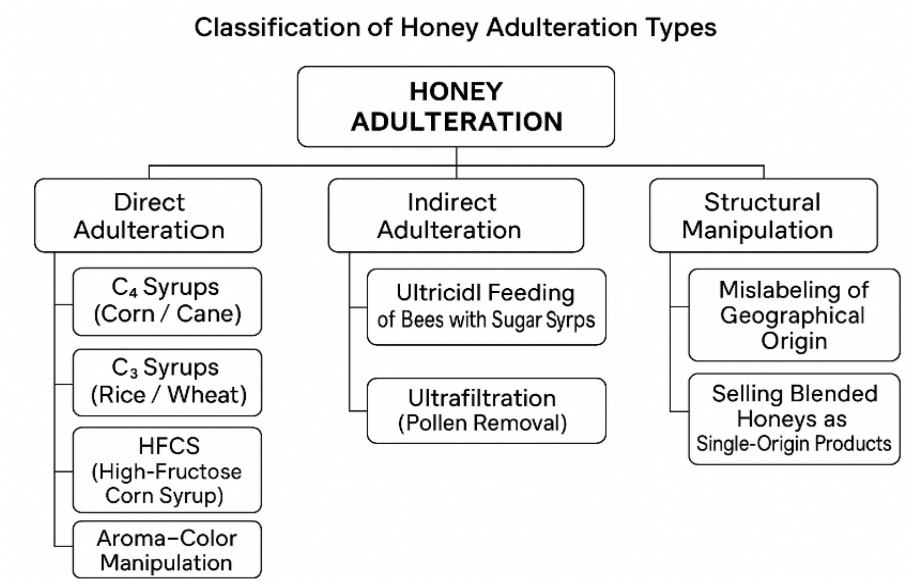

Figure 1. Classification of honey adulteration types and their major subcategories.

This diagram summarizes the main categories of honey adulteration reported in the scientific literature, including direct sugar-syrup adulteration (C_3/C_4 syrups and HFCS), indirect adulteration through artificial feeding, structural manipulations such as ultrafiltration and excessive heating, and labeling-related frauds such as misrepresentation of geographical origin or blending practices.

Table 1. Major categories of honey adulteration and their defining characteristics.

Fraud Category	Definition	Typical Indicators	Most Effective Detection Methods
Sugar Syrup Addition	Direct mixing of honey with corn, cane, rice or beet syrups.	Altered sugar ratios, reduced enzyme activity, $\delta^{13}C$ deviations.	IRMS (C_4 syrups), NMR profiling, LC-MS/MS (oligosaccharide markers).
HFCS Blending	Incorporation of high-fructose corn syrup to mimic natural sweetness.	Normal sensory profile despite biochemical dilution.	NMR metabolomics, IRMS + chemometric comparison.
Indirect Adulteration (Sugar Feeding)	Bees produce “honey-like liquid” after being fed commercial syrup.	Low diastase, altered protein signatures, atypical pollen distribution.	NMR fingerprints + melissopalynology combination.
Ultrafiltration / Pollen Removal	Removal of pollen to mask botanical or geographical origin.	Pollen-free honey; origin becomes unverifiable.	Microscopic pollen count, DNA barcoding.
Geographical Origin Fraud	Mislabeling country or flora of origin, or blending with premium honey.	Inconsistent mineral profile, pollen mismatch.	NMR databases, elemental fingerprinting, DNA barcoding.
Heat Manipulation / HMF Increase	Excessive heating to delay crystallization or disguise aging.	Elevated HMF, reduced diastase, aroma degradation.	HMF quantification, GC-MS volatiles, UV–Vis.
Aroma or Color Manipulation	Synthetic flavors, plant extracts, dyes or caramelization added.	Abnormal volatile peaks, unnatural color.	HS-SPME–GC-MS, spectroscopic screening.
Blending of Different Honeys	Mixing low-quality honey with high-value monofloral honey.	Intermediate chemical signatures; diluted markers.	NMR cluster analysis, machine learning classifiers.

a. Detection improves when multiple analytical methods are combined.
b. Indirect adulteration (bee feeding) is harder to detect due to partial metabolic transformation.
***c.** Removal of pollen is considered manipulation under Codex standards.*

Honey fraud is defined as altering the chemical, physical, or botanical properties of honey to deviate from its natural state or marketing it with false or misleading origin information. Current fraudulent practices include both direct chemical adulteration and supply-chain manipulations that obscure geographical or botanical identity (Shi et al., 2018; Zhang et al., 2023). As shown in Figure 1, honey adulteration can be grouped into several major categories, including direct syrup addition, indirect adulteration through artificial feeding, ultrafiltration, heat manipulation, flavor or color enhancement, and origin misrepresentation. The defining characteristics, typical indicators, and most effective analytical detection methods for these fraud types are summarized in *Table 1*. This section provides an overview of these common adulteration practices with supporting evidence from the scientific literature.

3.1. Adulteration by Addition of Sugar Syrup

The most common form of honey adulteration is the direct addition of syrups derived from plants such as corn, sugarcane, or rice to honey. This process leads to dilution of phenolic compounds, changes in electrical conductivity, decreased diastase activity, and deviations in $\delta^{13}C$ isotope values. While C_4 plant-derived syrups can be largely detected with the EA-IRMS method, the isotopic values of modern plant-derived syrups with C_3 are quite similar to those of natural honey, limiting the discriminatory power of IRMS analysis alone (L. Wu, Du, B., Vander Heyden, Y., Chen, L., Zhao, L., Wang, M., Xue, X., 2017). Therefore, advanced methods such as NMR and LC-MS/MS are used for reliable determination of syrup adulteration.

3.2. High Fructose Corn Syrup (HFCS) Blend

HFCS is a frequently used adulterant due to its proximity to the natural sugar composition of honey, its low cost, and its sensory similarity. Because HFCS-sweetened honey easily passes conventional quality tests, NMR metabolomic profiling and IRMS applications offer significant advantages in detection. These methods reveal the unique carbohydrate traces and isotopic shifts left by HFCS with high accuracy (Zábrodská & Vorlová, 2015).

3.3 Direct Sugar Feeding of Bees (Indirect Adulteration)

In this method, syrup is not added directly to the honey; because the bees are fed commercial syrup, the product they produce turns into a honey-like liquid. While very similar in appearance to natural honey, the enzyme activities, sugar profile, and isotopic structure are systematically different from natural honey. Because bee metabolism involves some biochemical transformation, detection becomes difficult. In determining indirect adulteration, the combined evaluation of NMR-based metabolic fingerprint analysis and pollen spectra provides the highest accuracy (Anklam, 1998).

3.4 Pollen Filtration (Ultrafiltration) and Origin Disguise

Ultrafiltration largely removes pollen from honey, making it nearly impossible to determine the product's botanical or geographic origin. Because pollen analysis (melissopalynology) is the primary method for origin verification, complete removal of pollen is defined by Codex as "unacceptable manipulation" (Codex Alimentarius Commission, 2019). A 2011 unscientific journalistic investigation published by Food Safety News reported that some commercial honey in the US was pollen-free; this finding sparked significant debate about the misuse of ultrafiltration.

3.5. Geographical Origin Fraud

High-commercial monofloral honeys, such as Manuka, Sidr, and Anzer, are among the most vulnerable to provenance fraud. Mixing lower-quality honeys with premium honeys or labeling them with the wrong country name is common practice. The combined use of NMR profiling, pollen analysis, elemental/mineral composition (mineral fingerprinting), and DNA barcoding methods for origin verification yields the most reliable results (Zhang et al., 2023)

3.6. Heat Process Manipulation and HMF Adulteration

High-temperature treatments applied to extend the shelf life of honey or delay crystallization lead to serious deterioration in the product's chemical structure. Excessive heat treatment leads to increased HMF levels, loss of volatile aromatic compounds, and decreased diastase activity. According to Codex criteria, HMF levels exceeding 40 mg/kg are a significant indicator of quality loss (Codex Alimentarius Commission, 2019). Therefore, HMF is a key indicator for both excessive heat treatment and color manipulation.

3.7. Aroma and Color Manipulation

Natural or synthetic flavors, plant extracts, caramelization products, or food dyes can be used to mimic the sensory properties of low-quality honeys compared to natural honeys. These processes significantly alter the volatile compound profile of honey. HS-SPME–GC-MS analyses are critical for determining aroma manipulation because they can detect aromatic peaks not present in natural honey with high sensitivity (Danieli & Lazzari, 2022; Ruoff & Bogdanov, 2004). Color manipulation is generally manifested by increased HMF, decreased enzyme activity, and deviations in UV-Vis absorbance values.

3.8. Blending Different Honeys (Blending Fraud)

Mixing lower-cost honeys with higher-quality products in specific proportions is one of the most economically profitable methods of counterfeiting. Such blends may appear similar to natural honey in terms of sensory and physicochemical properties. However, NMR-based cluster analyses, multivariate chemical profiling, and machine learning models can distinguish blends from natural honeys with high accuracy (Zhang et al., 2023).

4. MODERN ANALYTICAL METHODS IN DETECTING HONEY FRAUD

Table 2. Comparison of modern analytical techniques used to detect honey adulteration.

Analytical Method	Analytical Principle	Detectable Fraud Types	Strengths	Limitations
^{1}H-NMR Spectroscopy	Metabolic fingerprinting of sugars, acids and minor compounds.	Syrups (C_3 & C_4), botanical origin, geographical origin, blends.	Holistic, database-supported, high accuracy.	High cost; large databases required.
IRMS ($\delta^{13}C$)	Isotopic difference between plant sources.	Mainly C_4 syrups (HFCS, cane).	Reliable for C_4 fractions.	Limited for C_3 syrups (rice, wheat).
LC-MS/MS	High-resolution separation of oligosaccharides and phenolics.	C_3 syrups, rice markers, heat-induced compounds.	Very sensitive; detects low-level adulteration.	Requires skilled operators and standards.
GC-MS / HS-SPME	Volatile compound profiling.	Aroma manipulation, heat treatment.	Detects synthetic flavoring; detailed aroma map.	Limited for non-volatile adulterants.
Pollen Analysis	Microscopy of pollen grains.	Botanical & geographical origin; ultrafiltration.	Strong evidence of floral source.	Cannot detect syrups.
DNA Barcoding / Metabarcoding	Sequencing of plant DNA residues.	Botanical origin; verification of monofloral claim.	High taxonomic precision.	Heat-processed honey may lose DNA.
FTIR / Raman / UV–Vis	Vibrational and absorbance spectra.	Syrups, heat damage, color manipulation.	Rapid, non-destructive screening.	Requires chemometric modelling.
Machine Learning Models	Classification using large chemical datasets.	Almost all fraud types (depending on inputs).	High prediction power; recognizes patterns.	Needs large validated datasets.

a.NMR accuracy depends on strong reference databases.
b. *IRMS is reliable for C_4 syrups but limited for C_3 syrups.*
c. *LC-MS/MS detects low-level syrup markers with high sensitivity.*
d. *Spectroscopic screening methods require chemometric modeling.*

As honey fraud has become increasingly sophisticated, traditional authentication approaches that rely solely on basic physicochemical measurements are no longer sufficient. Modern verification now depends on advanced analytical techniques capable of generating comprehensive datasets at metabolic, isotopic, volatile, and molecular levels. These contemporary methods offer a more holistic view of honey composition and allow subtle forms of adulteration to be detected with far greater sensitivity. The key analytical tools commonly employed in current research together with their principles, strengths, and limitations are outlined in *Table 2*, providing an integrated comparison of the techniques most relevant to honey authenticity assessment.

4.1. Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR is central to current authentication systems due to its ability to holistically assess the metabolic structure of honey. 1H-NMR measurements reveal the chemical fingerprints of hundreds of honey components in a single analysis; this dataset is evaluated using multivariate statistical models (PCA, PLS-DA, etc.) to identify biases caused by fraud. Bertelli et al. (2010) conducted a study on over 1,000 samples, demonstrating that NMR can detect even small amounts of syrup. The Honey-Profiling® database, developed by Bruker, has become the reference standard for geographical and botanical origin verification.

4.2. Isotope Ratio Mass Spectrometry (IRMS)

IRMS measures carbon isotope ratios ($\delta^{13}C$) to separate honey and its protein fractions from C_3 and C_4 plant-derived syrups. A $\delta^{13}C$ difference between the protein and sugar fractions of honey exceeding a certain threshold indicates syrup adulteration. However, the isotopic profiles of C_3-derived syrups, such as rice and wheat, can overlap with natural honeys, limiting the use of IRMS alone (L. Wu, Du, B., Vander Heyden, Y., Chen, L., Zhao, L., Wang, M., Xue, X., 2017). Therefore, IRMS is currently mostly applied in conjunction with NMR or LC-MS/MS.

4.3. LC-MS/MS (Liquid Chromatography Tandem Mass Spectrometry)

LC-MS/MS can identify low levels of oligosaccharides in honey, characteristic syrup markers (kojibiose, maltotriose, certain traces of rice syrup), and structural deviations in phenolic compounds with high sensitivity. Martinello et al. (2022) were able to reliably detect only 5% rice syrup adulteration with LC-HRMS. Furthermore, a combination of targeted and untargeted metabolite analysis can assess both additives and heat treatment indicators.

4.4. GC-MS (Gas Chromatography- Mass Spectrometry)

GC-MS is a powerful method for identifying changes in the volatile compound structure of honey. Aroma manipulation, addition of botanical extracts, or excessive heat treatment create characteristic deviations from the natural volatile profile of honey. HS-SPME–GC-MS analyses; It can indicate counterfeiting by detecting anomalies in unique aromatic compounds such as linalool, benzaldehyde, and furfural derivatives (ElMasry et al., 2019; Ruoff & Bogdanov, 2004). Quantitative measurement of HMF and other thermal degradation products can also be performed with GC-MS.

4.5. Melissopalynology (Pollen Analysis)

Pollen analysis is the primary method for determining the botanical and geographical origin of honey. The pollen spectrum is critical in detecting counterfeiting because it provides direct evidence of the product's floral origin. Removing pollen structure through ultrafiltration is considered manipulation by the Codex Alimentarius Commission (Codex Alimentarius Commission, 2019). Therefore, the origin of pollen-free honey cannot be scientifically verified.

4.6. DNA Barcoding and Metabarcoding

DNA barcoding determines the floral structure of the product by analyzing plant DNA residues found in honey. Metabarcoding, on the other hand, allows for multi-species sequencing, offering higher taxonomic resolution compared to pollen analysis. Chen, Hu, and Dai (2023) reported success rates of up to 98% for monofloral honey verification using this method.

DNA-based methods are an important complementary tool for determining the origin of honey, particularly in ultrafiltered or blended honey.

4.7. FTIR, Raman, and UV–Vis Spectroscopies

These rapid screening techniques identify signs of adulteration by evaluating honey's functional groups, molecular vibrational properties, and absorption behavior. FTIR rapidly analyzes changes in the band structures of sugars and organic acids; Raman analyzes crystallization tendencies and polymerization levels; and UV–Vis rapidly analyzes HMF and color parameters. Combining spectroscopic data with chemometric models (PCA, PLS-DA, SVM) allows for the classification of natural and adulterated honey with 90–98% accuracy (X. Wu et al., 2022).

4.8. Chemometric and Machine Learning Approaches

High-dimensional datasets obtained from methods such as NMR, LC-MS/MS, FTIR, or GC-MS, combined with chemometric analysis and machine learning algorithms, significantly enhance forgery detection. Models such as PCA, HCA, LDA, SVM, Random Forest, and neural networks can process mixed chemical profiles and classify honey samples as natural, blended, or syrup-adulterated with high accuracy. These approaches are particularly effective at identifying low rates of adulteration compared to conventional methods.

5. WORLD HONEY TRADE, STANDARDS, AND REGULATORY FRAMEWORK

Honey is a bee product with high economic and cultural significance worldwide. According to FAO data, global honey production is approximately 2.1 million tons annually, concentrated in a wide geographic area stretching from Asia to Europe (FAO, 2023). While countries such as China, India, Turkey, and Argentina are prominent producers, countries such as the US, Germany, and Japan are strong players in terms of consumption and imports. However, this volatility in international trade also leaves the product vulnerable to counterfeiting. Differences in production costs across countries, import quotas, inequalities in quality control, and lax labeling practices are among the primary factors facilitating honey adulteration.

Therefore, many countries have established their own regulations defining the definition of honey, quality criteria, and labeling requirements; standards aimed at protecting the chemical integrity of honey have also been developed at the international level. The key regulatory frameworks that play a decisive role in honey trade are discussed below.

5.1. Codex Alimentarius Honey Standard

The Honey Standard (CODEX STAN 12-1981), first published by the Codex Alimentarius Commission in 2001 and last revised in 2019, is widely regarded as the foundational international reference for honey quality. The standard outlines several mandatory criteria, including a maximum moisture content of 20%, a hydroxymethylfurfural (HMF) limit of 40 mg/kg, a minimum diastase activity of 8 DN, and a combined fructose + glucose content of at least 60%. It also requires that honey preserve its natural pollen structure and strictly prohibits the use of additives or excessive filtration practices that could alter its composition. Despite its global relevance, the Codex standard is not sufficient to detect sophisticated modern adulteration techniques, as it does not prescribe detailed analytical methods for identifying C_3-plant-derived syrups or other advanced fraudulent practices (E. Schievano, Finotello, Uddin, Mammi, & Piana, 2016).

5.2. European Union Honey Directive (EU Honey Directive)

The European Union implements some of the world's most stringent regulatory measures to combat honey adulteration. Under the Honey Directive 2001/110/EC and its recent draft revisions, the EU enforces clear origin labeling, preservation of natural pollen structure, strict bans on additives, and the prevention of excessive heat treatment that may compromise honey quality. A large-scale EU market survey conducted in 2022 revealed that approximately 46% of imported honey samples were suspected of containing added sugar syrups (European Parliament, 2023). In response, the EU significantly strengthened its monitoring capacity and formally adopted the NMR Honey-Profiling® technique as a reference analytical tool, thereby standardizing the scientific approach to honey authentication across EU laboratories.

5.3. US FDA Standards and National Honey Association Practices

In the United States, the Food and Drug Administration (FDA) defines honey as a substance that must retain its pure and natural character, yet the country remains highly vulnerable to adulteration due to its substantial honey import volume. Between 2013 and 2022, large-scale enforcement actions by U.S. Customs and Border Protection uncovered significant amounts of imported honey adulterated with sugar syrups. In response to these challenges, the U.S. has intensified efforts to strengthen domestic market transparency through mandatory Country of Origin Labeling (COOL) practices, the recommended use of pollen analysis as a supportive authenticity tool, and rigorous inspections for antibiotic residues.

5.4. New Zealand Manuka Honey Regulations

Manuka honey is one of the most frequently counterfeited specialty honey varieties globally due to its high market value and widespread availability of counterfeit goods. Therefore, New Zealand implemented the world's most comprehensive authentication standard for Manuka honey in 2017. This standard is based on multiple authentication criteria, including quantitative measurement of five chemical markers specific to the Leptospermum scoparium species, detection of plant-specific DNA, and NMR metabolic profiling. This multi-layered structure has significantly increased transparency and traceability in the international Manuka honey trade.

5.5. Turkey: Turkish Food Codex Honey Communiqué

Turkey is one of the world leaders in honey production, and honey has a high cultural and economic value. The Turkish Food Codex Honey Communiqué has criteria largely aligned with the Codex. The HMF limit (40 mg/kg), the lower diastase limit, the requirement for the presence of pollen, and the ban on additives constitute the fundamental regulations. However, studies such as Guler et al. (2014), Tosun (2013) and Kivrak et al. (2016) have revealed that various forms of adulteration are still prevalent in the domestic market. Periodic inspections by the Ministry of Agriculture and Forestry are supported by recalls when counterfeiting is detected, but recording deficiencies in the supply chain have not been fully addressed.

5.6. Legislative Limitations and Global Harmonization Gaps

While honey standards from different countries have similar objectives, significant inconsistencies remain at the implementation level. In particular, a common international framework has not yet been established for the detection of C_3 syrups, the definition of ultrafiltration limits, and geographic origin declarations. The lack of clear legal implications for standardization of analytical methods, database sharing, and blockchain-based traceability solutions are among the current challenges facing the global honey trade. Therefore, the literature emphasizes the need for more comprehensive harmonization across countries, both in terms of analytical verification and supply chain management, to reduce counterfeiting.

6. BLOCKCHAIN-BASED TRACEABILITY SYSTEMS

6.1. The Need for Traceability in the Food Supply Chain

The honey supply chain has a multi-stage structure involving many actors, from production to consumer delivery. Beekeepers, cooperatives, packaging facilities, laboratories, logistics companies, and retailers are involved at different points in the chain. In systems involving so many actors, it is extremely difficult to fully and accurately record the product's origin, production conditions, and laboratory analyses. Fraud, particularly in honey, such as syrup adulteration, alteration of origin, excessive filtration, and label manipulation, make recording errors or intentional changes to information throughout the supply chain a significant problem. Therefore, modern food safety approaches require a robust traceability infrastructure in addition to chemical analyses. Blockchain technology offers a data integrity mechanism that can address this need (Galvez et al., 2018).

6.2. The Fundamental Mechanism of Blockchain Technology

Blockchain is essentially a distributed digital ledger system. Each data record is stored in units called blocks, and these blocks are cryptographically linked. When a new record is added to the system, it is verified by all parties in the network and cannot be changed retroactively once recorded. This structure increases the reliability of records for products susceptible to fraud, such as honey. For example, when harvest time, hive identification, migratory beekeeping information, storage temperatures, and analysis reports are stored on the blockchain, any attempt to delete or alter data at any stage is detected. This immutability makes blockchain a powerful tool for food traceability (Kshetri, 2018).

6.3. Blockchain Applications in the Honey Supply Chain

Although blockchain applications in the honey sector are still in an early phase of development, recent studies show that this technology can substantially improve transparency and authenticity control. When every step of the supply chain from the beekeeper to the packaging facility is recorded sequentially in a tamper-proof digital ledger, the true origin of the product becomes verifiable. Such a system enables the early detection of fraudulent practices, including misleading origin declarations or blending honeys from different countries, both of which are frequent issues in global trade. Integrating laboratory results into the blockchain further enhances the credibility of NMR or LC-MS/MS findings, since digitally signed analytical data cannot be modified by external parties. The essential data layers involved in blockchain-supported traceability, and their respective roles in fraud prevention, are summarized in *Table 3*. Pilot implementations of blockchain-based authentication have already been proposed for certain geographically indicated honeys, and wider adoption of this approach is expected in the coming years (Behnke & Janssen, 2020).

Table 3. Key data layers and functions of a blockchain-supported honey traceability system.

Traceability Layer	Recorded Information	Data Source	Contribution to Fraud Prevention
Hive-Level Data	Hive ID, beekeeper ID, location, migratory routes.	GPS-enabled IoT devices; beekeeper inputs.	Prevents false origin declarations.
Environmental & Bee-Colony Data	Temperature, humidity, hive weight, nectar flow patterns.	IoT sensors (scale units, climate sensors).	Early detection of abnormal feeding or environmental manipulation.
Harvest & Processing Records	Harvest date, extraction conditions, heating practices.	Apiary logs; timestamps; automated sensors.	Detects suspicious heating or filtration activities.

Laboratory Results	NMR, LC-MS/MS, IRMS, antibiotic residues.	Accredited laboratories; cryptographic signatures.	Ensures analysis reports cannot be altered.
Logistics & Storage Conditions	Transportation temperature, vibration, storage location.	IoT temperature loggers; RFID.	Identifies overheating, contamination or re-routing.
Retail & Consumer Interface	QR-code product history, digital certificate.	Blockchain front-end platforms.	Increases transparency and consumer confidence.

a. Blockchain ensures immutable records; accuracy depends on correct data entry.
***b.** IoT sensors automate measurements and reduce human error.*
***c.** Laboratory results stored on-chain cannot be altered late.*

6.4. Blockchain and IoT Integration: Monitoring Temperature, Weight, and GPS Data

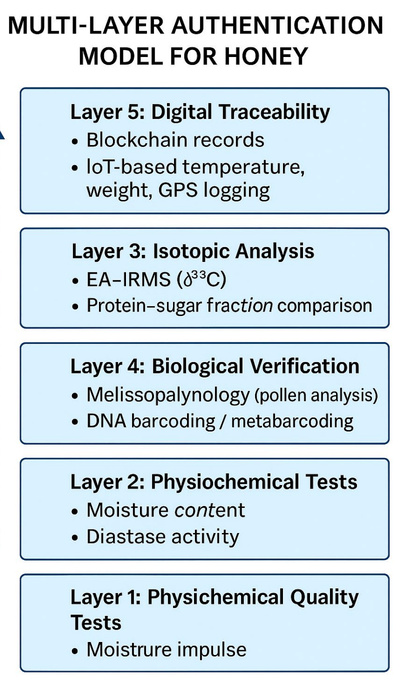

Figure 2. Multi-layer authentication model used for the detection of honey adulteration.

This diagram summarizes the complementary layers used in honey authentication, from basic physicochemical measurements to advanced analytical techniques (NMR, LC–MS/MS, EA–IRMS), alongside biological tools such as pollen analysis and DNA barcoding. The upper layer incorporates digital traceability technologies, including blockchain and IoT data streams, to enhance supply-chain transparency and support laboratory-based verification.

While blockchain is a powerful recording mechanism on its own, its effectiveness increases considerably when combined with IoT sensors that enable real-time data acquisition. As illustrated in *Figure 2*, integrating multiple layers of digital and analytical verification provides a more comprehensive framework for ensuring honey authenticity. IoT devices automatically capture information such as hive weight, temperature, humidity, GPS coordinates, and vibration levels during transportation, and these data are transferred directly to the blockchain without manual intervention. This automated flow significantly reduces the likelihood of human error or intentional data manipulation. Moreover, as shown in *Figure 3*, continuous monitoring of transportation conditions such as temperature fluctuations or deviations from predefined routes allows rapid detection of abnormalities. In migratory beekeeping systems, recording GPS-based movement paths also strengthens verification of floral sources and declared origins. Overall, IoT–blockchain integration forms a complementary architecture that enhances both honey security and supply-chain transparency (Feng, Wang, Duan, Zhang, & Zhang, 2020).

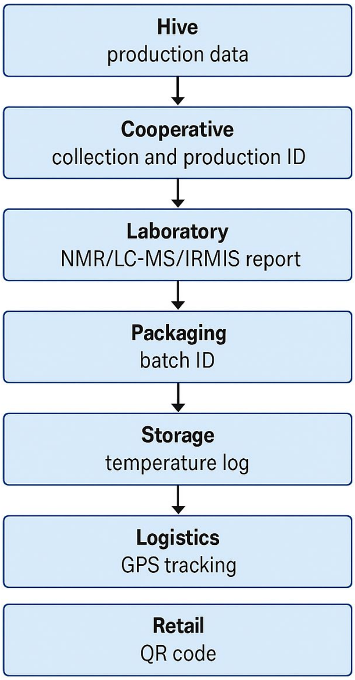

Figure 3. Blockchain-based traceability framework for honey authentication across the production and supply chain.

This diagram presents the integration of blockchain technology into the honey supply chain, where critical data from hive-level production records to laboratory analyses, packaging batches, storage conditions, and logistics tracking are stored as immutable blocks. The system enhances traceability, supports authenticity verification, and strengthens consumer confidence

6.5. Limitations and Implementation Challenges of the Blockchain Approach

While blockchain technology offers significant advantages in honey traceability, there are also several challenges in implementing it. Small-scale beekeepers' unfamiliarity with digital recording systems, high initial costs, and unequal access to sensor technologies across regions make implementation difficult. Furthermore, international legislation has not yet fully consolidated the legal validity of blockchain records. Another limitation is that manually entered data into the system is still susceptible to human error. Therefore, most researchers in the literature emphasize that using blockchain in conjunction with laboratory-based verification techniques is a more realistic approach (Galvez et al., 2018; Kshetri, 2018). For this implementation to be effective, the technology must be simplified, producer training increased, and national regulations clarified.

7. DISCUSSION

The increasing complexity of honey fraud, as revealed by scientific studies over the past decade, necessitates the simultaneous strengthening of both analytical capacity and supply chain audits. For example, Anklam (1998) and Bogdanov et al. (2008) early emphasized that the chemical structure of honey is highly variable, making it difficult to make a reliable decision with a single test. This view has gained further validity today with the proliferation of modern fraud types.

The emergence of plant-derived syrups, particularly C_3, is considered a significant factor limiting the discriminatory power of the EA-IRMS method. L. Wu, Du, B., Vander Heyden, Y., Chen, L., Zhao, L., Wang, M., Xue, X. (2017) reported that the $\delta^{13}C$ isotope values of rice syrup can be extremely close to those of natural honey, and therefore, IRMS offers high accuracy only in identifying C_4 syrups. Similarly, Zábrodská and Vorlová (2015) demonstrated that the protein fraction and the sugar fraction of honey should be evaluated together for a more reliable interpretation of IRMS results.

NMR spectroscopy stands out in the literature as one of the most comprehensive verification tools. Bertelli et al. (2010) demonstrated that metabolomic profiles generated with 1H-NMR can detect even low rates of syrup adulteration, and E. Schievano et al. (2016) confirmed that NMR has a high classification accuracy in both botanical and geographic origin verification. However, the main obstacles to widespread use of NMR are the high cost of equipment and the need for extensive reference databases. Hansen et al. (2024) states that, "without analytical standardization and database sharing, it is difficult for NMR to become a global reference method."

Mass spectrometry-based methods such as LC-MS/MS and GC-MS are particularly prominent in the literature for targeted counterfeiting detection. Martinello et al. (2022) demonstrated that oligosaccharides specific to rice syrup can be confidently detected even at 5% levels in LC-HRMS analyses. In GC-MS studies, Ruoff and Bogdanov (2004) reported that even small deviations in the aroma profile revealed attempts at artificial flavoring. These findings demonstrate that the combined use of volatile and non-volatile metabolite-based approaches increases detection power.

Various studies have indicated that counterfeiting is not solely driven by chemical manipulation, but that gaps in supply chain record keeping significantly contribute to this process. Behnke and Janssen (2020) stated that a significant portion of food fraud stems from information asymmetry in the supply chain, and therefore, technologies such as blockchain can offer a "second layer of verification supported by chemical analysis." Similarly, Galvez et al. (2018) reported that blockchain-based systems strengthen data integrity, particularly in complex international supply chains, and increase transparency throughout the process from producer to consumer. It is widely accepted in the literature that such digital verification systems complement analytical methods but are not sufficient to prevent counterfeiting on their own.

Regulatory incompatibilities also constitute an important aspect of the discussion. The European Union's 2022 report indicated that 46% of imported honey contained suspected syrup European Parliament (2023) and emphasized the need to harmonize analytical verification processes across all member states. In Turkey, local studies such as Guler et al. (2014), Kivrak et al. (2016), and Tosun (2013)have shown that adulteration in the market may be more widespread than previously thought. This suggests that laboratory capacity, field inspections, and regulatory enforcement must be addressed together.

Generally, the literature suggests that there is no single "perfect method" for combating honey fraud; rather, a multi-layered approach, where NMR, IRMS, LC-MS/MS, melissopalynology, DNA analysis, and spectroscopy complement each other, is the most appropriate approach. Furthermore, the ability of blockchain and IoT technologies to support analytical verification by providing transparency throughout the supply chain is a key trend emphasized by current studies. In today's conditions where counterfeiting is increasingly diversifying, it seems inevitable to integrate both laboratory-based methods and digital traceability systems into a more holistic model.

8. CONCLUSION

The findings presented in this review demonstrate that honey fraud is not merely a matter of chemical manipulation; it is a multifaceted problem compounded by factors such as incomplete records, uncertainty of origin, and poor control throughout the supply chain. Analytical methods developed in recent years, particularly high-resolution techniques such as NMR, LC-MS/MS, GC-MS, and IRMS, have made it possible to identify different types of fraud more precisely. These techniques have provided significant advances in areas such as the detection of syrup additives, the identification of flavor and color manipulations, and the verification of botanical and geographic origin. However, the literature generally emphasizes that no single method can reliably distinguish all types of fraud (Bertelli et al., 2010; Martinello et al., 2022; L. Wu, Du, B., Vander Heyden, Y., Chen, L., Zhao, L., Wang, M., Xue, X., 2017).

The increasing complexity of fraud necessitates the evaluation of laboratory analyses in conjunction with supply chain data. Blockchain and IoT-based traceability systems offer significant support to the analytical verification process by enabling more transparent and verifiable recording of the stages honey goes through from production to consumer delivery. However, for these systems to be widely adopted, issues such as producer training, technical infrastructure, legal compliance, and cost must be addressed.

The harmonization of national and international regulations also plays a critical role in combating counterfeiting. While standards such as Codex Alimentarius, the EU Honey Directive, and the Turkish Honey Communiqué provide a basic framework, more detailed criteria are needed, particularly for current types of counterfeiting, such as the detection of modern C_3 syrups and over-filtration. Therefore, the future honey verification approach appears to be a two-tiered structure based on both robust analytical capacity and data-driven supply chain management.

In general, reducing honey counterfeiting will be possible through a holistic verification system that complements laboratory-based chemical analyses, supply chain records, and digital traceability solutions. Supporting scientific advances with models that are compliant with legislation, have practical application, and can be easily adopted by stakeholders is of great importance for both protecting beekeepers' labor and maintaining consumer confidence.

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CHAPTER V

THE EFFECTS OF PESTICIDES ON THE MICROBIOTA OF HONEYBEES

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DOI: https://www.doi.org/10.5281/zenodo.18138242

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1. INTRODUCTION

Without pollination, the continuity of plants cannot be ensured. Therefore, honeybees play a vital role in sustaining life on Earth through their role in pollination (Jankielsohn, 2018).

Colony collapse disorder (CCD) is a phenomenon that has emerged as a cause for concern in bee colonies and is attracting considerable attention. Colony collapse disorder is a situation that occurs when adult worker bees disappear within a short period of time without any dead bees being found in the hive (Hristov et al., 2021). Although many different environmental factors are thought to contribute to this condition, no definitive cause has been identified (Singh & Rana, 2025). A notable feature of this process is that worker bees leave the hive, abandoning the queen and her brood, and do not return (Ganie et al., 2024). In CCD cases, a very high level of decline in colony population is observed, and it is reported that these losses occur more rapidly compared to other causes of colony collapse (VanEngelsdorp et al., 2009). Considering the role of honeybees in plant survival and agriculture, their continuous decline poses a significant risk factor for global food security (Marshman et al., 2019).

Today, progress in many areas, particularly industry, technology, and health, has accelerated alongside population growth. This rapid population growth has increased the demand for food, necessitating progress in the agriculture and livestock sectors. Pests slow down progress in this area. As a common point in protecting against harmful organisms and diseases, the use of pesticides has become widespread throughout the world (Lazarević-Pašti et al., 2025).

In recent years, the spread of harmful organisms and disease agents in honeybees, increased pesticide use, changes in climate conditions, the decline in plants that serve as sources of nectar and pollen, and intensive agricultural activities have caused serious declines in both wild and farmed honeybee populations (Stanimirović et al., 2019).

The presence of pesticide and antibiotic residues in agricultural products causes significant problems worldwide. One such problem is the issue faced by honeybees, which are among the most important pollinators. Residues cause adverse effects both for individual bees and for the colony. Honeybee products, which are also important for human health, are affected by residues and pose a risk to human health. Antibiotic residues lead to the emergence of resistant pathogenic microorganisms, while pesticide residues cause genetic changes and cellular damage (Kumar & Kumar, 2019; Lima et al., 2020).

Pesticides have been reported to cause significant harm to bees. Exposure of honeybees to pesticides mostly occurs through ingestion of residues found on plants and in water. Another important route of exposure is the use of pesticides to protect honeybees from varroa and other parasites (Sanchez-Bayo & Goka, 2016).

A healthy gut microbiota is one of the most important defense systems of honeybees. Stress factors such as poor nutrition, parasites, diseases, and pesticides have negative effects on the immune system and microbiota. This section will examine the effects of pesticides on the gut microbiota of honeybees.

2. DIGESTIVE SYSTEM IN HONEYBEES

The digestive system of honeybees is a channel structure that extends from the mouth to the anus and consists of specialized sections. The digestive system of honeybees has a specialized structure that interacts with the gut microbiota. The digestive system is divided into three sections: the foregut, midgut, and hindgut, which perform the functions of storing, digesting, and absorbing nutrients. The mouth of honeybees is designed to absorb liquid food and nectar. The proboscis (tongue) structure plays the most important role at this point. The first stage of digestion is carried out by enzymes secreted in the salivary glands. The esophagus, honey stomach, and proventriculus form the foregut. No digestion occurs in the honey stomach (crop), where nectar is temporarily stored, but food is distributed among the colony members. After this section, food is transferred to the midgut for digestion. Before food passes from the honey stomach to the midgut, it is regulated in the proventriculus. Solid particles are retained, while liquid food is allowed to pass into the midgut. The main section where digestion occurs is the midgut, where digestive enzymes are secreted. The peritrophic membrane, which acts as a barrier against pathogenic microorganisms, is also located in the midgut. The ileum and rectum form the hindgut. A significant portion of the gut microbiota is located here (Chapman et al., 2013; Faux, 2021).

3. GUT MICROBIOTA IN HONEYBEES

The microbiota concentrated in the distal part of the intestines is acquired within the first few days after emerging from the pupal stage of the honeybee (*Apis mellifera*) through contact with other adult worker bees. The social interaction of honeybees enables the direct transfer of microbiota components (Kwong et al., 2017). Microbiota contributes to honeybees in terms of nutrition, growth, endocrine signaling, resistance to pathogenic microorganisms, and the immune system. Studies have shown that disturbances in the balance of microbiota negatively affect the quality of life of honeybees (Zheng et al., 2018). Although the presence of species within the microbiota is generally similar, differences may exist between hives, colony individuals, larvae, and worker bees. The microbiota of honeybees has a limited, simple, and specialized structure (Martinson et al., 2011). The gut microbiota of honeybees consists of microaerophilic and facultative anaerobic bacteria, making it similar to mammalian microbiota, but it is much simpler than mammalian microbiota. Therefore, honeybee microbiota is important as a model for research and has great potential (Engel et al., 2016).

Only nine bacterial species are dominant in the microbiome of honeybees, consisting of five basic bacterial species and four rare bacterial species (Martinson et al., 2012). It has been reported that approximately 1% of the current population consists of yeast, 27% of gram-positive bacteria, and 70% of gram-negative bacteria (Tootiaie et al., 2021). The core bacterial species include *Gilliamella apicola* and *Snodgrassella alvian* from the *Proteobacteria* phylum among gram-negative bacteria, Lactobacillus Firm-4 and Lactobacillus Firm-5 from the *Firmicutes* phylum among gram-positive bacteria, and the *Bifidobacterium asteroides* species cluster belonging to the *Actinobacteria* phylum. *Proteobacteria* species such as *Gluconobacter* species, *Frischella perrara*, *Bartonella apis,* and *Parasaccharibacter apium* are rare species (Moran et al., 2012). Studies indicate that the microbiota population may vary depending on the food consumed, the season, the age of the bee, the caste, and the geography (Castelli et al., 2022). The queen bee gut microbiota shows long-term microbial succession associated with mating, environmental conditions, and colony adaptation processes (Copeland et al., 2022).

The ileum and rectum form the hindgut. Gram-negative bacteria are abundant in the ileum, with *S. alvi* found in the lumen and along the wall of the ileal folds, and *G. apicola* found on the intestinal wall (Martinson et al., 2012). *F. perrara* is concentrated in the pyloric region (Engel et al., 2015). Gram-positive bacteria *Lactobacillus* Firm-4, *Lactobacillus* Firm-5, and the *B. asteroides* group colonize the rectum (Alatawy et al., 2020).

The gut microbiota provides benefits for honeybees in terms of important metabolism and overall health. These benefits are observed at both the individual and colony levels. The bee microbiome establishes symbiotic relationships with the host, exhibits protective properties against pathogenic microorganisms, can play a role in immune functions, and can contribute to colony resistance (Koch & Schmid-Hempel, 2011). Studies have observed that microbiota transfer and monoclonization experiments stimulate the honeybee immune system (Emery et al., 2017). The gut microbiota can affect honeybee health by influencing host immune responses. Antimicrobial peptides (AMPs) are fundamental components of the honeybee immune system's defense against pathogens. AMPs are synthesized during fungal, protozoan, and bacterial infections, disrupting the structure of microorganisms to exert their antimicrobial effect. In a study examining the effects of the microbiota on AMP synthesis, AMP synthesis against infection was observed, and it was concluded that the honeybee microbiota triggers immune responses (Mojgani et al., 2025).

The F. perrara bacterium, which is part of the honeybee microbiota, is present in the pyloric epithelium and triggers the crusting phenotype (Engel et al., 2015). Transcriptome analyses have shown that F. perrara colonies stimulate the honeybee immune system. Studies have shown that the *F. perrara* bacterium can stimulate the immune response ( ) even in the presence of other gut microbiota colonies (Schmidt et al., 2023). Research indicates that this bacterium is effective in the pyloric region for gut health and hemostasis (Kwong et al., 2017).

Lactobacillus kunkeei is a fructophilic lactic acid bacterium most commonly found in the gastrointestinal tracts of honeybees. *L. kunkeei* has been shown to support honeybee health through its antifungal effects (Iorizzo et al., 2020).

The metabolic activities of bacteria in the ileum and rectum are greatly influenced by the host's diet (components obtained from honey, nectar, and pollen). Nucleosides, organic acids, quinates, various sugars, and sugar acids are also additional nutrient sources for the microbiota. The pollen wall consists of the pollen membrane, exine, and intine layers (Oh, 2023). The intine layer, which contains cellulose, hemicellulose, and pectin, can be broken down by *Gilliamella apicola* in the ileum. In a study where polysaccharide degradation genes were identified in cultured genome sequences and metagenomic data, it was stated that *Gilliamella* and *Bifidobacterium* bacteria digest hemicellulose and pectin in honeybee intestines, while other gut microbiota species cannot degrade polysaccharides. This is quite important for honeybees to obtain amino acids from pollen containing cellulose, hemicellulose, and lignin (Zheng et al., 2019).

Lactobacillus species and *Bifidobacterium asteroides* species are predominant in the rectum. These bacteria can break down aromatic compounds such as flavonoids, phenolamides, and ω-hydroxy acids derived from exin found in the pollen wall and exin layer (Kešnerová et al., 2017).

Studies have reported that the honeybee microbiota contributes to honeybee weight gain. According to metatranscriptome and metagenome analyses, bacteria in the microbiota break down and ferment saccharides. This fermentation, as part of the honeybee metabolism, produces short-chain fatty acids that can be considered a nutrient for the honeybee. This process contributes to honeybee weight gain. The same study indicated that the core bacteria of the honeybee microbiota are active in the organism and play a crucial role in the production of organic acids using molecules derived from plants (Lee et al., 2018).

Lactic acid bacteria, which constitute an important group of the honeybee gut microbiota, play a role in food digestion and immune system stimulation. These bacteria can also exert an antagonistic effect against harmful microorganisms in the digestive system (Iorizzo et al., 2020). Dysbiosis in honeybees can lead to a weakened or disrupted immune system (Raymann & Moran, 2018).

Inadequate and unbalanced nutrition negatively affects the balance of the microbiota in the intestines, increasing mortality rates in honeybees and increasing their susceptibility to disease. This resulting dysbiosis negatively affects the functioning of genes related to growth and development in worker bees during the early developmental period, leading to problems with honeybee growth (Goulson et al., 2015).

Another important factor that can cause disruption in the honeybee microbiome is the widespread use of antibiotics. Studies have shown that exposure to antibiotics results in dysbiosis in honeybees. As a result, susceptibility to opportunistic pathogenic microorganisms increases, negatively affecting the health of honeybees.

It has been demonstrated that antibiotic applications can cause long-term changes in both the total density and microbial composition of the honeybee gut microbiome. In bees exposed to antibiotics, survival rates were found to be significantly reduced, both under natural colony conditions and in laboratory experiments where they were deliberately exposed to opportunistic bacterial pathogens. The findings indicate that antibiotic-induced dysbiosis negatively affects honeybee health, particularly through increased susceptibility to common opportunistic pathogens (Raymann et al, 2017; Deng et al., 2022).

4. PESTICIDES AND THEIR EFFECTS ON HONEYBEES

Pesticides are defined as chemical substances used to control or eliminate harmful organisms such as insects, rodents, fungi, and unwanted weeds. These compounds play an important role in agricultural production because they reduce yield losses, support continuity in food production, and contribute to obtaining economically accessible, high-quality products. However, while pesticide use is thought to provide certain short-term benefits, it has serious and lasting negative effects on ecosystem balance in the long term (Ahmad et al., 2024).

The toxicity level of a pesticide on honeybees varies depending on physicochemical parameters such as the chemical's vapor pressure and solubility in water, the type of commercial formulation, the biological characteristics of the target bee population, environmental factors, and the period during which the pesticide is applied (Mullin et al., 2010). A study comparing the toxicity of different pesticide formulations on honeybees found that powder formulations exhibited significantly higher lethality compared to liquid-based preparations, and it was noted that this was due to the anatomical structure of the honeybee (Johansen, 1977).

Pesticides are among the most important factors in the decline of honeybee colonies (Decourtye et al., 2013). Among pesticide groups, insecticides are among the most debated chemicals due to their effects on honeybees. Insecticides that pose a serious threat to honeybees are generally classified into five main groups: chlorinated compounds, organophosphates, carbamates, pyrethroid derivatives, and neonicotinoids (Belzunces et al., 2012). The adverse effects of insecticides and herbicides on honeybees have been reported, and the use of highly toxic pesticides in agriculture has been banned (Möhring et al., 2020; Ofosu et al., 2023).

The widespread use of synthetic insecticides has been noted to be quite risky for bee health, and insecticides have generally been evaluated in studies conducted on honeybees. In this study, the effects of fungicides, herbicides, and other non-specific insecticides on honeybees were evaluated. The study found that fungicides are not as harmful as insecticides, but when combined, fungicides are at least as harmful as the combination of fungicides and insecticides. Herbicides and other groups were also found to have more harmful effects than fungicides (Iwasaki & Hogendoorn, 2021).

Honeybees can be directly exposed to insecticides on flowering plants where they collect nectar and pollen to sustain their lives, and they can also indirectly affect other individuals in the colony through contaminated nectar and pollen that they carry back to the hive. This situation leads to an increase in forager bee deaths, a decrease in the queen bee's egg production, and a decline in royal jelly production. In cases of heavy exposure, it can result in colony collapse (Sanchez-Bayo & Goka, 2016). Dead bees accumulating intensely in front of the hive and on the landing board are among the most important indicators of pesticide poisoning. Furthermore, among insects directly or indirectly exposed to insecticides, honeybees show higher sensitivity to these substances due to their physiological characteristics (Hardstone & Scott, 2010). Honeybees that have survived the winter, are older, and are malnourished exhibit a more sensitive structure to pesticides compared to younger individuals. One of the main reasons for this increased sensitivity is the decrease in vitellogenin levels in the hemolymph of these individuals, which plays an important role in the immune and antioxidant defense of bees (Johnson, 2015).

Chemical analyses conducted on honeybees and bee products have shown that many pesticide residues from various sources are present in honeybees and their products. The most common residues come from acaricides used to combat *Varroa destructor*. Antimicrobials used to control bacterial and microsporidian diseases also leave residues. Fungicides used on flowering plants are also carried back to the hive by honeybees. While the aforementioned pesticides may not be highly toxic to bees individually, their combined effects can reach serious levels of toxicity (Johnson et al., 2013).

Neonicotinoids, which are widely used in agriculture today, have a systemic structure that allows them to be absorbed by plants and transported within their tissues. This enables them to exert a powerful toxic effect on insects that feed on these plants, and they are considered extremely harmful to honeybees. Furthermore, while only a limited portion of the applied neonicotinoids is taken up by the plant, a significant portion is released into the environment, causing adverse effects on the ecosystem (Kiljanek et al., 2016).

5. EFFECTS OF PESTICIDES ON THE MICROBIOTA OF HONEYBEES

The gut microbiota, which is limited in number but of considerable importance to honeybees, possesses a specialized bacterial community. It plays important roles in the digestion of food, the regulation of the immune system, the formation and suppression of a defense line against pathogens, and detoxification (Raymann & Moran, 2018). Exposure of honeybees to pesticides seriously affects the balance of bacteria present here, endangering the health of honeybees and the colony (Kakumanu et al., 2016).

In a study investigating the effects of chronic low-level exposure to pesticides on the gut microbiota of honeybees, it was observed that while the core gut bacteria were unaffected, a few rare bacterial species were impacted by pesticides. However, the study concluded that even mild exposure to pesticides could directly alter the physiological homeostasis of newly mated honeybees, and that this effect would be even more pronounced if dysbiosis occurred in the bees (Almasri et al., 2022).

Commonly used insecticides such as neonicotinoids, organophosphates, and pyrethroids can have toxic effects on bacteria in the gut microbiota. These negative effects of insecticides cause changes in the microbiota, leading to a decrease in the number of basic bacterial species. This process causes dysbiosis, suppressing symbionts of beneficial species such as *Lactobacillus, G. apicola,* and *S. alvi* (Brandt et al., 2016).

In a study conducted to observe the effects of exposing honeybees to four different neurotoxic insecticides under in vivo conditions, a significant decrease was observed in the *Lactobacillus* and *Bifidobacterium* bacterial populations present in the honeybee microbiome (Rouzé et al., 2019).

Glyphosate, the most widely used herbicide in weed control worldwide, acts by inhibiting the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme in plants and certain microorganisms, which is vital in the shikimate pathway. Genomic analyses have revealed that nearly all of the core bacteria in the gut microbiota of honeybees possess genes encoding the EPSPS enzyme targeted by glyphosate. Therefore, glyphosate negatively affects the gut microbiota of honeybees. Experimental data have demonstrated that worker bees exposed to glyphosate at doses encountered in environmental field conditions experience a significant decline in both the proportion and number of dominant bacterial species in their guts (Motta et al., 2018).

The honeybee microbiome can play a role in breaking down certain pesticide sources and reducing their toxic effects. As a result of the microbiome being damaged and its density decreasing due to intense pesticide exposure, the detoxification level of honeybees decreases, and the toxic effects of pesticides become more severe.

One of the important functions of the microbiota is its contribution to the immune system. Disruption and dysbiosis in the microbiota indirectly affect the immune system of honeybees. As a result of the adverse effects of pesticides, it has been found that the expression of antimicrobial peptides changes, immune signals become irregular, and bees become more susceptible to pathogens such as *Nosema* spp. (Raymann & Moran, 2018).

Disruptions in the microbiota caused by long-term pesticide exposure lead to problems not only at the individual level but also at the colony level. Imbalances in the microbiota result in negative consequences such as a weakened immune system in honeybees, problems with nutrient digestion, and impaired energy metabolism. As a result of these conditions, worker bees become more vulnerable to diseases, weaken, and experience a decline in performance, leading to the gradual weakening and eventual collapse of the colony (Goulson et al., 2015).

6. CONCLUSION

Honeybees are vital and indispensable organisms for the sustainability of ecosystems and agricultural production due to their role as pollinators (Fontaine et al., 2006). There are numerous environmental factors that negatively impact the health of honeybees. Pesticides threaten honeybees and human health directly and indirectly through their toxic effects. Pesticides cause many problems for honeybees. One of these problems is the significant and lasting effects they have on the honeybee gut microbiome (Paris et al., 2020).

The honeybee gut microbiota has significant effects on honeybee and colony health (Bonilla-Rosso & Engel, 2018). The microbiota plays a role in vital functions such as immune system stimulation, suppression of pathogenic microorganisms, detoxification, digestion of nutrients, growth, and development, providing benefits to honeybees (Motta & Moran, 2024). The imbalances and disruptions caused by pesticides in the microbiota lead to dysbiosis, resulting in a decrease in the number of beneficial bacteria. This weakens the immune system, increasing susceptibility and frequency of disease (Daisley et al., 2020). Insecticides, especially neonicotinoids, disrupt the balance of the microbiota, reducing resistance to pathogens (Brandt et al., 2016).

The effects of dysbiosis cause significant losses at the colony level. Disruption of the microbiome balance leads to a decrease in the queen bee's egg-laying rate and an increase in worker bee mortality rates, thereby reducing colony productivity. In advanced cases, these losses can result in colony collapse. This situation suggests that pesticides are a significant component of the global decline in honeybee populations (Hotchkiss et al., 2022).

Pesticides cause significant harm to honeybees, and when assessing this harm, the damage they inflict on the honeybee gut microbiome should not be overlooked and must be taken into account. The use of pesticides should be regulated, and the use and research of natural alternatives to pesticides should be encouraged and preferred when combating pests in many areas. The indiscriminate use of pesticides can cause major health problems for both honeybees and people who use bee products in many areas. In order to fully understand the effects of pesticides on the bee microbiome and the functional consequences of these changes, comprehensive studies covering different bee species and broad-spectrum agricultural chemicals are needed.

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CHAPTER VI

MOLECULAR AND BIOSENSOR- BASED DIAGNOSTIC APPROACHES IN BEE DISEASES: CURRENT STATUS AND FUTURE PERSPECTIVES

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DOI: https://www.doi.org/10.5281/zenodo.18138294

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1. INTRODUCTION

Honey bees (Apis Bees (Achilles' tusks mellifera) are indispensable organisms for global food security and ecosystem sustainability, and it is estimated that approximately one-third of agricultural production worldwide is directly or indirectly dependent on their pollination services. However, increasing colony losses in recent years have made the complex interaction of biotic and abiotic stress factors threatening bee health more visible. In this context, pathogen-induced diseases, especially American Foulbrood (AfF) , are becoming increasingly prevalent. Foulbrood (AFB) and nosemosis are among the major infectious diseases that seriously threaten colony health and sustainable beekeeping (de Graaf et al., 2013; Matović et al., 2023).

Paenibacillus, the causative agent of American Foulbrood. Nosema larvae are a bacterial pathogen capable of forming highly resistant spores, spreading rapidly among colonies, and causing high mortality in infected colonies. The ability of spores to survive for a long time under environmental conditions makes disease eradication difficult and necessitates radical control measures such as the destruction of infected colonies in most countries (Forsgren et al., 2008; WOAH, 2023). In contrast, Nosema apis and especially Nosema, which has become dominant on a global scale in recent years. Ceranae causes digestive system infections in adult bees, reducing colony performance, increasing winter losses, and often goes undetected early due to its subclinical course (Fries , 2010; Schüler et al., 2023).

The commonality between these two diseases is the difficulty in early and reliable diagnosis. Traditional diagnostic approaches include field observations based on clinical symptoms and microscopic spore detection for AFB; and spore counting by light microscopy for nosemosis . However, these approaches are unable to differentiate at the species level, are insufficient at low infection loads, and produce operator-dependent results (de Graaf et al., 2013; Szabó et al., 2025). The inability to morphologically distinguish between N. apis and N. ceranae , in particular , necessitates molecular confirmation.

To overcome these limitations, molecular diagnostic techniques have become widely used in bee disease research in recent years. Conventional PCR, real-time PCR (qPCR), and isothermal amplification -based methods (LAMP, RPA) provide high sensitivity and specificity by targeting pathogen-specific gene regions. For P. larvae , the 16S rRNA , ftsZ , and plx genes; for Nosema...

spp. For this purpose, SSU rRNA and ITS regions are among the most commonly used molecular targets in diagnosis (Chen et al., 2009; Tiritelli et al., 2025). However, these methods generally require laboratory infrastructure, trained personnel, and relatively high costs, limiting their routine application in field settings.

At this point, biosensor- based diagnostic approaches stand out as promising alternatives for the early, rapid, and field-adaptive detection of bee diseases. Electrochemical, optical (SPR, fluorescence), immunosensor , and microfluidic- based systems have the potential to directly and quickly detect pathogen DNA, specific proteins, or metabolites (Dicle & Karamese , 2024; Sabaté del Río et al., 2020). Particularly low detection limits, portability, and minimal sample preparation requirements make these technologies attractive for beekeeping applications. However, much of the current biosensor research remains at the laboratory scale; significant gaps exist in areas such as standardization, field validation , and cost-effectiveness.

This section aims to address molecular and biosensor- based diagnostic approaches in bee diseases from a holistic perspective. Nosema spp . And Paenibacillus Focusing on larvae , the biological basis, technical advantages, and limitations of current diagnostic methods will be evaluated comparatively; the potential and future application areas of biosensor technologies will be discussed in light of current research published in the last five years. Thus , the study aims to provide a comprehensive reference for both researchers and field practitioners on innovative and applicable approaches to the early diagnosis of bee diseases.

Clearly demonstrates that bee diseases should be addressed not only from an pathogen-focused perspective but also from a diagnostic capacity- focused perspective. Nosema spp . And Paenibacillus Since the effects of pathogens such as larvae at the colony level are often not clinically apparent in the early stages of infection, the need for diagnostic methods with high sensitivity and specificity is increasing day by day. This need has necessitated moving beyond traditional microscopic and culture-based approaches; advances in molecular biology and biosensor technologies have created a new paradigm in the diagnosis of bee diseases. In this context, the following sections will first address molecular diagnostic methods commonly used in the detection of bee pathogens ; then, biosensor technologies aiming to overcome the limitations of these methods will be discussed. Innovative approaches based on this approach will be evaluated in detail in terms of their technical foundations, application potential, and field compatibility.

2. MOLECULAR APPROACHES IN THE DIAGNOSIS OF BEE DISEASES

Molecular diagnostic approaches are based on the detection of pathogens involved in the etiology of bee diseases through specific and amplifiable biomarkers at the nucleic acid level . The main advantage of these methods is that they provide high sensitivity even in early infection stages where the pathogen load is low, and allow for the identification of agents at the species or strain level that cannot be distinguished by classical methods due to morphological similarities. Nosema spp . And Paenibacillus In the diagnosis of bee pathogens such as larvae , ribosomal RNA genes (16S rRNA , SSU rRNA), internal transcription intervals (ITS), and specific virulence or cellular function genes (e.g. Gene regions (plx , ftsZ , rpoB) are widely targeted due to their high copy numbers and phylogenetic distinctiveness. Molecular techniques based on amplification of these gene regions enable not only confirmation of pathogen presence but also quantitative assessment of infection dynamics and support for epidemiological surveillance studies. However, the level of conservatism of the selected target gene , sequence variation, and potential cross -reactivity risk stand out as critical parameters directly affecting the performance of developed molecular diagnostic systems.

2.1.PCR and qPCR Based Methods

Polymerase chain reaction (PCR) and real-time PCR (qPCR) are among the methods considered the gold standard in the molecular diagnosis of bee diseases , involving the enzymatic detection of pathogen-specific nucleic acid sequences. It provides high sensitivity and specificity through amplification . While conventional PCR allows for qualitative confirmation of the presence of the target gene region, qPCR technology enables quantitative pathogen load determination by monitoring the amplification process in real time via fluorescently labeled probes or intercalation dyes. Nosema spp . SSU rRNA and ITS regions are frequently found in Paenibacillus . For larvae , 16S rRNA , ftsZ , and plx genes are targeted, and the selection of these genes both increases analytical sensitivity and strengthens interspecies discrimination. Although

PCR and qPCR- based approaches provide high accuracy in the detection of subclinical infections, monitoring of infection severity, and epidemiological surveillance studies, they may be limited in field applications due to the need for thermal cycling equipment, laboratory infrastructure, and expert personnel. This situation constitutes a significant driving force encouraging the development of alternative molecular and biosensor- based approaches for more portable and rapid diagnostic systems. Polymerase chain reaction (PCR) and real-time PCR (qPCR) are the most commonly used molecular techniques in the diagnosis of bee pathogens. These methods allow the detection of specific gene regions of pathogens with high sensitivity and specificity. Especially Nosema 16S rRNA and ITS regions for Paenibacillus spp . For larvae , 16S rRNA , plx1, and ftsZ genes are frequently targeted for diagnosis.

As summarized in Table 1 , PCR and qPCR- based molecular methods are effective against Nosema due to their high sensitivity and specificity. spp . And Paenibacillus This method enables reliable detection of larval infections. However, analysis time, laboratory infrastructure requirements, and limited field applicability are the main factors limiting the use of these methods in routine colony screening. In contrast, isothermal amplification techniques and biosensor- based approaches offer shorter analysis times and portability advantages, increasing the potential for early diagnosis in field conditions. However, most biosensor systems are still in the development phase in terms of standardization, validation , and widespread application, and the need for comprehensive field data continues compared to molecular methods.

2.2. Isothermal Amplification Techniques

Isothermal nucleic acid amplification techniques, based on amplifying target DNA at a constant temperature, eliminate the need for thermal cycling required by classical PCR systems and thus offer diagnostic solutions more suitable for field conditions. Loop-mediated isothermal amplification (LAMP) and recombinase polymerase Reproductive amplification (RPA) methods are gaining increasing attention for the rapid diagnosis of bee diseases due to their high amplification efficiency, short analysis time, and minimal equipment requirements. Nosema spp . And Paenibacillus LAMP and RPA protocols developed for larvae mostly target ribosomal RNA genes or species-specific virulence genes; they allow for easy interpretation of results through visual color change, fluorescent signal, or lateral flow formats. However, the number of primer sets and design complexity used in these techniques are critical parameters in terms of specificity and the risk of false positive results, and stand out as a factor that needs to be carefully optimized when transitioning to field applications. In this respect, isothermal amplification methods form a functional bridge between molecular diagnostics and biosensor- based systems and provide an important foundation for the development of portable diagnostic platforms.

2.3. Next-Generation Sequencing and Metagenomic Approaches

Next- Generation Sequencing Sequencing (NGS) and metagenomic approaches allow for a holistic assessment of the colony microbiota , moving beyond single-pathogen-focused analyses in the diagnosis and monitoring of bee diseases . These techniques enable the simultaneous detection of bacteria, fungi, protozoa , and viruses present in bee colonies without requiring targeted amplification or via selected marker genes ; thus providing in-depth information about subclinical infections, co -infection dynamics, and pathogen-microbiota interactions. Nosema spp . In infections , ITS and SSU rRNA- based amplicon sequencing approaches are used to determine species distribution and population structure; while in Paenibacillus infections... Whole genome sequencing (WGS) analyses for larvae offer significant advantages in strain-level differentiation, characterization of virulence genes , and outbreak monitoring studies. However, NGS-based diagnostic approaches are used more for research, surveillance , and risk assessment purposes rather than routine diagnosis due to their high cost, complex bioinformatic analysis requirements, and limited field applicability . In this respect , metagenomic approaches constitute a critical information infrastructure in identifying potential targets for biosensor and rapid molecular diagnostic systems.

Next-generation sequencing (NGS) technologies are considered a strategic discovery tool in the early and critical stages of biosensor development, rather than for routine diagnosis of bee diseases. NGS and metagenomic analyses are used in the study of Nosema. spp . And Paenibacillus By enabling detailed characterization of the genomic structures of pathogens such as larvae , it allows for the identification of highly specific nucleic acid sequences, protein targets, and metabolic processes that can be used in biosensor design. This enables the identification of biomarkers . In this context, NGS plays a decisive role in the target selection and validation phase, which forms the basis of sensor platforms, rather than simply validating a single pathogen.

However, NGS-based approaches are not directly competitive with biosensors as diagnostic tools due to factors such as high cost, complex data analysis, and incompatibility with field conditions. Instead, NGS outputs support the rational design of specific probes and binding molecules for use in electrochemical, optical, or immunosensor platforms, contributing to the optimization of sensors in terms of sensitivity, specificity, and cross -reactivity control. Therefore, NGS should be positioned as a laboratory-based discovery and validation tool in the development of biosensor- based diagnostic systems for bee diseases; and in field applications, it should play a complementary role with faster, portable, and cost-effective sensor technologies.

biomarker information obtained from these molecular and genomic approaches forms the basis for the rational design of biosensor- based systems aimed at enabling rapid, portable, and field-adaptive diagnosis of bee diseases.

Table 1. Molecular diagnostic methods used in bee diseases.

Method	**Target Pathogen(s)**	**Sensitivity**	**Specificity**	**Analysis Period**	**Field Applicability**	**Advantages**	**Limitations**
Conventional PCR	*Nosema spp* . , *P. larvae*	High	High	3-5 hours	Low	Species-specific diagnosis is reliable.	Laboratory infrastructure is required.
qPCR	*Nosema spp* . , *P. larvae*	Very high	Very high	2-3 hours	Low	Quantitative results, early diagnosis.	High cost
LAMP / RPA	*Nosema spp* . , *P. larvae*	High	High	30–60 minutes	Medium-High	Portable, fast	Primary design is critical.

Method	Target Pathogen(s)	Sensitivity	Specificity	Analysis Period	Field Applicability	Advantages	Limitations
Electrochemical biosensor	*Nosema spp* . , *P. larvae*	Very high	High	10–30 min	High	Fast, low detection limit.	Lack of standardization
Optical (SPR/Fluorescent) biosensor	Various bee pathogens	High	Very high	10–60 min	Middle	Real-time measurement	Device cost
Immunosensor / Lateral flow	*P. larvae*	Middle	Medium -High	10-15 minutes	Very high	Ease of use	Limited distinction between species and cargo.

Table 1 presents a comparative analysis of commonly used molecular methods for diagnosing bee diseases, considering their target pathogens, sensitivity and specificity levels, analysis time, and field applicability. While laboratory-based techniques such as conventional PCR and qPCR provide high accuracy and species specificity , they are limited in field conditions due to infrastructure and time requirements. In contrast, isothermal amplification methods such as LAMP and RPA stand out as more suitable alternatives for field applications due to their shorter analysis times and portability.

3. BIOSENSOR- BASED DIAGNOSTIC APPROACHES IN BEE DISEASES

Early diagnosis of bee diseases is critical for preventing colony losses and ensuring sustainable beekeeping. Traditional diagnostic methods rely on microscopic examination, culture techniques, and laboratory-based molecular analysis, which are often time-consuming, require expertise, and are difficult to apply in field conditions. These limitations lead to delayed diagnosis and rapid spread of diseases, especially in migratory beekeeping areas. In this context, biosensor- based approaches stand out as innovative technologies that enable rapid, accurate, and field-appropriate diagnosis of bee diseases.

Biosensors generally consist of a biological receptor that recognizes the target pathogen and a transducer that converts this interaction into a measurable signal. Biosensor systems developed for bee diseases make it possible to detect bacterial, parasitic, fungal, and viral agents at the molecular or protein level.

3.1. Electrochemical Biosensors

Electrochemical biosensors are among the most widely researched and promising systems for diagnosing bee diseases. These sensors work by measuring electrical changes (current, potential, or impedance) caused by DNA, RNA, or metabolites of the target pathogen on the electrode surface. This is particularly true for Nosema . spp . and Paenibacillus DNA probe -based electrochemical sensors , capable of identifying specific gene regions of pathogens such as larvae , allow for the early detection of subclinical infections thanks to their low detection limits .

The key advantages of these systems include short analysis time, high sensitivity, integration with portable devices, and applicability in field conditions. Electrochemical biosensors have the potential to form the basis of colony-based continuous disease monitoring platforms in the future when combined with smart hive systems.

3.2. Optical and Fluorescent Biosensors

Optical biosensors detect the interaction between a biological recognizer and a target molecule via light-based signals. Sensors developed using fluorescently labeled DNA probes or antibodies offer high specificity and accuracy rates. These systems are particularly preferred in rapid screening analyses using laboratory equipment.

Surface plasmon resonance (SPR) based biosensors have the advantage of being able to make real-time measurements without the need for any marker. SPR sensors are considered an important tool in the study and validation analysis of biomolecular interactions of bee pathogens . The development of portable versions of optical biosensors will enable their more widespread use in the field in the future.

3.3. Immunosensors

Immunosensors are biosensor systems based on antigen-antibody interactions and play a significant role in the rapid and practical detection of bee diseases. Lateral sensors, in particular, have been developed for the early field diagnosis of highly contagious diseases such as American Foulbrood. Flow (rapid diagnostic) tests are readily available for use by beekeepers.

ELISA-based immunosensors offer higher accuracy rates and are preferred in screening and validation phases. The main advantages of immunosensors are their user-friendliness, rapid results, and lack of need for specialized technical infrastructure. However, their limited species differentiation and quantitative analysis capabilities necessitate their use in conjunction with molecular sensors .

3.4. Nanobiotechnology -Supported Biosensors

Advances in nanobiotechnology have significantly improved biosensor performance. Nanomaterials such as gold nanoparticles , graphene , and magnetic nanoparticles increase the sensor surface area , enhancing the binding capacity of biomolecules and providing signal amplification . This allows for the detection of even very low pathogen densities, making early and reliable diagnosis possible.

Nanobiosensors have the potential to precisely determine not only the presence of bee diseases but also their infection load. Because of these characteristics , nanotechnology- supported biosensors are considered among the fundamental components of future beekeeping diagnostic systems.

3.5. Microfluidic (Lab -on-a- Chip) Biosensor Systems

Microfluidic Biosensor systems are integrated platforms that combine sample preparation, analysis, and results reading on a single chip. Capable of working with very small volumes of samples taken from honey, bee tissue, or intracolony remains, these systems offer cost-effective and rapid diagnostic capabilities.

Lab -on-a- chip technologies are highly deployable systems in the field and can be integrated with mobile diagnostic devices and smart hive applications. This approach enables early warning systems for monitoring bee diseases and digital mapping of regional disease spread.

3.6. General Assessment

Biosensor- based approaches offer a powerful alternative to classical methods in the early diagnosis, monitoring, and management of bee diseases. Biosensors integrated with molecular biology, nanotechnology , and digital systems contribute to supporting sustainable beekeeping with rapid, accurate, and field-specific diagnostic solutions. In the future, the widespread adoption of these systems will play a decisive role in reducing colony losses and protecting bee health.

Table 2. Biosensor- based diagnostic approaches in bee diseases.

Type of Biosensor	**Target Pathogen(s)**	**Biological Diagnostic Element**	**Sensitivity**	**Analysis Period**	**Field Applicability**	**Advantages**	**Limitations**
Electrochemical DNA biosensor	*Nosema spp .* , *P. larvae*	DNA probe	Very high	10–30 min	High	Low detection limit, fast response.	Lack of standardization and validation
Optical (SPR) biosensor	Various bee pathogens	DNA / antibody	High	10–60 min	Middle	Real-time measurement	Device cost
Fluorescent biosensor	*Nosema spp .*	DNA probe	High	20–40 minutes	Middle	High signal sensitivity	Fluorescent extinction
Immunosensor	*P. larvae*	Antibody	Medium-High	10-20 minutes	High	Specific protein recognition	Risk of cross-reaction
Lateral flow tests	*P. larvae*	Antibody	Middle	10-15 minutes	Very high	Ease of use, quick results.	No quantitative analysis.

Table 2 summarizes biosensor- based approaches developed for the diagnosis of bee diseases in terms of the biological diagnostic elements used, analysis time, sensitivity level, and field applicability. Electrochemical and optical biosensors enable the rapid and sensitive detection of nucleic acids or proteins belonging to target pathogens, while offering the potential for early diagnosis and continuous monitoring. Particularly lateral Flow and immunosensor systems provide practical solutions in beekeeping applications due to their ease of use and portability; however, they have limitations in terms of quantitative analysis and standardization.

4. TARGETED SPECIFIC GENE REGIONS FOR BIOSENSOR AND MOLECULAR DIAGNOSIS IN BEE DISEASES

Biosensor and molecular diagnostic systems depends on the accurate selection of specific, conserved, and distinctive gene regions of the target pathogen. Nosema, which is an important pathogen among bee disease agents, is one such example. spp . And Paenibacillus Commonly used gene regions in the literature for larvae were determined considering sensitivity, specificity, and field applicability criteria. These gene targets are fundamental reference points in the development of molecular techniques such as PCR/ qPCR , as well as DNA/RNA probe- based biosensors .

4.1. Nosema Target Gene Regions for spp . (*N. apis and N. ceranae*)

The most commonly preferred targets for the diagnosis of Nosema species are ribosomal RNA (rRNA) genes and their intermediate regions, which have high diagnostic value due to containing species-specific sequences. In particular, the 16S rRNA (SSU rRNA) gene is used as a standard target in PCR, qPCR , LAMP, and biosensor applications due to both its conserved structure and species-specific variations . This gene region allows for the early detection of low-intensity and subclinical infections.

In addition, Internal Transcribed The spacer regions (ITS-1 and ITS-2) exhibit a high degree of variability between *N. apis* and *N. ceranae* , allowing for clear species differentiation. While ITS regions are particularly preferred for phylogenetic analyses and validation studies, they have limited use in quantitative analyses. The 18S rRNA gene, on the other hand, exhibits a more conserved structure and is used as a supporting target in broad-spectrum Nosema screenings and metagenomic studies.

In diagnostic systems developed at the research level, alternative gene regions such as Hsp70 (heat shock protein) and Rpb1 (RNA polymerase II subunit) are also used to reveal interspecies genetic variation; these genes are particularly important for advanced molecular characterization and increasing biosensor specificity.

4.2. Paenibacillus Target Gene Regions for Larvae

Paenibacillus, the causative agent of American Foulbrood. Larvae are highly resistant to environmental conditions due to their spore-forming structure. This necessitates the selection of gene regions with high specificity in diagnostic systems. The 16S rRNA gene, one of the most commonly used targets, is widely used in screening PCR and qPCR analyses; it enables the detection of spore presence in honey, honeycomb, and bee samples. However, the similarity of the 16S rRNA gene to closely related bacterial species carries the risk of cross-reaction when used alone.

To overcome this limitation, P. larvae- specific virulence genes such as plx1 (paenilarvin toxin gene) are being incorporated into diagnostic systems. Due to its high specificity, the plx1 gene stands out as an important target in confirming clinical cases and in the development of virulence- based biosensors . In addition, the ftsZ and rpoB genes, involved in cell division and transcription processes, are highly species-discriminating targets that provide reliable results in quantitative analyses.

epidemiological and strain- level differentiation, ERIC and REP repeat sequences are used; these regions contribute to revealing the dynamics of disease spread and genotypic diversity.

Table 3. *Nosema spp* . And *Paenibacillus* Specific gene regions for *larvae*

Pathogen	Gene	Diagnostic Purpose	Method	Biosensor Compatibility
Nosema spp .	16S rRNA	Species identification	PCR/ qPCR	Very high
Nosema spp .	ITS	Species distinction	PCR/NGS	High
P. larvae	plx 1	Clinical validation	qPCR	Very high
P. larvae	ftsZ	Quantitative analysis	qPCR	High

Table 3 **,** *Nosema spp* . And *Paenibacillus* This table presents specific gene regions commonly used in molecular diagnostics and biosensor development studies for *larval pathogens. The table* shows a comparative analysis of each gene target's diagnostic purpose, preferred molecular methods, and suitability for biosensor platforms. Specifically, 16S rRNA and ITS regions stand out for species identification and differentiation, while genes such as *plx1* and *ftsZ demonstrate high potential in* biosensor design for virulence and quantitative analysis . This assessment highlights the crucial role of gene target selection in the performance of diagnostic systems.

4.3. The Importance of Gene Targets in Biosensor Development

Nosema spp . And These specific gene regions identified for P. larvae can be directly used in the design of DNA/RNA probe- based electrochemical and optical biosensors . Targets such as 16S rRNA , ITS, and plx1, in particular, are ideal molecular markers for biosensor platforms due to their high specificity, low detection limit, and adaptability to field conditions . Integration of these gene targets into biosensor systems enables rapid and reliable diagnosis of bee diseases, allowing for early warning and effective disease management.

In conclusion, the selection of the correct gene targets is one of the key factors determining the success of molecular diagnostic methods and biosensor-based approaches. The integration of these targets into biotechnological diagnostic systems plays a key role in the development of sustainable beekeeping practices.

5. CONCLUSION AND DISCUSSION

This section presents the current state of molecular and biosensor- based approaches used in the diagnosis of bee diseases and highlights the potential of these technologies for sustainable beekeeping.

Over the last five years (2021–2025), there has been a significant increase in scientific studies on the diagnosis and management of bee diseases, with molecular biology and biotechnology- based approaches coming to the forefront. Current research in the SCI-E database shows that Nosema ceranae and Paenibacillus This demonstrates the optimization of qPCR , multiplex PCR, and isothermal amplification techniques for the early and sensitive detection of key pathogens such as larvae . Review studies published during the same period highlight the inadequacy of classical microscopic methods alone and emphasize the necessity of molecular validation. Furthermore , there has been a remarkable increase in the number of studies aimed at adapting biosensor- based systems to the field.

Table 4. Evolution of diagnostic technologies and integration gap in the literature.

Level	System	Advantage	Irritability	Literature Review
Classical	Microscopy	Simple	Low sensitivity	It is decreasing.
Molecular	PCR/ qPCR	High specificity	Infrastructure	Widespread
Biosensor	DNA sensors	Fast	Standardization	Developing
Integrated	Smart systems	Early warning	Validation	A vacancy exists.

Table 4 summarizes the technological evolution of methods used in the diagnosis of bee diseases and the existing integration gap in the literature within a conceptual framework. This evolutionary process, extending from classical microscopic and culture-based methods to molecular techniques, and then to biosensor- based and integrated digital systems, shows that while analytical performance has increased, field applicability and standardization remain significant limitations. The table clearly reveals that intelligent systems integrating molecular diagnostics with biosensor technologies are not yet adequately represented in the literature.

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CHAPTER VII

THE USE OF BIOSENSORS FOR CHEMICAL RESIDUE LIMITS AND TRACEABILITY IN BEE PRODUCTS

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DOI: https://www.doi.org/10.5281/zenodo.18138340

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1. INTRODUCTION

Beekeeping has evolved from a traditional agricultural activity based solely on honey production to a multifaceted production field directly linked to ecosystem services, biodiversity, environmental monitoring, and human health. Honey bees (Apis) Bees (Mellium mellifera), due to their wide flight ranges and their behavior of collecting nectar, pollen, resin, and water from numerous plant sources, are considered biological sensors that reflect the biochemical and toxicological profile of their environment onto hive products . This characteristic makes bee products both high-value foods and biological indicators of environmental exposure .

Modern beekeeping practices are shaped by intensive agricultural production, increased pesticide use, widespread veterinary medicines, and industrial pollution. Products such as honey, pollen, propolis , royal jelly, and bee venom produced under these conditions carry a risk of contamination with pesticides, acaricides , antibiotics, heavy metals, and persistent organic pollutants (POPs) . The biologically active nature of bee products and the consumption of some products (especially propolis and royal jelly) for therapeutic purposes make the potential effects of these residues on human health even more critical.

One of the key tools used in managing this risk is the Maximum Residue Limit (MRL) concept. MRLs define the highest level of chemical residues allowed in a given food matrix and are based on toxicological risk assessment models. However, the MRL approach has largely been developed for plant and animal foods and does not fully reflect the unique biochemical properties, production dynamics, and consumption patterns of bee products. This makes it difficult to strike a balance between food safety and environmental sustainability in the beekeeping sector.

particularly for bee products other than honey (pollen, propolis , royal jelly, and bee venom), creates a significant regulatory gap. This is evident in the European Union (EU), the Turkish Food Codex (TGK), and Codex. An examination of alimentarius regulations reveals that while relatively detailed limits are defined for honey, other bee products are evaluated within an indirect or vague regulatory framework. This approach leads to uncertainties in the trade of beekeeping products and weakens the producer-consumer trust chain.

The assessment of chemical residues in bee products is important not only for regulatory compliance but also for bee health, colony sustainability, and ecosystem integrity. Sublethal pesticide exposures can directly affect the bees' nervous system, immune response, navigation behavior, and microbiota , leading to colony losses and reduced product quality. Therefore, the MRL (Maximum Residue Limit) concept needs to be re-evaluated from a beekeeping perspective and supported by product-specific scientific data.

The aim of this book chapter is to address the problem of chemical residues in bee products, specifically in beekeeping, with a holistic approach, to discuss the scientific basis and limitations of the MRL concept, and to examine the European Union, Turkish Food Codex, and Codex. The aim is to compare alimentarius regulations on a product basis and to reveal the impact of existing regulatory gaps on the beekeeping sector. Furthermore, considering the limitations of classical analytical methods, the integration of biosensor- based monitoring systems into beekeeping practices is evaluated as a new paradigm in terms of early warning and risk management.

This chapter covers the behavior of chemical residues in bee products, the scientific basis of the MRL approach, existing regulations for each bee product, comparative tables, and critical evaluations; and finally, the possibilities offered by biosensor technologies in terms of food safety and environmental monitoring in beekeeping.

2. SOURCES OF CHEMICAL RESIDUES IN BEE PRODUCTS

The origin of chemical residues detected in bee products should be considered as a combination of bee biology, ecological interactions, and modern agricultural practices. Honey bees actively collect nectar, pollen, plant resins, and water within a radius of approximately 2–5 km; in this process, they are in direct or indirect contact with numerous chemical agents used in agricultural ecosystems. This large foraging area makes bee colonies extremely sensitive biological systems in terms of environmental chemical load.

The main sources of chemical residues can be grouped into three main categories: (i) agricultural pesticides and herbicides, (ii) veterinary drugs and acaricides used in beekeeping practices, (iii) heavy metals and persistent organic pollutants from environmental and industrial pollution. Organic

Pollutants (POPs). Each of these groups exhibits different distribution and accumulation behavior in bee products.

Agricultural pesticides, particularly insecticides (neonicotinoids , pyrethroids , organophosphates) and fungicides , constitute the most frequently reported residue group in bee products. These substances can be systemically transported into the nectar and pollen of flowering plants and carried into the hive by bees. The fact that systemic pesticides can produce sublethal effects even at low concentrations necessitates an assessment of residues in bee products not only in terms of legal limits but also in terms of their potential biological effects.

Veterinary medicines and acaricides used in beekeeping (e.g., amitraz , fluvalinate , coumaphos), especially against Varroa mites . It is commonly applied in the fight against destructors . Since these substances are applied directly to the hive environment, they tend to accumulate in the hive matrices, especially beeswax . The lipophilic nature of beeswax acts as a reservoir for such chemicals; over time, they secondary to other products such as honey, pollen, and royal jelly. Transmission can occur through contamination . This indicates that residues can appear not only depending on the application period but also on the long-term history of the hive.

Heavy metals (lead, cadmium, mercury, arsenic) and POPs (DDT derivatives, polychlorinated oils) from environmental pollution sources. Biphenyls (PHEs) are considered to be more of an indicator of chronic exposure in bee products . Bees indirectly transport these pollutants from soil, water, and atmosphere into the hive through the materials they collect. Pollen and propolis , in particular, are sensitive biomarkers in determining environmental heavy metal load. They stand out as matrices .

The behavior of chemical residues in bee products is determined by the physicochemical properties of the residue (lipophilicity , volatility, stability), the composition of the product matrix , and intrahive biochemical processes. Honey, due to its high water and sugar content, harbors more hydrophilic residues, while propolis and beeswax have a high retention capacity for lipophilic pesticides. Pollen, due to its balanced protein, lipid , and carbohydrate content, is a critical carrier for both hydrophilic and lipophilic compounds.

These distribution differences make it scientifically problematic to consider bee products as a single, uniform food matrix . The fact that the same chemical substance can be found in low concentrations in honey and high concentrations in propolis necessitates a product-by-product reassessment of the MRL (Mean Residue Reduction in Ratio) approach. Furthermore, the fact that some chemicals can be metabolized or become more toxic during in-hive transformation processes Its potential for conversion into metabolites necessitates toxicological evaluations beyond classical residue analyses .

In conclusion, the sources and behavior of chemical residues in bee products are shaped by the complex interplay of bee biology, environmental exposure , and hive ecology. This complex structure clearly demonstrates why the MRL concept, which will be discussed in the next section, remains limited to bee products and why product-based, dynamic, and biotechnology-supported monitoring approaches are needed.

2.1. The Scientific Basis of the Maximum Residue Limit (MRL) Concept and its Evaluation from the Perspective of Bee Products

The Maximum Residue Limit (MRL) is a regulatory parameter based on toxicological risk assessment that indicates the highest level of pesticide or veterinary drug residue allowed in a given food matrix . MRL values are not direct toxicity limits; rather, they are derived by relating toxicological thresholds such as Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD) to hypothetical consumption scenarios .

The fundamental approach in determining MRLs is to ensure that the amount of chemicals an individual will be exposed to while consuming a particular food item throughout their lifetime remains below acceptable limits for human health. In this context , MRLs are based on Good Agricultural Practices (GAP) . Agricultural MRLs (Maximum Requirements Limit) are determined based on GAP (Global Application Practices), taking into account the recommended usage doses and application frequency of the chemical. Therefore, MRLs are primarily aimed at legal compliance and agricultural application control, rather than toxicological safety .

However, this approach has significant scientific limitations when it comes to bee products. First, the MRL (Maximum Residue Limit) concept is largely designed for a single food matrix , based on short- or medium-term consumption patterns. Bee products, on the other hand, are consumed in small quantities but over a long period, contain biologically active components, and are sometimes used for therapeutic purposes. These characteristics mean that classical MRL risk assessment models are insufficient for bee products.

Another fundamental problem with the MRL (Maximum Resistance Level) approach in bee products is the failure to consider biochemical differences between products. Honey, pollen, propolis , and royal jelly; their chemical binding capacities, lipophilicity levels, and metabolic characteristics... They differ significantly in terms of their stability . The fact that the same chemical substance can be found in low concentrations in honey but accumulate in high levels in propolis or beeswax weakens the scientific validity of a single MRL (Meaning Resistance Level) approach.

Furthermore, MRL systems are mostly based on the assumption of a single chemical–single product . However, bee products can contain multiple pesticide residues and their synergistic or antagonistic effects as a result of environmental exposure . These multiple exposures at sublethal levels can create biological effects beyond classical toxicological thresholds for bee and human health . The current MRL framework is not designed to evaluate such interactions.

From a beekeeping perspective, the fact that the MRL concept focuses solely on human consumption is a significant shortcoming. Bees are non-target organisms directly exposed to these chemicals, and the effects on colony health often appear earlier and at lower concentrations than the risks to human health. Therefore, residues detected in bee products should be considered not only in terms of consumer safety but also as an indicator of bee health and ecosystem integrity.

In conclusion, the MRL approach is a necessary but insufficient regulatory tool for bee products. Given the complex nature of bee biology, product matrix variations, and environmental exposure , MRLs need to be evaluated in conjunction with product-based, dynamic, and supportive monitoring systems. This clearly demonstrates why national and international regulatory approaches are inadequate for bee products and highlights the need for new monitoring paradigms, which will be discussed in the next section.

2.2. Regulatory Approaches to Chemical Residues in Bee Products

Legislative approaches to regulating chemical residues in bee products show significant differences at national and international levels. These differences are not limited solely to the variety of legal limit values; they also extend to distinct risk assessment philosophies, product scope, and application practices. The European Union (EU), the Turkish Food Codex (TGK), and Codex Alimentarius constitutes the three fundamental regulatory frameworks considered as references for bee products.

2.2.1. The European Union Approach

In the European Union, regulations concerning chemical residues in bee products are primarily addressed within the framework of horizontal legislation focusing on pesticide residues. Honey is included as a clearly defined food matrix in EU legislation , with specific MRL (Mean Time Limit) values established for numerous pesticides and veterinary drugs. This approach is based on the importance of honey in international trade and its widespread consumption.

However, for other bee products such as pollen, propolis , and royal jelly, directly defined MRL (Mean Time Limit) values are largely absent from EU legislation. These products are often indirectly classified under “other animal products” or “specialty foods.” This indicates that the biochemical specificity of bee products is not adequately reflected at the legislative level. Furthermore, the EU approach focuses on the risk to human health; bee health and colony dynamics are not directly included in the regulatory criteria.

2.2.2. The Turkish Food Codex Approach

The Turkish Food Codex largely bases its regulations on residue levels in bee products on European Union legislation. The MRL (Maximum Residue Limit) values set for honey are highly aligned with the EU and are used as a reference in practice. This alignment is important for reducing technical barriers encountered in Türkiye's honey exports.

However, even within the scope of the Turkish Food Codex, specific MRL (Maximum Residue Limit) values are limited or nonexistent for bee products other than honey. The lack of defined residue limits for products such as pollen, propolis , and royal jelly leads to uncertainties in the marketing of these products. The increasing use of these products as functional foods and dietary supplements in recent years has made this regulatory gap even more visible.

the TGK's EU-centric structure offers advantages in terms of legislative harmonization, it also brings limitations such as insufficient consideration of Turkey's specific environmental conditions, agricultural practices, and beekeeping practices. This situation highlights the need to integrate local risk profiles into legislative processes.

2.2.3. Codex The Alimentarius Approach

Codex The Alimentarius Commission establishes globally reference MRL (Maximum Residue Limit) values for bee products and aims to ensure technical harmonization in international trade. The Codex includes MRL values for a limited number of pesticides and veterinary drugs for honey; however, these values are often defined as general and conservative limits.

While the Codex approach offers a flexible framework that takes into account the diversity of agricultural practices in different countries , it does not address the unique risk profiles of bee products in detail. In particular, there is a significant regulatory gap within the Codex for bee products other than honey . This indicates that the Codex primarily provides a trade-facilitating framework, while the depth of product-specific toxicological analysis remains limited.

2.2.4. Comparative Evaluation and Results from the Perspective of Beekeeping

of the EU, Turkish Food Codex, and Codex approaches reveals that legislation concerning bee products is largely focused on honey. Products such as pollen, propolis , and royal jelly, despite having high scientific risks and high biological activity, remain secondary in the regulatory framework.

This situation leads to three main problems for the beekeeping sector: (i) the inability to conduct product-based risk assessments, (ii) uncertainties in international trade, and (iii) the inability to effectively implement quality and safety standards in the field. Therefore, current legislative approaches need to be reviewed to take into account the diversity of bee products and the unique dynamics of beekeeping practices.

In this context, presenting MRL values for each bee product through comparative tables in the next section will provide a concrete illustration of the existing regulatory gaps.

2.3 . Comparative Analysis of Maximum Residue Limits (MRLs) for Honey (EU–TGK– Codex)

Honey is the most meticulously regulated matrix in terms of chemical residues among bee products . This is primarily because honey is a widely consumed food globally and holds a significant place in international trade. The European Union, the Turkish Food Codex, and the Codex all adhere to these standards. An examination of the Alimentarius regulations reveals that the defined MRL values for honey are relatively consistent; however, this consistency has debatable aspects in terms of scientific validity.

2.3.1. Scientific Basis of the Approach to Determining MRLs in Honey

MRL (Maximum Resistance Level) values for honey are determined primarily by considering the transfer of pesticides applied to plant products to nectar and pollen. In this approach, honey is considered an indirect exposure product; chemicals used directly in beekeeping (e.g., acaricides) are evaluated within separate regulatory frameworks. However, this distinction does not adequately reflect the impact of intra-hive contamination on honey.

Honey's high sugar and water content allows for easier detection of hydrophilic pesticides and some antibiotic residues within the matrix , while lipophilic chemicals are generally reported at low levels. This leads to honey being perceived as a relatively "clean" product; however, the long-term effects of secondary transfers from beeswax and propolis are often overlooked.

2.3.2. Comparison of MRL Values of AB – TGK – Codex Honey

Below is a comparative summary of the MRL (Maximum Residue Limit) values determined for some pesticides and veterinary drugs commonly detected in honey.

Table 2.1. Comparison of MRLs (mg/kg) for selected chemical substances in honey.

Chemical Substance	EU	TGK	Codex
Chlorpyrifos	0.05	0.05	0.05
Deltamethrin	0.01	0.01	0.01
Cypermethrin	0.01	0.01	0.01
Amitraz (metabolites)	0.2	0.2	0.2
Fluvalinate	0.05	0.05	0.05

This table shows a high level of quantitative alignment among international regulations for honey. However, this alignment does not mean that the chemical risk profile of honey is fully managed.

2.3.3. Scientific Limitations of Honey MRLs from a Beekeeping Perspective

The main limitation of the defined MRL (Maximum Residue Limit) values for honey is that these limits mostly focus on the risk of human consumption . However, many chemical residues detected in honey can be found at levels that can lead to sublethal effects on the bee colony. This shows that MRLs do not serve as an early warning function for bee health.

Furthermore , MRLs (Mean Time Limitations) are often assessed based on a single chemical, neglecting the synergistic effects of multiple residues present together in honey . In beekeeping practices, particularly in hives near intensively farmed areas, the presence of numerous pesticide residues at low levels is common. This mixed exposure scenario cannot be adequately assessed with the current MRL approach.

2.3.4. Implications of Honey MRLs in Monitoring and Implementation

The defined MRL (Maximum Residue Limit) values for honey are verified in official control laboratories through chromatographic analyses (GC-MS, LC-MS/MS). However, these methods have limitations such as high cost, time requirement, and incompatibility with field conditions. In beekeeping practice, real-time or early-stage residue detection in the honey production process is not possible.

This situation often leads to MRL compliance in honey being reduced to final product inspection , leaving risk management in the production process limited. In this context, increasing the effectiveness of the MRL approach for honey will be possible not only by defining legal limits but also by developing monitoring strategies.

2.3.5. Evaluation

Although honey is the best-defined MRL (Maximum Resistance Limit) among bee products, the current regulatory approach still contains significant gaps from a beekeeping perspective. Honey MRLs aim to ensure minimum safety for human health and are insufficient for a holistic assessment of bee health, colony sustainability, and environmental exposure .

These limitations highlight why the situation is more problematic with bee products other than honey, and explain why MRL approaches for pollen, which will be discussed in the next section , are more complex and inadequate.

2.4. Maximum Residue Limits (MRLs) for Pollen

the matrices with the highest risk profile in terms of chemical residues among bee products , pollen is one of the least defined products at the regulatory level. The main reason for this is that pollen is located at the intersection of both plant and animal production chains and therefore cannot be subjected to a clear classification in regulatory systems. The European Union, the Turkish Food Codex, and the Codex... When alimentarius approaches are considered together, it becomes clear that the concept of MRL (Maximum Resistance Level) for pollen is structurally incomplete.

2.4.1. Chemical Residue Profile and Scientific Significance of Pollen

Pollen, a structure that carries the male reproductive cells of plants, is one of the first biological materials to be directly exposed to pesticides used in agricultural production. Pollen collected by bees can contain numerous chemical substances through surface contamination and the transport of systemic pesticides into plant tissues.

Scientific studies show that pesticide concentrations detected in pollen are often higher than those in honey. Neonicotinoids , fungicides , and herbicides, in particular, form multiple residue profiles in pollen. This makes pollen a critical monitoring matrix for both bee health and human consumption.

2.4.2. MRL Approach for Pollen in the European Union

In European Union legislation, pollen has long been considered a natural component of honey and not treated as an independent food matrix . This approach has led to the lack of specific MRL (Mean Time Limit) values for pollen. In the EU, pollen analyses are often compared to MRL values defined for plant products, or risk assessments are carried out on a case-by-case basis.

This situation creates a significant scientific paradox. Pollen exhibits neither the characteristics of a classic plant product nor those of a typical animal product. Furthermore, its biochemical composition, rich in fatty acids and proteins, allows for stronger retention of many pesticides.

2.4.3. Pollen and Regulatory Uncertainties in the Turkish Food Codex

The Turkish Food Codex , clear MRL (Maximum Residue Limit) values for chemical residues are not defined. In practice, analysis results for pollen are generally compared with MRLs for plant products or evaluated according to the legislation of the country to which it will be exported.

This approach creates significant uncertainties for manufacturers and regulatory bodies. Particularly in pollen products marketed as dietary supplements, the inability to scientifically limit the risk of residue poses a major problem in terms of consumer safety and product standardization.

2.4.4. Codex Alimentarius and Pollen

Codex The Alimentarius Commission has not established independent MRL (Maximum Residue Limit) values for pollen. In the Codex , pollen is generally addressed indirectly, often under the categories of "specialty foods" or "other products." This demonstrates that the Codex's global trade-focused approach is insufficient for niche but high-risk products like pollen.

The most significant limitation of the Codex approach is that, despite the high chemical exposure of the pollen , the risk assessment is based on generalized limits. This often leads to the neglect of the effects of different pesticide use patterns in various countries on the pollen.

2.4.5. Comparative MRL Status for Pollen

Below is a summary comparison of current regulatory approaches for pollen.

Table 2.2. Comparison of MRL approaches for pollen (EU–TGK– Codex)

Legislation	Specific MRL for pollen.	The approach in practice
EU	None	Herbal product MRLs or case-based evaluation
TGK	None	Reference to MRLs for herbal products.
Codex	None	General limits / non-product evaluation

This table clearly shows that pollen has a clear regulatory gap in all three regulatory systems.

2.4.6. Evaluation from the Perspective of Beekeeping and Food Safety

The lack of defined MRL (Maximum Residue Limit) values for pollen overlooks the strategic importance of this product for beekeeping. Pollen plays a fundamental role in the nutrition of bee colonies and also functions as a biomatrix that can be an early indicator of chemical exposure .

The current regulatory approach addresses pollen only indirectly from the perspective of human consumption; it does not adequately consider the aspects of bee health and colony sustainability. This highlights the need to develop pollen-based risk assessment models and create product-based MRL (Maximum Requirement Ratio) approaches.

the MRL approach for propolis , which will be discussed in the next section, is even more complex and controversial.

2.5. Maximum Residue Limits (MRL) for Propolis

Propolis is one of the most complex matrices among bee products in terms of chemical residues and has the highest accumulation potential . Collected from resinous plant sources , propolis contains a high percentage of lipophilic compounds and, due to this property, adsorbs many pesticides and veterinary drugs more strongly than honey and pollen . Despite this, there are no specific MRL (Maximum Residue Limit) values defined at the regulatory level for propolis.

2.5.1. Chemical Structure and Residue Dynamics of Propolis

The main components of propolis are flavonoids , phenolic acids, beeswax fractions, and essential oils. This lipophilic structure makes propolis particularly resistant to fat-soluble chemicals such as pyrethroids , organochlorine compounds, and some fungicides. This leads to a concentration in its matrix.

Scientific studies show that some pesticides detected at low levels in honey from the same hive environment can be found in much higher concentrations in propolis . This makes propolis both an indicator of long-term environmental exposure and a base for the accumulation of intra-hive contamination.

2.5.2. Propolis in EU Legislation

In European Union legislation, propolis is not a clearly defined food matrix like honey . Propolis is often indirectly addressed under the headings of "bee products" or "other animal products". This approach prevents the establishment of specific MRL (Maximum Residue Limit) values for propolis and makes product-based risk assessment difficult.

In the EU, propolis analyses are mostly carried out for scientific research purposes; official controls use case-by-case assessments or indirect references to MRLs (Maximum Residue Limits) established for honey. This does not offer a scientifically valid risk management approach.

2.5.3. Turkish Food Codex and Propolis

Turkish Food Codex Propolis is considered a dietary supplement and a natural product. However, specific MRL (Maximum Residue Limit) values for chemical residues have not been defined for propolis. In practice, residue analyses of propolis products are mostly carried out according to the demands of the export target countries or interpreted within the framework of general food safety principles.

This situation creates significant uncertainty, particularly for domestic producers, making product standardization and quality control more difficult. Furthermore, the extensive use of propolis in pharmaceutical and cosmetic products necessitates an assessment of residue risk not only from a food safety perspective but also from a public health standpoint.

2.5.4. Codex Alimentarius and Propolis

Codex The Alimentarius Commission has not defined any specific MRL (Maximum Residue Limit) values for propolis . The Codex approach views propolis as a niche product with a limited share in global trade and therefore does not offer a detailed regulatory framework.

However, given the increasing use and biological activity of propolis in the international market, it can be said that the current approach of the Codex falls short of scientific requirements. In particular, the potential for accumulation of lipophilic pesticides in propolis necessitates product-based risk assessment.

2.5.5. Comparative Regulatory Status for Propolis

Table 2.3. Comparison of MRL approaches for propolis (AB–TGK– Codex)

Legislation	Specific MRL for propolis	The approach in practice
EU	None	Case-based assessment
TGK	None	General principles of food safety.
Codex	None	No regulations in place.

This table shows that propolis has a significant regulatory gap in all three regulatory systems.

2.5.6. Assessment from the Perspective of Beekeeping and Risk Management

propolis contradicts its high biological activity and potential for residue accumulation. The current regulatory approach treats propolis solely as a secondary bee product, failing to holistically assess bee health, environmental exposure , and long-term human consumption risks.

This situation reveals that propolis can be used as a biomarker , particularly in monitoring intra-hive contamination, and that product-based MRL (Maximum Residue Limit) models should be developed.

royal jelly , which will be discussed in the next section, requires a different MRL (Maximum Residue Limit) assessment approach due to its high biological activity despite its low production volume.

2.6. Maximum Residue Limits (MRLs) for Royal Jelly

Royal jelly Royal jelly is a special matrix among bee products that has the highest biological activity, the lowest production quantity, and lies on the border between functional food and pharmaceutical products. Playing a fundamental role in the queen bee's diet, this product is rich in proteins, free amino acids, fatty acids (especially 10-hydroxy-2-dekenoic acid; 10-HDA), vitamins, and hormone-like compounds. This unique chemical structure makes royal jelly a product that requires separate evaluation in terms of chemical residues.

2.6.1. Chemical Structure and Residue Carrying Potential of Royal Jelly

Royal jelly, despite its high water content, is a complex matrix capable of binding both hydrophilic and lipophilic chemicals due to its free fatty acid and protein fractions . In particular, antibiotic residues and some pesticide metabolites can bind to the protein structure of royal jelly and become stable.

Scientific studies show that most residues detected in royal jelly originate directly from beekeeping practices (e.g., veterinary drugs used to combat diseases within the hive). In this respect, royal jelly stands out as an indicator of management practices rather than environmental exposure .

2.6.2. The MRL Approach for Royal Jelly in the European Union

In European Union legislation, royal jelly is not classified in the same category as honey. However, specific MRL (Mean Time Limit) values have not been defined for royal jelly either. In EU practice, royal jelly is generally treated within the scope of "other animal products," and residue assessment is carried out according to the MRL values established for honey or the zero-tolerance approach for antibiotics.

A zero-tolerance policy is applied to royal jelly, particularly for antibiotics such as chloramphenicol , nitrofurans , and streptomycin . This approach has been adopted considering the therapeutic potential of royal jelly and vulnerable consumer groups (children, the elderly, immunocompromised individuals).

2.6.3 . Turkish Food Codex and Royal Jelly

the Turkish Food Codex , product-specific MRL (Maximum Residue Limit) values for chemical residues are not provided. In practice, a zero-tolerance approach, in line with the EU, is adopted for antibiotic residues in royal jelly.

However, the lack of specific threshold values for pesticide residues in royal jelly leads to differing interpretations and uncertainties in application during official inspections. This complicates quality control processes, especially in production intended for export.

2.6.4. Codex Alimentarius Perspective

Codex The Alimentarius Commission has not established specific MRL (Maximum Residue Limit) values for royal jelly. The Codex approach considers royal jelly a product with limited volume in global trade and therefore does not offer detailed risk assessment models.

This situation demonstrates that the Codex standards are scientifically inadequate for products with high biological activity, such as royal jelly. In particular, chronic exposure and potential biological effects are not adequately addressed within the current framework of the Codex .

2.6.5. Comparison of Legislative Aspects for Royal Jelly

Table 2.4. Comparison of MRL approaches for royal jelly (AB–TGK– Codex)

Legislation	Specific MRL for royal jelly	Basic approach
EU	None	Zero tolerance for antibiotics.
TGK	None	Implementation in line with the EU.
Codex	None	No specific regulation.

2.6.6. Evaluation from the Perspective of Beekeeping and Public Health

Royal jelly has a unique risk profile in terms of chemical residues due to its high biological activity despite low consumption rates. Current MRL (Material Residue Limit) systems, with their classical risk assessment approach based on consumption rate, do not adequately reflect the biological effects of royal jelly.

Therefore, the MRL (Maximum Residue Limit) concept for royal jelly needs to be re-evaluated from a functional food and pharmaceutical product perspective, rather than a classic food safety approach. This approach will strengthen both the standardization of beekeeping practices and consumer safety.

2.7. Maximum Residue Limits (MRLs) for Pollen and Bee Bread (Perga)

Pollen and bee bread (perga) are considered the matrices among bee products that reflect environmental chemical exposure most directly and rapidly . Pollen collected directly from plant sources by bees carries a high risk of contact with pesticides, heavy metals, and industrial pollutants used in agricultural activities. Therefore , pollen and perga are critical biological indicators for both bee health and environmental monitoring.

2.7.1. Biochemical Structure and Residue Dynamics of Pollen and Perga

Pollen is a biological material rich in proteins, free amino acids, lipids, vitamins, and minerals. This complex structure makes pollen a strong binding matrix for both hydrophilic and lipophilic chemical residues. In particular, systemic pesticides (e.g., neonicotinoids) can be transported within plant tissues and directly transferred to the pollen.

Perga is formed when pollen is fermented by bees in the hive. While the biochemical transformations carried out by lactic acid bacteria and yeasts in this process can lead to the breakdown of some chemical compounds, this process is ineffective against many pesticides and heavy metals. Therefore, perga is a cumulative record of long-term and chronic environmental exposure.

2.7.2. MRL Approach for Pollen and Perga in the European Union

Although European Union legislation considers pollen a different product from honey, specific MRL (Mean Time Limit) values are not defined for pollen and perga . In EU practice, pollen is mostly evaluated in reference to MRLs established for plant products . However, this approach is scientifically insufficient when pollen collected by bees is intended for direct human consumption.

The sublethal effects of neonicotinoid pesticides on pollen , in particular, have led to stricter regulations on bee health in the EU; however, these regulations have not established specific MRLs (Mean Time Limits) for pollen products offered to consumers.

2.7.3. Turkish Food Codex and Pollen/ Perga

The Turkish Food Codex defines pollen as a bee product, but it does not include product-specific MRL (Maximum Residue Limit) values for chemical residues. In practice, the residue assessment criteria applied to honey or general food safety limits are used for pollen products.

for perga in the Turkish Food Codex . This makes it difficult to evaluate and regulate perga as a commercial product. However , perga can have a higher residue accumulation potential compared to pollen.

2.7.4. Codex Alimentarius Perspective

Codex The Alimentarius Commission has not established specific MRL (Maximum Residue Limit) values for pollen and perga . The Codex approach considers these products to be of limited importance in global trade and therefore does not offer detailed risk assessment models.

This approach overlooks the scientific value of pollen and perga in terms of environmental monitoring. Especially in areas where agricultural chemicals are widely used, pollen and perga residues can be used as important indicators of ecosystem health.

Comparison of Legislative Procedures for Pollen and Perga

Table 2.5. Comparison of MRL approaches for pollen and perga (AB–TGK–Codex)

Legislation	Private MRL	The approach in practice
EU	None	Indirect reference to MRLs of plant-based products.
TGK	None	Analogy to honey MRLs
Codex	None	No regulations in place.

2.7.6. Assessment of Beekeeping from the Perspective of Environment and Food Safety

Pollen and perga are not only food products but also biological records of environmental exposure in terms of chemical residues . Residue levels in these products directly reflect the chemical load of the ecosystem in which bees live.

Therefore, the MRL (Maximum Requirement Ratio) approach for pollen and perga needs to go beyond classical consumer safety models and be integrated with environmental risk assessment, bee health, and ecosystem integrity. These products hold significant potential for early warning systems and biosensor- based monitoring approaches in beekeeping.

2.8. Maximum Residue Limits (MRL) for Beeswax

is considered the matrix in which chemical residues accumulate most intensely and for the longest periods among bee products . Beeswax, used by bees for honeycomb construction, forms a strong adsorption medium for pesticides, mite control chemicals, and environmental pollutants due to its highly lipophilic structure . This property makes beeswax both the primary reservoir of intra-hive contamination and a secondary source of contamination for other bee products.

2.8.1. Physicochemical Structure and Residue Accumulation of Beeswax

Beeswax exhibits a hydrophobic structure composed of long-chain fatty acids, esters, alkanes, and alcohols. This structure allows certain pesticides, particularly organochlorine pesticides, pyrethroids , and some fungicides, to remain stable within the beeswax for extended periods. These residues, accumulating in the beeswax, can eventually transfer to other bee products such as honey, royal jelly, and propolis.

Studies show that older honeycombs contain significantly higher levels of pesticide residue compared to newer ones, and these residues can persist in the hive for years. This reveals that beeswax is not just a product, but also functions as a chemical memory within the hive .

2.8.2. MRL Approach for Beeswax in the European Union

In European Union legislation, beeswax is not considered a food intended for direct human consumption. Therefore, specific MRL (Maximum Residue Limit) values for beeswax are not defined. However, beeswax is of particular importance in terms of risk management in the EU because it indirectly affects the safety of honey and other bee products.

In EU practices, residues in beeswax are assessed, particularly in the context of monitoring veterinary drugs used in varroa mite control (e.g., flumethrin , coumaphos). High residue levels detected in beeswax are considered indicative of intra-hive contamination and necessitate a review of production practices.

2.8.3. Turkish Food Codex and Beeswax

The Turkish Food Codex, beeswax is considered not as food, but as beekeeping material and industrial raw material. Therefore, MRL (Maximum Residue Limit) values are not defined for beeswax. However, the use of beeswax in food packaging, cosmetics, and pharmaceuticals raises an indirect public health risk in terms of chemical residues.

In TGK (Turkish Beekeeping Association) practices, the control of beeswax-derived residues is ensured not directly through legislation, but through good beekeeping practices and risk-based assessments carried out during official inspections.

2.8.4. Codex Alimentarius and Beeswax

Codex The Alimentarius Commission has not established specific MRL (Maximum Requirement Level) values for beeswax either. The Codex approach does not consider beeswax as a product directly consumed in global food trade and therefore does not offer a regulatory framework.

Given the central role of beeswax in hive contamination , the Codex's current approach is insufficient for assessing the integrity of the bee product chain.

2.8.5. Comparison of Legislative Procedures for Beeswax

Table 2.6. Comparison of MRL approaches for beeswax (AB–TGK–Codex)

Legislation	MRL for beeswax	Approach
EU	None	Indirect monitoring (hive hygiene)
TGK	None	Good beekeeping practices
Codex	None	No regulations in place.

2.8.6. Evaluation from the Perspective of Beekeeping and Residue Management

Beeswax is one of the most critical risk factors in beekeeping due to the long-term accumulation of chemical residues in the hive. These residues affect not only the current production season but also the safety of products in subsequent years.

Therefore, even though an MRL (Maximum Residue Limit) has not been defined for beeswax, this matrix should be considered as a central monitoring parameter in the bee product chain. Beeswax is an ideal target matrix for biosensor- based monitoring systems and holds great potential for the early detection of intrahive chemical load.

2.9. Limitations of the MRL Approach in Bee Products and the Need to Transition to Biosensor- Based Monitoring Systems

The Maximum Residue Limit (MRL) approach has been used for many years as a fundamental regulatory tool in ensuring food safety. However, considering the biological, chemical, and ecological characteristics of bee products, it is clear that classical MRL systems have significant structural limitations for these products. Bee products are not merely food items; they are a holistic reflection of environmental exposure , bee health, and agricultural practices.

2.9.1. Key Limitations of the MRL Approach for Bee Products

The most fundamental limitation of MRL (Metal Residue Residue) systems is their reliance on a static and post-product inspection approach. In current systems, residue analysis is mostly carried out at the harvest or marketing stage, providing limited information about the source and temporal dynamics of chemical exposure . However, residue formation in bee products constantly varies depending on seasonal, regional, and management factors.

Another significant limitation is that MRL values are mostly determined based on a single chemical. However, in bee products, multiple pesticide residues and their synergistic effects are frequently involved. This indicates that the current MRL approach cannot adequately represent complex exposure scenarios.

Furthermore, MRL systems do not directly assess bee health. The presence of a chemical in a bee product below legal limits does not mean it is harmless to the bee colony. Factors such as sublethal effects, behavioral disorders, and immune suppression fall outside the scope of current MRL systems.

2.9.2. The Need for Dynamic and Continuous Monitoring in Bee Products

Bee products have the potential to be early indicators of environmental chemical load. Matrices such as pollen, perga , and beeswax reflect residue accumulation even in the initial stages of exposure . This characteristic shows that bee products can be used not only for end-product control but also for preventive and proactive monitoring systems.

At this point, while classical laboratory-based analytical methods (GC-MS, LC-MS/MS) provide high accuracy, they are time-consuming, costly, and have limited applicability in field conditions. Effective risk management in beekeeping requires field-applicable, rapid, and continuous monitoring systems.

2.9.3. Integration of Biosensor Technologies into MRL Systems

Biosensors are analytical systems composed of a combination of biological recognition elements (enzymes, antibodies, aptamers, cells) and physicochemical transducers. These systems have the ability to detect target chemicals with high sensitivity and specificity.

Biosensors in bee products offers an innovative solution that can overcome the fundamental limitations of the MRL (Material Reduction in Weights and Measures) approach. Thanks to biosensors:

• Residues can be traced before harvest and during the production process,

• Multiple chemical exposures can be assessed simultaneously.

• bee health and chemical exposure can be established.

Biosensor Application Potential Based on Bee Products

biosensors developed for honey enable the rapid detection of pesticide and antibiotic residues. Optical and fluorescence- based biosensors for pollen and perga can be used for early monitoring of environmental exposure . Lipophilic biosensors such as beeswax and propolis ... Matrices, in turn, are ideal targets for biosensor- based monitoring systems in terms of tracking long-term accumulations.

In this context, biosensors are becoming a strategic tool not only for monitoring bee products in terms of regulatory compliance, but also for monitoring ecosystem health.

2.9.5. The Need for Legislative and Scientific Transformation

Current EU, Turkish Food Codex, and Codex regulations treat the MRL (Maximum Residue Limit) approach as a static legal threshold. However, the MRL concept for bee products needs to be transformed into a dynamic risk management system supported by biosensor data.

This transformation will promote sustainable production in the beekeeping sector; reduce colony losses through early detection of environmental pollutants; and strengthen consumer confidence.

3.BIOSENSOR- BASED RESIDUE MONITORING SYSTEMS IN BEE PRODUCTS

3.1. Scientific Basis for the Need for Biosensors in Residue Monitoring in Bee Products

Current approaches to monitoring chemical residues in bee products largely rely on laboratory-based, post-sample and static analysis systems. While methods such as gas chromatography -mass spectrometry (GC-MS) and liquid chromatography -mass spectrometry (LC-MS/MS) offer high accuracy, they have significant limitations in terms of time, cost, expertise requirements, and field applicability.

prevent the fulfillment of the need for early warning and continuous monitoring, particularly in a highly environmentally sensitive and dynamic production system like beekeeping. Yet, bee products contain not only the ultimate result of chemical exposure but also the biological signals that permeate the process. In this context, biosensor technologies stand out as a critical tool for preventive risk management that goes beyond the MRL (Material Risk Reduction) approach.

Biosensors are based on the principle of biologically recognizing the target analyte and converting this interaction into a measurable signal. Thanks to these characteristics, biosensors have the potential to radically transform the residue monitoring paradigm in beekeeping by offering fast, portable, low-cost systems capable of performing multiple analyses.

3.2. Biosensor Applications Specific to Bee Products

3.2.1. Biosensor Systems for Honey

Honey is the most studied bee product in terms of biosensor applications. Thanks to electrochemical biosensors:

- Organophosphate and pyrethroid pesticides,
- Antibiotic residues,
- Some heavy metals

It can be detected quickly under field conditions.

These systems enable the rapid assessment of MRL (Maximum Requirement Level) compliance before honey is brought to market.

3.2.2. Biosensor Approaches for Pollen and Perga

Pollen and perga are of strategic importance for biosensor applications as early indicators of environmental exposure . Optical and fluorescence- based biosensors :

- Neonicotinoid residues,
- Systemic fungicides ,
- Heavy metals

It offers high sensitivity, allowing the effects of agricultural chemicals on bee colonies to be monitored before harvest .

3.2.3. Biosensor Systems for Propolis and Beeswax

Propolis and beeswax are ideal matrices for monitoring long-term chemical accumulation. Using piezoelectric and electrochemical biosensors :

- Medications used in the fight against Varroa mites,
- Persistent organic pollutants,
- Lipophilic pesticides

high precision.

Since these products constitute a biological archive of the chemical load inside the hive, biosensor data are extremely valuable for long-term risk analysis.

3.2.4. Biosensors for Royal Jelly

Biosensors developed for royal jelly focus particularly on antibiotic residues. Thanks to immunosensors and aptamer- based systems, substances requiring zero tolerance can be detected with low detection limits.

3.3. Integration of Biosensors with MRL Systems

Biosensor technologies play a complementary and strengthening role in the MRL approach. Through this integration:

- The risk of residue before harvest can be determined.
- Multiple residue exposures can be assessed.
- Regulatory compliance processes can be accelerated.

The integration of biosensor data into official monitoring systems by EU and Turkish Food Codex regulations in the future will support sustainable quality management in beekeeping.

3.4. Future Perspective: Smart Beehives and Digital Monitoring

of biosensors with Internet of Things (IoT) and artificial intelligence-based data analysis systems makes the concept of a smart beehive possible. Thanks to these systems:

- Chemical exposure can be monitored in real time.
- Bee health can be assessed at an early stage.
- MRL violations can be prevented before they occur.

4. CONCLUSION AND DISCUSSION

Biosensor- based monitoring systems for bee products offer a dynamic and preventive food safety paradigm that transcends the limitations of the classical MRL (Material Requirement Ratio) approach. These systems directly impact not only consumer health but also bee health, environmental sustainability, and the future of the beekeeping industry.

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CHAPTER VIII

THE RESPONSE OF HONEY PRODUCTION TO SHOCKS: AN INVESTIGATION WITHIN THE FRAMEWORK OF UNIT ROOT TESTING

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DOI: https://www.doi.org/10.5281/zenodo.18138440

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1. INTRODUCTION

Beekeeping (apiculture), throughout history, has been more than just a source of food for humankind; it has held a strategic place in the continuity of agricultural production and rural development models. The economic dimensions of beekeeping encompass various aspects, including income generation, job creation, market development, and value chain integration (Prodanović, 2024). With its significant sectoral volume encompassing natural compound products such as honey, beeswax, royal jelly, bee venom, pollen, and bee gum, the beekeeping sector plays a crucial role in rural development in many countries around the world (Sokhai and Mardy, 2024).

Today, beekeeping stands out as an economically significant agricultural activity due to its increasing production capacity, diversified product range, and multifaceted contributions. As a strategic branch of agricultural production, beekeeping is notable for its honey and other bee products, which contribute to a balanced and healthy diet for humans due to their high nutritional value. Furthermore, the pollination activity of bees plays a vital role in the sustainability of the ecosystem and the increase of agricultural productivity. In these respects, beekeeping creates employment for rural populations, increases income levels, and facilitates access to healthy food, particularly in developing countries, thus occupying a privileged and strategic position among agricultural activities both economically and ecologically (Burucu and Bal, 2017). Turkey possesses significant potential for beekeeping thanks to its natural environmental characteristics, geographical location, and climatic diversity. The unique topographical structure of Anatolia, consisting of different altitudes and landforms, allows for variations in the flowering periods of plants depending on the region; this ensures the continuity of nectar and pollen sources for a large part of the year. These ecological advantages, combined with rich and diverse vegetation, create a suitable environment for beekeeping activities to be carried out in almost every region of the country and for the sector to expand and develop (Çevrimli and Sakarya, 2018). Beekeeping holds a special place among agricultural activities due to its characteristics such as supporting plant production, providing economic returns in a short time, being able to be carried out with low capital requirements, and not being dependent on land ownership. The relatively low operating costs, the need for less labor compared to other branches of agriculture, the ability to preserve products for a long time, and the ability to sell them with high added value in the market increase the economic attractiveness of beekeeping. With these characteristics, beekeeping is an important source of employment and income for rural areas, especially in developing countries, as well as contributing to healthy nutrition (Uzundumlu et al., 2011).

Historically, beekeeping is considered one of the oldest agricultural activities in human history. Beekeeping and honey production, one of its main outputs, create significant economic value today, going beyond being an independent agricultural production area. In recent years, with the increasing awareness of human health, the demand for natural and healthy products has risen; accordingly, a broad industry encompassing honey and bee products has developed. In addition to honey, products such as royal jelly, pollen, beeswax, and propolis are among the high value-added bee products due to their unique nutritional and functional properties; the contribution of bees to agricultural production through pollination further increases the global importance of beekeeping. Advantages such as being able to start with low capital, obtaining products from the first year, and not requiring advanced expertise make beekeeping an accessible and sustainable investment area for agricultural entrepreneurs (Budak, 2023). Beekeeping is an agricultural activity mostly carried out by small-scale family businesses and, in this respect, has strategic importance in terms of rural development. Globally, the sector is constantly evolving with the diversification and expansion of the uses of bee products. Examining regional practices, it is observed that in the United States, beekeeping activities are predominantly based on pollination services, with millions of bee colonies used in almond production, providing significant economic contributions to beekeepers. In Far Eastern countries, the medicinal and complementary uses of bee products are prominent, while in Europe, beekeeping is approached more from a nutritional and food consumption perspective (Saner et al., 2018).

The increasing global demand for food and the emergence of honey as a more natural and healthier product compared to synthetic sweeteners are increasing interest in beekeeping activities. In line with these developments, many countries are implementing various policies and incentive mechanisms to support and develop beekeeping. The effective implementation of these supports contributes to the expansion of the beekeeping market and lays the groundwork for sustainable growth in the sector in the future (Bogdanov, 2008).

The economic impacts of beekeeping can be considered primarily along two main axes: Beekeeping is a sector that creates economic value through the production, processing, and trade of honey and other bee products. Furthermore, it has indirect effects on agricultural production, quality, and welfare through the pollination activity carried out by bees in nature (FAO, 2020). Direct economic impacts of beekeeping: The most visible economic output of beekeeping activities is hive products. According to FAO (Food and Agriculture Organization of the United Nations) data, the global honey trade has reached a market volume of billions of dollars (FAO, 2022).

Table 1 shows data on the top twenty honey-producing countries among 115 countries as of 2023. China has the highest honey production in the world. The country closest to China in honey production is Turkey. Among the G7 countries, only the USA is included in the ranking. When considering the G20 countries, Argentina, Brazil, China, South Korea, India, Canada, Mexico, and Russia are included in the ranking.

Today, improving the skills of beekeepers is crucial for enhancing the economic impact of beekeeping. To this end, factors that encourage young people to engage in beekeeping should be identified. More emphasis should be placed on practical field work to enable young people to increase production. Projects can be developed to increase practical applications to ensure the efficient progress of the production process. Focusing solely on bees and beekeepers is insufficient for achieving efficiency in the sector. Good coordination with stakeholders in the sector is also necessary. While development in the beekeeping sector is improving day by day, beekeeping businesses need to change for production to proceed correctly. In this context, a solution to the accommodation problem of migratory beekeepers is needed, particularly to alleviate financing difficulties. Accommodation problems hinder income and economic well-being. An inventory of the sector should be compiled to create an accommodation structure. Besides the accommodation sector, yield per colony is another problem. Choosing appropriate bee breeds is important for increasing yield per colony (Aksoy et al., 2022).

Table 1: Top 20 Countries with the Highest Honey Production Worldwide as of 2023

Serial No	Country Name	Honey Production - Tons	Serial No	Country Name	Honey Production - Tons
1	China	472221.1	11	Ukraine	57919.0
2	Türkiye	114886.4	12	Canada	41643.0
3	Ethiopia	84591.0	13	Tanzania	31613.1
4	Iran	80389.0	14	South Korea	29467.8
5	Argentina	73395.3	15	Vietnam	24657.0
6	India	70850.2	16	Angola	23458.9
7	Russia	64511.0	17	Kenya	17151.0
8	Brazil	64189.0	18	Central African Republic	16714.0
9	USA	62855.0	19	Uzbekistan	15835.1
10	Mexico	58033.2	20	Chile	12184.4

Source: FAOSTAT

Businesses need to pay attention to risk factors in order to achieve an efficient production process. Accordingly, risks that businesses may encounter at all stages, from establishment to the market launch of products, should be thoroughly analyzed. For the beekeeping sector, existing risks and uncertainties should be analyzed, and measures should be taken accordingly. Environmental issues, in particular, are among the leading risk factors directly affecting this sector. Because honey bees are dependent on/sensitive to nature, climate change and global warming pose a risk to the sector. Another risk factor is diseases and pests. Furthermore, the untimely and unannounced use of pesticides by farmers is also a risk factor. Like other sectors, beekeeping is profit-oriented. Therefore, economic, financial, and marketing risks should also be considered. Businesses should prioritize efforts to increase efficiency to reduce or prevent these risks. Producers can coordinate among themselves through cooperatives. This allows them to sell their products at a fixed price and increases their standard of living. Record-keeping is crucial during risk analysis. Branding and advertising efforts are very important for businesses to reach a wider audience for product sales. Income can increase thanks to these promotional activities (Varalan and Çevrimli, 2023). Businesses should plan to produce and sell different bee products in addition to honey production in order to take measures to reduce risks and prevent potential losses. In terms of production and marketing, transitioning to an organized production model will strengthen producers in the market. Considering ecological richness, it is important to spread sustainable and conscious beekeeping (İnci et al., 2022).

2. EMPIRICAL APPLICATION

In this study, 17 countries[2] were selected as the sample group (excluding the EU group of G-20 countries and Indonesia). This group was chosen because it includes both developed and developing countries. The common period for which data is available for these countries is 1992-2017.

The results of heterogeneity and cross-sectional dependence tests for 17 countries for the period 1992-2017 are given in Table 2. In this study, considering the T>N feature, the Bias-adjusted CD test and the $\tilde{\Delta}$ and $\tilde{\Delta}$_adj homogeneity tests developed by Pesaran and Yamagata (2008) were performed. Since the probability values were <0.05, the H0 hypothesis was rejected, and cross-sectional dependence and heterogeneity were detected.

Table 2: Cross-Sectional Dependence and Homogeneity Test Results

Bias-adjusted CD test		$\tilde{\Delta}$		$\tilde{\Delta}_{adj}$	
Statistics	Probability Value	Statistics	Probability Value	Statistics	Probability Value
34.870 ***	0.000	**4.327*****	0.000	**4.601*****	0.000

Note: The *** in the table represents a 1% significance level.

Since cross-sectional dependence and heterogeneity were detected, the unit root property of the series was examined using the Cross-Sectional Extended Im, Peseran and Shin (CIPS) panel unit root test. This test was developed by Peseran (2007), and its H_0 hypothesis states the existence of a unit root. The unit root test results for the constant model are given in Table 3.

[2] To avoid duplicate data usage, the EU country group was excluded from the G-20 countries. Indonesia was also excluded from the analysis due to the lack of data available for it.

According to the results obtained, since the probability value is $0.000 < 0.05$, the H_0 hypothesis is rejected, and it is concluded that the series is stationary at the level. This result shows that when a shock occurs to honey production, this shock is temporary, meaning that honey production can return to its previous level.

Table 3: Panel Unit Root Test Result for CIPS Constant Model

Variable	T-bar TestStatistics	Probability Value
Honey Production	-2.876	0.000

Note: The number of lags in the analysis was taken as 1, and *** in the table represents a 1% significance level.

3. CONCLUSION

The beekeeping sector, with its high value-added products such as honey, pollen, royal jelly, and propolis, as well as its contribution to pollination in plant production, constitutes one of the cornerstones of both economic and ecological sustainability. As emphasized in the study, its low capital requirement and family labor-based structure place the sector in a strategic position, especially in terms of rural development and employment. However, the sector's ability to fully transform its existing potential into economic prosperity depends on the effective management of risk factors such as climate change, diseases, and marketing deficiencies. In this context, organizing producers through cooperatives, focusing on branding activities, and diversifying products will increase the resilience of businesses to risks and raise their income levels. The empirical section of the study, which analyzed 17 countries for the period 1992-2017, revealed an important finding regarding the dynamic structure of the sector. The CIPS panel unit root test results showed that the honey production series is stationary, proving that the effects of external shocks to the sector are temporary and that production can quickly return to its previous equilibrium level. In order to preserve the sector's natural resilience to shocks and to maintain its effectiveness in the global market, especially for leading producers such as Turkey and China, it is of great importance to encourage young producers, solve structural problems such as accommodation, and disseminate modern techniques in the field.

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ISBN: 978-625-378-539-0