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International Congress on Bee Sciences

Third International Congress on Bee Sciences Proceedings Book

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ONLINE

FREE OF CHARGE

15 September 2024

ISBN: 978-605-71368-9-3

Third International Congress on Bee Sciences Full Text Book

https://www.beeandlifecongress.com/ (biohealthcongress@gmail.com)
Editor: Ulaş ACARÖZ
Published: 14.09.2024
ISBN: 978-605-71368-8-6

Editor's Note

The Third International Congress on Bee Sciences was successfully held online. We are delighted to have offered this event free of charge. It was an honor to bring together experts from various fields of bee sciences. This Congress allowed to exchange of innovative ideas and fostering the development of new research and collaborative projects. With 44 invited speakers representing 30 countries and a scientific committee composed of nearly 300 distinguished scientists from over 65 countries, the congress truly reflected global participation. We extend our heartfelt thanks to everyone who contributed and supported the event. We look forward to seeing you at our next congress.

Warm regards from Turkey!

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The Role of *Varroa* Mites in the Transmission of Deformed Wing Virus

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Abstract:
Honey bees have a crucial economic and biological role in the ecosystem due to their unique and balanced pollinator roles. Recently, there have been major losses in honey bee populations worldwide. *Varroa* mites are regarded as a significant threat to honey bees. The main cause of global colony losses today is believed to be the combination of *Varroa* mites (*Varroa destructor*) and viruses. The presence of *Varroa destructor* (*V. destructor*) in bee colonies significantly impacts colony stress and raises the likelihood of colony losses caused by viral infections. Deformed Wing Virus (DWV), a very common virus that infects honey bee larvae and adults, is the most frequent virus in honey bee colonies that are exposed to *Varroa* mites. Therefore, it is believed that *Varroa* mites play a significant role in the transmission of DWV. DWV can cause wing deformities in bees, preventing them from flying. Furthermore, bees that are infected with DWV have a reduced body size and shorter survival time. *Varroa* mites are present in colonies with wing deformities. Wing deformities may develop in pupae depending on the viral load injected by the mite. Several acaricides that contain both synthetic and organic compounds are used to fight against *Varroa* mites. However, development of resistance in *Varroa* mites makes drugs ineffective. The objective of this study is to summarize the current scientific knowledge on *Varroa* mites contribution to the transmission of DWV.

Keywords: honey bees, *Varroa* mites, deformed wing virus, transmission

1. Introduction

Honey bee (*Apis mellifera* L.,) is one of the most important economical insects, both by its various products such as honey, propolis, pollen, and royal jelly, and by contribution to the pollination of agricultural plants. Honey bee-mediated pollination is important for agricultural production. The value of insect pollination worldwide is $153 billion annually, and honey bees are the primary pollinators (Gallai et al. 2009, Calderone 2012). Specifically, the honey bee service provides biotic pollination that enhances the quality or quantity of fruit or seeds in approximately 70% of 1330 tropical crops and 85 % of 264 crops cultivated in Europe (Aizen et al. 2009). However, massive colony losses observed in many parts of the world in recent years, and it has been gaining growing public interest around the world (Tang et al. 2023, Insolia et al. 2024; Lamas et al. 2024).

The rate of colony losses in the USA has reached 30.7% in 2018, whereas European colony losses were 11.22% in 2014 (Barroso-Arévalo et al. 2019). Colony health and survival are influenced by multiple biotic and abiotic factors, including mites, viral and bacterial diseases, bee genetics, forage quality and availability, weather, management practices, and agrochemical exposure (Cornman et al. 20212, Locke et al. 2014, Glenny et al. 2017).

Studies in beekeeping have revealed that viruses are the primary cause of the heavy losses in bees (Tantillo et al. 2015; Hristov et al. 2020, Ullah et al. 2021). Although there are more than 18 viruses that have been described, Deformed wing virus (DWV), Black queen cell virus (BQCV), Acute bee paralysis virus (ABPV), Chronic bee paralysis virus (CBPV), and Sacbrood virus (SBV) are the most common viruses in honey bees, (Tantillo et al. 2015, Ding et al. 2016, Beaurepaire et al. 2020). DWV has been found to be the most commonly detected bee virus worldwide by numerous studies of honey bee viruses (Zhang et al. 2012, Tehel et al. 2016, Paxton et al. 2022). Furthermore, DWV is the most frequent virus in honey bee colonies that are exposed to *Varroa* mites. It is believed that *Varroa* mites play a significant role in the transmission of DWV (Posada-Florez et al. 2019, Posada-Florez F et al. 2020). Therefore, objective of this study is to summarize the current scientific knowledge on *Varroa* mites contribution to the transmission of DWV.

2. Results and Discussion

The DWV has a single, positive-stranded RNA genome, and it belongs to the genus *Iflaviridae*, order *Picornavirales* (Forzan et al. 2017, Valles et al. 2017). There are four variants of DWV including DWV-A, DWV-B (also known as Varroa destructor virus-1), DWV-C, and DWV-D.

Numerous recombinants have also been identified among these variants (Mordecai et al. 2016, Brettell et al. 2017, de Miranda et al. 2022). DWV sequences that are available from different parts of the world have a 98% to 99% identity. This is in line with the suggested recent global expansion of the virus and the restricted evolutionary divergence (Lanzi et al. 2006). DWV infects honey bee larvae and adults, and it is strongly linked to distinctive wing deformations, paralysis, abdominal bloating, and rapid death in emerging adult bees (Lanzi et al. 2006).

DWV can be transmitted both horizontally and vertically (Amiri et al. 2018, Martin and Brettell, 2019). Furthermore, DWV can be transmitted biologically through *Varroa* mites. The virus is transferred to this ectoparasitic mite by means of the hemolymph of an infected-bee and it is spread while feeding on another bee (Lanzi et al. 2006, Ongus et al. 2006). Both DWV-A and DWV-B variants are transmitted by the mite. The Eastern honeybee *Apis cerana* is obligate host to the mite *Varroa destructor* (*V. destructor*). At the beginning of the 20th century, *V. destructor* became prevalent in the Western honey bee *A. mellifera*, reaching Western Europe and then North America about 30 years ago (Rosenkranz et al. 2010). A recent statement mentions that DWV-B can be replicated within the *V. destructor*, but DWV-A is not able to be replicated. It is suggested that DWV-B may transmit more rapidly than DWV-A when vectored by *V. destructor* mites. DWV-B had the ability to infect the mite's intestinal epithelium and salivary glands, but DWV-A was unable to infect it. Therefore, mite saliva has the potential to transmit at least DWV-B (Gisder and Genersch, 2021). In a recent field study at the colony level, a manipulation of *V. destructor* infestation in the presence of DWV-A and DWV-B resulted in DWV-B reaching higher titres and dominating DWV-A (Norton et al. 2021). However, Posada-Florez et al. (2019) has suggested that DWV-A can replicate in mites. Further research is required to gain clarity on the ability of DWV-A to replicate in *V. destructor*.

Negative effects of DWV infection are typically observed when the virus is transmitted by the ectoparasitic mite *V. destructor* (Highfield et al. 2009, Dainat et al. 2012a). The DWV levels in bees that are infested with *Varroa* are 100 to 1000 times higher than those that are not infested with *Varroa* (Ryabov et al. 2014, Wilfert et al. 2016). Over-wintering bees are vulnerable to DWV infection and colonies collapse during winter when the *Varroa* infestation levels exceed 2000-3000 mites per colony during the autumn season. The reason is that the new generation produced at this time is not enough to replace the infected over-wintering bees that die (Martin 2001).

Colony loss caused by *V. destructor* and DWV is a major factor, particularly because both the mite and the DWV have a double effect on infestation. *V. destructor* is closely associated with the honeybee life cycle, influencing the brood by feeding on it and spreading viruses (Gisder et al. 2009, Moore et al. 2011, Mondet et al. 2014). Mites vectoring DWV and introducing it directly into the pupa's body during feeding could lead to significantly higher titers in developing honeybees (Moore et al. 2011, Ryabov et al. 2014). Their combined efforts have a greater effect on the health of the honeybee colony and can even lead to its collapse (Martin 2001, Dainat et al. 2012b).

3. Conclusion

In conclusion, there are multiple factors that can lead to the loss of honeybees. *Varroa* mites and DWV play a key role in declining colonies. Colony collapse can be predicted by the load of DWV and levels of *Varroa*, which are both linked to colony strength. Preventing colony losses can be achieved by using drugs to fight *Varroa* mites and taking necessary precautions.

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Bioaccessibility and Bioavailability of Bee Pollen Bioactives

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Abstract:
Bee pollen, a natural honeybee product, is a valuable source of nutrients and bioactive compounds. Bee pollen exhibits antioxidant, anti-inflammatory, anti-carcinogenic, antimicrobial, anti-diabetic, anti-atherosclerotic, immunomodulatory, hepatoprotective, and cardioprotective properties. To provide health benefits, these compounds must be bioaccessible and bioavailable. Bioavailability is defined as both the uptake and metabolic use of a compound. Bioaccessibility, which is a first step of bioavailability, is the amount of nutrients available for absorption that is released from the food matrix in the gastrointestinal tract. Bee pollens contain chlorogenic acid, caffeic acid, syringic acid, coumaric acid, sinapic acid, ferulic acid, abscisic acid, cinnamic acid, epicatechin, rutin, myricetin, quercetin, naringenin, apigenin, kaempferol, rhamnetin, and galangin. The bioaccesibility of total phenolics and total flavonoids for bee pollen was 4-150% and 1-50%, respectively. Myricetin was the most bioaccessible compound, followed by quercetin and cinnamic acid. Phenylamides were the most affected by gastrointestinal digestion, reaching complete digestibility by the end of the intestinal phase. Significant variations were noted in the bioactive and bioaccessibility characteristics of the pollen samples based on the variety, geographic location, and year of collection; these variations affected the pollens' chemical composition, which in turn affected the phenolic profile.

Keywords: bee pollen, bioaccessibility, bioavailability, phenolics, bioactives

1. Introduction

In recent times, there has been a growing interest in natural products for maintaining a healthy and balanced diet, driven by concerns over synthetic food and food additives. In this context, research efforts have focused on identifying natural functional products with high nutritional value and beneficial effects on health, including bee products like bee pollen (Aylanc et al. 2021). The chemical makeup of these bee products can fluctuate depending on factors such as the plant's origin, its nutrient content, the environmental conditions in the region, the soil, and the the methods of collection, storage, and processing. Furthermore, these variabilities frequently influence the diversity and quantity of phytochemicals, including phenolic compounds that are resposible for their biological activities.

The protective role of phytochemicals that are part of our diet is becoming an increasingly important area of human nutrition research. Althogh the majority of biological effects of bioactive compounds are attributed to their high antioxidant potential, it is known that these compounds are more than just antioxidants. It is important to note that simply consuming a diet rich in phytochemicals does not guarantee their effectiveness. These compounds need to be be bioaccessible and bioavailable to exert their health benefits.

2. Bee pollen: Bioactives and health effects

Bee pollen, a versatile natural product collected by bees for its rich source of proteins and nutrients, contains a variety of bioactive compounds (Rodríguez-Pólit et al. 2023). Bee pollen is a product of the hive composed of the pollen grains from flowers, nectar and salivary secretion from bees, which beekeepers can gather without disrupting the hive (El Ghoizi et al. 2023).

The nutritional composition of bee pollen is shown in Figure 1. The composition of bee pollen can differ based on factors such as its geographic and botanical origin, the time of collection, climate, and environmental conditions (Kacemi et al. 2023; Ozkan et al. 2018). Bee pollen may have anti-diabetic, anti-carcinogenic, anti-inflammatory, antioxidant, anti-atherosclerotic, antimicrobial, immunomodulatory, hepaprotective, and cardioprotective effects (Aylanc et al. 2021; Rodríguez-Pólit et al. 2023; Kacemi et al. 2023). The potential benefits of bee pollen in the treatment of malnutrition, digestive health, metabolic disorders, and other biological processes, which could be beneficial in restoring homeostasis, as well as its effects on cardiovascular diseases, were identified (Kacemi et al. 2023).

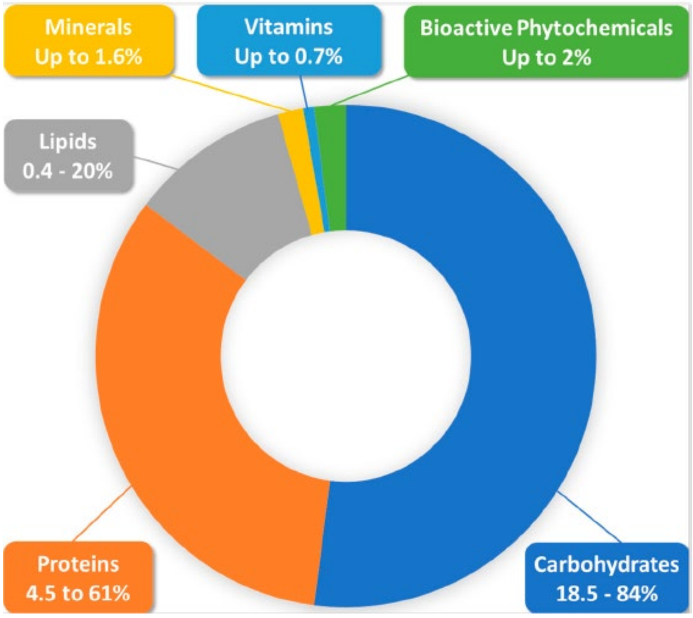

Figure 1. The nutritional composition of bee pollen (Kacemi et al. 2023)

3. Bioaccessibility and bioavailability

Bioavailability can be defined as the uptake and metabolic use of a nutrient, encompassing the bioaccessibility, absorption, tissue distrubution, and bioactivity concepts. The amount of an ingested nutrient that is available for absorption in the gut after digestion is defined as bioaccessibility. Bioaccessibility is the first step of bioavailability (Aylanc et al. 2021). In order to assess the bioaccessibility of food, the gastrointestinal system is typically simulated using *in vitro* methods, with the use of Caco-2 cells sometimes employed as a follow-up (Aylanc et al. 2021; Mutlu et al. 2023).

4. Bioaccessibility and bioavailability of pollen phenolics

Bioaccessibility of total phenolics and flavonoids in bee pollens are shown in Table 1 and Table 2, respectively. There is considerable variation in the bioaccessibility values of total phenolics, depending on the pollen type, origin, and digestion method.

Table 1. Bioaccessibility of total phenolics in bee pollens

Origin	Digestion Method	Bioaccessibility (%)	Reference
Portugal	Infogest (Minekus et al. 2014)	31	Aylanc et al., 2021
Turkey	McDougall et al. 2005	4.0-7.3	Ozkan et al. 2018
Turkey	Infogest (Minekus et al. 2014)	18-72	Akpinar Bayazit et al. 2023
Turkey	Vitali et al. 2009	37-78	Dulger Altiner et al. 2020
Turkey	Infogest (Minekus et al. 2014)	45-150	Mutlu et al. 2023
China	Infogest (Minekus et al. 2014)	105-127	Yuan et al. 2024
Chile	Ah-Hen et al. 2018	93-102	Bridi et al. 2022

Table 2. Bioaccessibility of total flavonoids in bee pollens

Origin	Digestion Method	Bioaccessibility (%)	Reference
Portugal	Infogest (Minekus et al. 2014)	25	Aylanc et al., 2021
Turkey	McDougall et al. 2005	1.1-5.9	Ozkan et al. 2018
Turkey	Infogest (Minekus et al. 2014)	4-10	Akpinar Bayazit et al. 2023
China	Infogest (Minekus et al. 2014)	27-50	Yuan et al. 2024

5. Conclusion

Significant variations were observed in the bioactive and bioaccessibility characteristics of the pollen samples, which were dependent on the variety, geographic location, and year of collection. These variations affected the pollen samples' chemical composition which in turn affected the phenolic profile and bioaccessibility.

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Evaluation of Bioactive Properties of Various Propolis Mixtures Used as Functional Food

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Abstract:
Propolis, gathered by bees from tree buds and bark, is augmented with wax, pollen, and saliva secretions. Propolis has been traditionally used in folk medicine for its antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory effects. Recently, in order for people to benefit from the beneficial effects of propolis on health, functional foods have been produced and consumed by adding it to various foods in different proportions. The aim of this study was to determine the bioactive effects (phenolic content, flavonoid content and antioxidant activity) of different propolis mixtures from Turkey (Erzurum province). For this purpose, eight commercial propolis mixtures were obtained from pharmacies and marketplaces in 2023. Folin-Ciocalteu method was used for the determination of total phenolic content (TPC), while aluminium chloride colorimetric test was used for the determination of total flavonoid content (TFC). In addition, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay was used to evaluate the antioxidant activity of propolis mixtures. Significant variations in both phenolic and flavonoid content and antioxidant activity were observed among propolis mixture samples ($P \leq 0.05$). It was found that the antioxidant activities of propolis mixture samples increased with increasing amounts of TPC and TFC. The highest TPC, TFC and DPPH scavenging activity was found in propolis drops (30%) and the lowest values were found in propolis mouth spray. These results provide valuable information about the antioxidant potential and health-promoting properties of commercial propolis products.

Keywords: antioxidant activity, propolis, total phenolic content, total flavonoid content

1. Introduction

Bee-derived products have played a significant role in human and animal well-being for centuries. Alongside their historical consumption as food, these products have been utilized for their therapeutic properties due to their active components (Dumitru et al. 2022). There has been a growing global interest in natural food products with functional benefits for enhancing human health in recent years (Diaz et al. 2020). While honey is typically the most recognized bee product, others like pollen, royal jelly, beeswax, bee venom, and propolis are also classified as essential bee products today.

In recent years, there has been a growing interest in utilizing natural products with potential health benefits as functional foods (Yeerong et al. 2024). Among these, propolis, a resinous substance collected by bees from various plant sources, has garnered attention for its diverse bioactive compounds and therapeutic properties (Sforcin 2016). Propolis has been traditionally used in folk medicine for its antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory effects (Dhifi et al. 2020).

Propolis, gathered by bees from tree buds and bark, is augmented with wax, pollen, and saliva secretions. Within the hive, bees employ propolis for both sanitization purposes and as a construction material (Wagh 2013). Notably, propolis serves as a vital contributor to bolstering the immune systems of both humans and animals (Hayriye 2018). Propolis composition can vary significantly depending on the geographic location, botanical sources, and environmental factors, leading to a wide array of bioactive constituents (Anjum et al. 2019). Phenolic compounds and flavonoids are among the primary bioactive components identified in propolis, contributing to its antioxidant properties and health-promoting effects (Socha et al. 2015).

Given the increasing interest in propolis as a potential functional food ingredient, there is a need for comprehensive studies to evaluate its bioactive properties when formulated into mixtures suitable for consumption. Understanding the antioxidant capacities, phenolic, and flavonoid contents of propolis mixtures is crucial for assessing their potential health benefits and optimizing their use in functional food products.

This study aims to determine the bioactive properties of propolis mixtures used as functional foods. Through analytical techniques such as antioxidant capacity assays and quantification of phenolic and flavonoid contents, we seek to elucidate the potential health-promoting effects of these formulations. By characterizing the bioactive profile of propolis mixtures, this research contributes to the broader understanding of propolis as a valuable natural ingredient in functional food development and its potential role in promoting human health and well-being.

2. Materials and Methods

Sample Collection and Preparation:

For this study, a total of 8 commercial propolis blends were sourced from various pharmacies in Türkiye (Erzurum) during the year 2023. To ensure diversity, care was taken to select samples with differing compositions. The composition of each product was determined based on the information provided on their packaging. Subsequently, the acquired propolis mixtures were transported to the laboratory in their original packaging, safeguarded against sunlight exposure, and promptly subjected to analysis.Before the analyses each propolis mixture was carefully removed from its original packaging and stored in airtight containers to prevent moisture absorption.

Preparation of Extracts:

Approximately 0.5 g of each propolis sample was accurately weighed and transferred into separate 50 mL centrifuge tubes. For TPC determination: 20 mL of 80% ethanol (v/v) was added to each tube, followed by sonication for 30 minutes at room temperature. For TFC determination: 20 mL of 80% methanol (v/v) was added to each tube, followed by sonication for 30 minutes at room temperature. After sonication, the samples were centrifuged at 3000 rpm for 10 minutes to obtain the supernatant.

Determination of Total Phenolic Content (TPC):

Firstly, the Folin-Ciocalteu reagent was prepared by diluting it with distilled water according to the manufacturer's instructions. Then, aliquots (0.5 mL) of each propolis extract were transferred into separate test tubes. To each test tube, 2.5 mL of diluted Folin-Ciocalteu reagent was added, followed by the addition of 2 mL of 7.5% sodium carbonate solution. The mixtures were vortexed and allowed to stand at room temperature in the dark for 90 minutes. The absorbance of the resulting blue-colored complex was measured at 765 nm using a UV-visible spectrophotometer. A standard calibration curve was prepared using gallic acid solutions of known concentrations (0-100 µg/mL). The total phenolic content of each propolis sample was calculated based on the gallic acid equivalent (GAE) using the calibration curve.

Determination of Total Flavonoid Content (TFC):

Aliquots (0.5 mL) of each propolis extract were transferred into separate test tubes. To each test tube, 2 mL of 2% aluminum chloride solution was added, followed by the addition of 3 mL of 5% sodium acetate solution. The mixtures were vortexed and allowed to stand at room temperature for 40 minutes. The absorbance of the resulting yellow-colored complex was measured at 415 nm using a UV-visible spectrophotometer. A standard calibration curve was prepared using quercetin solutions of known concentrations (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/mL). The total flavonoid content of each propolis sample was calculated based on the quercetin equivalent (QE) using the calibration curve.

DPPH Assay:

The antioxidant activity of propolis mixtures was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, following the protocols described by Liu et al. (2008) and Kılıç Altun and Aydemir (2022). A stock solution of 0.6 mM DPPH in methanol was prepared, and a working solution was obtained by diluting the stock solution with methanol to achieve an absorbance of 1.1 at 517 nm. Reagent mixtures consisting of a sample (7.5 µL) and DPPH stock solution (150 µL) dissolved in methanol were incubated in darkness for 30 minutes at room temperature in 96-well plates. Absorbance readings were taken at 517 nm against methanol as a blank. The results were quantified in terms of Trolox equivalent antioxidant capacity (TEAC) expressed as milligrams of Trolox equivalent per milliliter of dry weight.

3. Results

The phenolic content, flavonoid content, and antioxidant activity of the propolis mixtures are given in Table 1.

Table 1. Total phenolic substance, total flavonoid contents and antioxidant capacities of propolis mixtures

No	Sample definition	Total phenolic (mg GAE/100 g dry weight)	Total flavonoid (mg QE/100 g dry weight)	DPPH(mg TE/mL dry weight)
1	Propolis 10%	47.2±3.2	30.3±3.5	123.1±9.8
2	Propolis 20%	62.2±5.8	45.8±3.9	190.2±11.8
3	Propolis drops (15%)	50.8±4.6	29.7±2.8	145.5±7.6
4	Propolis drops (30%)	96.4±7.8	66.1±4.3	281.6±13.2
5	Propolis tablet 1	25.3±0.3	20.7±2.1	65.9±4.6
6	Propolis tablet 2	40.6±2.6	25.8±5.5	122.1±10.3
7	Propolis mouth spray	3.7±0.7	2.6±0.2	11.3±2.2
8	Propolis syrup for children	7.1±0.9	2.2±0.2	16.7±0.8

4. Discussion

Significant variations in both phenolic and flavonoid content and antioxidant activity were observed among propolis mixture samples ($P \leq 0.05$). It was found that the antioxidant activities of propolis mixture samples increased with increasing amounts of TPC and TFC. This can be explained by the high content of bioactive compounds in hawthorn vinegar. Indeed, it has been well documented that phenolics and flavonoids contribute to antioxidant capacity (Nikmaram et al. 2018). It has been previously reported that there is a correlation between TPC and TFC in propolis and antioxidant activity (Narimane et al. 2017; Asem et al. 2020). The high antioxidant activity of these bioactive compounds in propolis has been attributed to their ability to donate hydrogen atoms and electrons of an aromatic hydroxyl group to a free radical and the possibility of localising charges in the aromatic ring double bond system (Irigoiti et al. 2021). In previous studies, the bioactive compound content and antioxidant activity of propolis were reported to be high, but showed lower or higher activity compared to the results of the present study (Narimane et al. 2017; Sulaeman et al. 2019; Asem et al. 2020; Segueni et al. 2021; Özkök et al. 2021). The observation of these differences is due to the fact that the bioactive compounds contained in propolis change its bioactive properties due to the different bioactive compounds it contains, as well as DPPH and ABTS concentrations, standard substance concentrations and spectroscopic methods can significantly contribute to these differences.

Propolis has many biological effects depending on the amount and type of active compounds it contains, and the amount of phenolic compounds in it is also the most important indicator of the biological activity of propolis. The chemical composition of propolis varies depending on the region, plant, season, colony, and propolis collection technique, thus the phenolic compound composition of each propolis varies (Karlıdağ and Genç 2019). In addition, the variety of extraction methods, solvents used for extraction, sample preparation methods and analytical methods used may also explain these differences (Rodríguez-Carrasco et al. 2018).

Propolis has a wide range of applications due to its antioxidant and pharmacological properties. Recently, the use of propolis as a food ingredient has increased due to the current consumer trend towards functional foods. These new trends are expanding the consumer market and creating new business opportunities for propolis added to foods. Today, the use of propolis in the food industry is mainly aimed at delaying the effects of microbial and oxidative agents. As seen in the current study, propolis has been added to products in different proportions to provide functionality to these products (Stojanović et al. 2020) According to the analysis results, it was seen that as the proportion of propolis added to the products increased, its biological activity also increased (Table 1). It was found that the antioxidant activities of the propolis blend samples increased with increasing amounts of TPC and TFC. The highest TPC, TFC and DPPH scavenging activity was found in propolis drops (30%) and the lowest values were found in propolis mouth spray. The low level of these properties in the mouth spray can be explained by the low propolis content.

5. Conclusion

As a result, significant differences in TPC, TFC and antioxidant activity were observed according to the proportion of propolis contained in the propolis mixture samples. The highest TPC, TFC and DPPH scavenging activity was found in propolis drops (30%) and the lowest values were found in propolis mouth spray. As a result, the samples with the highest polyphenol content showed the strongest antioxidant activity. These results provide valuable information on the antioxidant potential and health-promoting properties of commercial propolis products. Further research is needed to identify the main compounds responsible for the antioxidant properties of commercial propolis products.

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Bee Venom Production: Pros and Cons

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Abstract:
Honeybee venom is a valuable bee product that is highly valued and has different biological and therapeutic properties. In recent years, bee venom has been used in cosmetics, pharmacology, veterinary applications and Apitherapy in the world. Bee venom production has been gaining value in recent years in our country, which is in an important position for beekeeping due to all these features and the fact that it can provide additional income to the beekeeper from a one-way production system. Bee venom production, quantity, and quality of the product obtained in production are under the influence of many parameters. The quantity and quality of bee venom production is affected by technical factors such as production method, production material, production period, production season, feeding of colonies, colony strength, beekeeper's knowledge and experience as well as environmental factors such as temperature, climate change, pollen and nectar resources, pesticide use. When all these criteria are considered, it is seen that venom production has advantages and disadvantages for the honeybee itself, the beekeeper and the final product. Determining these pros and cons and making production management plans are important in improving the production and quality of bee venom, which is used especially in human and animal health.

Keywords: honeybee, beekeeping, bee venom, production, apitherapy

1. Introduction

As an integral part of the ecosystem, honeybees produce valuable and biologically useful bee products. Each bee product has its own therapeutic properties and is used in different ways in the treatment of various diseases. Apitherapy is a medical complementary practice scientifically accepted as "the whole of the applied use methods of the protective and therapeutic properties of bee products in human, animal and plant health”. Bee products such as honey, pollen, royal jelly, propolis and bee venom are important products for human health. The antimicrobial, antioxidant, anti-inflammatory, anti-cancer, immunomodulatory, anti-inflammatory, anti-cancer, immunomodulatory effects of these products in research have led researchers to apitherapy and contributed to the development of the usage areas of these products in alternative medicine day by day. Bee venom is one of the bee products used in apitherapy. The use of bee venom dates back more than 6000 years (ancient Egypt). The Greeks and Romans also used bee products for medicinal purposes. Bee venom is also a valuable and high value-added product. Bee venom production is a system that is still becoming widespread in our country. However, its production requires special knowledge and experience. The quality of bee venom is very important for its use in apitherapy. Considering that honeybees are completely dependent on the environment, all environmental factors affect both the life of honeybees and the quantity and quality of the products they produce. Therefore, it is important to know the impact of environmental factors, the production system, the processing, and storage processes of the product and to know the pros and cons of bee venom production in order to improve production.

2. Honeybee venom

Among the many insect species, only a few are capable of defending themselves by injecting venom during a sting. Bee venom, also known as apitoxin; is produced in the venom glands in the abdominal cavity of the bee and stored in the venom sac (Kolayli and Keskin, 2020). Bee stinger and bee venom is an effective defensive weapon of bees. Honeybee venom is a clear liquid that dries quickly even at room temperature, has an attractive pungent fragrance, a bitter taste, and is a hydrolytic combination of proteins with a pH 4.5 to 5.5. Bee venom is a unique mixture of proteins, enzymes and biogenic amines, used in medicine, pharmacy and cosmetology with therapeutic effects and anti-aging potential (Łukasiewicz, 2021). Main peptide Mellitin which makes up about 50-60% of bee venom, appears to be the main biological agent, and the use of bee venom in apitherapy is mostly related to the function of this substance. Pharmacologically, bee venom has effects that increase blood circulation, rejuvenate the skin, increase smooth muscle contraction, bactericide, regulate heart rhythm and functions, increase blood flow in the heart and brain vessels, increase adrenaline level, protect against radiation, relieve pain before menstruation period in women, and lowers blood pressure. Besides, bee venom is a potential complementary medicine candidate for SARS-CoV-2 Infections (Kazosi et al., 2020).

3. Bee venom production and its importance

Bee venom production is a complex process influenced by various factors, including genetic, environmental, and physiological elements. Understanding these factors is crucial for optimizing venom yield in a sustainable and ethical manner. With the emergence of the adult bee from the pupa, the venom glands become active and are able to secrete venom in as little as three days. Even if a bee stings several times, it is not possible for it to discharge all the venom in its venom sac. Only 0.5-1.0 µl of venom can be extracted from a single bee. Therefore, the amount of dry venom that can be extracted from a bee is less than 0.1 µg.

Although the oldest method used in the extraction of bee venom is the collection of venom from individual bees by squeezing the bees, this method is not preferred. The method that remains valid today is the classical electroshock method of extracting bee venom. The electroshock method is a device consisting of a wire grid placed in the hive, a permeable surface fixed to the bottom of the grid and a compartment in which the venom is collected. In this device, the honeybee, which takes the electric current given to the hive at intervals (usually every 30 minutes) as an external threat, injects its venom by injecting its stinger into the glass surface when it meets the wire grid. This method of collection does not harm the bees. At the end of the collection process, the bee venom accumulated on the surface and in the glass is dried. While the collected venom has a clear appearance at the beginning of the process, it turns into a white crystallized powder at the end of the process. The amount of venom collected from a bee is approximately equal to 0.1 µg dry weight. Assuming that there are 10,000 bees in a hive, the amount of venom collected at the end of a 30-minute session will only be about 1grams of pure dried venom. Bee venom, which has a very important place in Apitherapy worldwide, is recognized as a highly effective alternative treatment option due to the large number of active substances in its composition with different effects. The production and use of bee venom in medicine has been increasing in recent years. Apitherapy, which includes bee venom treatment, has been accepted as alternative medicine or complementary medicine in America, Canada, China and many European countries. However, its production and use in our country is quite limited. Bee venom production in our country is carried out in low quantities, especially to contribute to scientific studies. However, bee venom is a very special product that has an important place in the world market. Besides, order to get good results in apitherapy applications, one of the most important conditions is the issue of appropriate products. The lack of standardization of the products used in apitherapy is one of the main factors that make it difficult to conduct clinical studies on apitherapy. More studies and updates are needed on the subject. In any case, one of the issues that cannot be ignored is the "quality" of these products.

3. Factors effecting bee venom quality

It is crucial to ensure the safety and effectiveness of honey bee venom for human and animal health in the production chain. Otherwise, it may cause harm rather than benefit. It is crucial to ensure the safety and purity of bee products before their usage to mitigate any potential risks to human health. The quality of bee venom is influenced by many factors. These factors could be classified as;

Pollen, nectar & water sources (geographical conditions)
Colony strength and population level
Age of the queen
Bee species and ecotypes
Feeding (pollen)
Collecting methods
Collecting periods
Season and weather conditions
Storage
In addition to these factors impact of the beekeeper should not be ignored. Location of the apiary, quantity and quality of pollen, nectar and water sources, colony strength and population level, beekeepers' knowledge and experience, hygiene and sanitation of equipment's and gears, pest and disease control applications, avoiding residues and contamination, ensuring the purity of bee products, harvesting period is crucially important for bee venom production.

It is also important that the colony is not harmed during the bee venom collection process. In the appropriate machine and season, in general, in order to preserve the quality of the poison and keep the colony as strong as possible, the method in which the device is left in the hive for 15-20 minutes and the harvesting process is repeated at intervals of 10-15 days is mostly preferred (Çaprazlı and Kekeçoğlu, 2021).

During spring, honeybee colonies experience heightened foraging activity as floral resources become abundant. This increased foraging correlates with elevated venom production, as worker bees actively engage in defensive behaviors to protect the expanding colony (Bogdanov, 2015). Winter is a period of reduced activity for honeybee colonies, marked by lower temperatures and limited foraging opportunities. Bee venom production is minimal during winter, reflecting the colony's focus on survival and energy conservation rather than active defense (Bogdanov, 2015; Simone-Finstrom and Spivak, 2012).

Studies have indicated that the diversity and composition of pollen sources influence the chemical composition of bee venom. Bees foraging on a variety of pollen sources may exhibit variations in venom constituents, potentially impacting the therapeutic properties of the venom. Bees foraging on specific plant species or sources may incorporate unique compounds from pollen into their venom. The chemical diversity of pollen sources contributes to the complexity of bee venom, potentially influencing its therapeutic properties (Bogdanov, 2015).

The exposure to pesticides can affect various aspects of honeybee biology, including foraging behavior, navigation, and overall colony health, which may consequently influence bee venom production. Sublethal exposure to pesticides, even at concentrations below lethal levels, can have profound impacts on honeybee colonies. These effects may include decreased brood survival, impaired communication, and compromised immune function, all of which may contribute to changes in venom production (Alkassab and Kirchner, 2018; Sánchez-Bayo et al., 2016). Studies have shown that exposure to neonicotinoids can impair the ability of worker bees to locate and collect food resources, potentially affecting the overall defensive behavior and venom production within the colony.

Chronic exposure to pesticides over an extended period can result in cumulative effects on honeybee colonies. Long-term exposure may lead to sustained stress, compromised immune function, and overall colony decline, potentially influencing the ability of worker bees to produce venom (Kessler et al., 2015; Wu-Smart and Spivak, 2016).

In addition to environmental impacts, attention should be paid to hygiene and sanitation in the production and processing process, the use of appropriate techniques, ensuring the cold chain in the storage period, ensuring traceability and proper analysis. In the application process, it should be ensured that bee venom applications are carried out by physicians or professionals, that no application is made without allergic tests and that the appropriate dosage and method is applied.

Besides, according to the results of a survey of beekeeper’s honeybee venom producing tendency conducted by Varol et al. (2022), Marketing problems and lack of information on production and harvesting are the most important problems affecting bee venom production, and producers stated that they are willing to produce bee venom if a suitable market condition is provided.

4. Bee venom production: Pros and Cons

A growing field in apiculture and biotechnology, bee venom production offers a complex mix of advantages and disadvantages. Positively, bee venom, or apitotoxin, have shown encouraging medicinal potential, including anti-inflammatory, anti-tumor, and antibacterial qualities. These qualities make it an important aspect of complementary medicine, especially in the treatment of multiple sclerosis, arthritis, and chronic pain. Bee venom production can also give beekeepers another source of income, expanding the financial advantages of beekeeping beyond honey and other byproducts. Nevertheless, there are difficulties associated with the bee venom collection method. Bees may become stressed or harmed during the extraction process, which could have an influence on their longevity and general health as well as the production of the hive. In addition, the method needs to be handled carefully to prevent major bee mortality and to maintain the sustainability of bee populations, which are already in danger due to a variety of environmental factors. The market for bee venom is relatively small and specialized. Also the quality and potency of bee venom can vary significantly based on factors such as the bee species, extraction method, and geographic location. This lack of standardization can lead to inconsistent product quality, undermining consumer trust and complicating marketing efforts. The sustainable and ethical production of bee venom depends on striking a balance between these advantages and disadvantages, which calls for continued research and developments in extraction technology to reduce harm to bees while optimizing medicinal benefits.

Pros	Cons
Therapeutic Uses: Bee venom, or apitoxin, has been found to have numerous medicinal properties.	**Complex Extraction Process: Extracting bee venom is a complex and delicate process that requires specialized knowledge and equipment. Incorrect methods can lead to poor-quality venom or harm to bees.**
Pharmaceutical Applications: Research is ongoing into the use of bee venom in developing new drugs and treatments. Its potential in cancer therapy, neurological disorders, and immune system modulation highlights its importance in modern medicine.	**Stress and Harm: The process of extracting bee venom can stress and harm bees. Even though modern methods aim to minimize harm, the act of venom extraction can lead to physical damage or increased mortality rates in bees. Stressed or harmed bees may be less productive, impacting the overall health and productivity of the hive. This can lead to decreased honey production and other bee-related products.**
Biomedical Research: Bee venom is a subject of extensive research for its potential biomedical applications. Understanding its properties and effects can lead to breakthroughs in treating various diseases and conditions, contributing to scientific knowledge and medical advancements.	**Regulatory and Legal Issues: Bee venom production and its use in products are subject to strict regulatory controls. Compliance with these regulations can be time-consuming and costly. Different countries have varying legal frameworks regarding the use and sale of bee venom products, posing challenges for international trade and market expansion.**

Complementary Medicine: Bee venom is used in apitherapy, a branch of alternative medicine that uses bee products for health benefits. Apitherapy practitioners utilize bee venom to treat various ailments, contributing to the growing field of natural and holistic health practices.	**Limited Awareness and Information: Beekeepers have limited knowledge for bee venom production and there is limited consumer awareness about the benefits and uses of bee venom.**
Economic Benefits: For beekeepers, bee venom production can provide an additional source of income beyond traditional products like honey, beeswax, and propolis. This diversification can enhance the economic sustainability of beekeeping operations.	**Market Volatility: The market for bee venom is niche and can be volatile. Prices for bee venom products can fluctuate, making it a potentially unstable source of income for beekeepers.**
High-Value Product: Bee venom is a high-value product in the pharmaceutical and cosmetic industries. Its rarity and the complexity of extraction contribute to its premium pricing, offering substantial profit margins for producers.	**Economic Challenges: The initial setup costs for bee venom extraction equipment can be high. Additionally, maintaining the equipment and ensuring the health of the bee colonies can be costly.**
Bee Health and Colony Sustainability: While there are concerns about the impact of venom extraction on bee health, advancements in humane extraction methods are being developed. Sustainable bee venom production practices can promote better bee management and awareness about the importance of bee conservation. Besides, sustainable venom production practices can contribute to the overall health and maintenance of bee colonies, indirectly supporting broader ecological systems.	**Ethical Concerns: Ethical concerns about the welfare of bees during venom extraction are significant. Ensuring that bees are not harmed or killed in the process is a major challenge and a point of contention among animal rights advocates and environmentally conscious consumers.**

6. Conclusion

Bee venom is an important bee product that holds significant importance in various contexts including traditional medicine, apitherapy, cosmetics, pharmacology, and scientific research. For all these purposes, it is crucially important to ensure the production and quality of bee venom. It is important to remember that bee venom production is a production system that is under the influence of certain environmental and technical factors and must be carried out with technical knowledge and attention. Due to the short shelf life and special storage conditions of bee venom as a bee product used in the pharmaceutical and cosmetic industry, it was deemed more appropriate to produce this product through contract beekeeping model and to support it with appropriate training material on production, harvesting and storage conditions.

On the other hand, the fact that quality standard studies on this product have not yet been completed, constitutes an important obstacle to the marketing of this product, and it is likely that a classification and pricing mechanism will be established according to quality once standards are determined. Knowing and understanding the pros and cons of bee venom production will be advantageous for both bees and producers in the development and regulation of this valuable bee products production.

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Profiling of the endogenous phenolic contents of honey from different geographical origins in Türkiye by LC-MS/MS

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Abstract
Honey, an important animal product that we know from ancient sources dating back thousands of years, continues to be a matter of curiosity today with its extensive biological properties and characteristic chemical composition. This study aimed to determine the phenolic content of honey obtained from different regions of Türkiye. A total of 99 honey samples were collected from beekeepers in 34 different provinces of Türkiye and 25 phenolic compounds were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The highest phenolic component measured was hydroxycinnamic acid in honey samples taken from Eastern Anatolia (1036.05±177.61 ng/g), Central Anatolia (1009.43±127.99 ng/g), Marmara (1193.25±172.66 ng/g), Aegean (1478.83±159.99 ng/g), and Black Sea (2048.31±332.81 ng/g) regions while in honey samples from the Mediterranean (1143.82±1377.23 ng/g) and Southeastern Anatolia (1485.36±817.22 ng/g) regions was vanillic acid. Alizarin, myricetin, protocatechuic acid, thymoquinone, and 2-hydroxy-1, 4-naphthoquinone could not be detected among these phenolic compounds in honey samples. The results of the study once again emphasize the effect of geographical differences and the flora from which honey is derived on the phenolic content of honey and provides valuable information about the phenolic content of honey produced in various regions of Türkiye, which has a wide variety of flora and honey types.

Keywords: honey, bee, phenolic content, LC-MS/MS, Türkiye

This research was funded by Harran University Harran Scientific Research Project Department with project number 18027.

Introduction

Honey, a natural product of bees, has been esteemed throughout history for its multifaceted medicinal and nutritional properties. Its composition varies greatly depending on floral origin, geographical location, and processing methods, rendering it a subject of continuous scientific investigation (Machado De-Melo et al., 2018). Among its diverse constituents, phenolic compounds have garnered significant attention due to their potent antioxidant, antimicrobial, and anti-inflammatory properties, contributing to the health-promoting effects associated with honey consumption (Cianciosi et al., 2018).

Phenolic compounds constitute a significant class of secondary metabolites found in honey, contributing not only to its sensory characteristics but also to its medicinal and nutritional properties (da Silva et al., 2016). These compounds encompass a wide range of chemical structures, including flavonoids, phenolic acids, and polyphenols, which are derived from the nectar and pollen of various floral sources (Tafere, 2021). Phenolics are renowned for their potent antioxidant activity, scavenging harmful free radicals and mitigating oxidative stress in the body (Crozier et al., 2009). Moreover, they exhibit antimicrobial properties, aiding in the preservation of honey and protecting against microbial spoilage (Estevinho et al., 2008). Additionally, phenolic compounds possess anti-inflammatory effects, contributing to honey's therapeutic potential in alleviating inflammation-related conditions. The presence of phenolics in honey has been correlated with its anti-cancer, anti-diabetic, and cardiovascular health-promoting properties, making them pivotal components in understanding honey's multifaceted health benefits (Cianciosi et al., 2018; Ranneh et al., 2021). Furthermore, phenolic composition serves as a marker for honey authenticity and quality, facilitating the differentiation between monofloral and multifloral honeys and providing insights into their geographical and botanical origins (Becerril-Sánchez et al., 2021). In essence, the phenolic constituents of honey play a crucial role not only in its sensory appeal but also in its therapeutic efficacy and nutritional value, underscoring their importance in the comprehensive evaluation of honey as a functional food and natural remedy (Lucheze et al., 2017).

Türkiye, with its rich floral diversity and unique geographical characteristics, stands as an intriguing region for the exploration of honey phenolics. Multifloral honey, derived from the nectar of various plant species, represents a particularly complex matrix with the potential for a diverse array of phenolic constituents (Can et al., 2015). Understanding the phenolic profile of Türkiye's multifloral honey holds promise not only for elucidating its health benefits but also for promoting its value in domestic and international markets.

This study aims to comprehensively evaluate the phenolic content of multifloral honey sourced from Türkiye, employing advanced analytical techniques such as liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). By characterizing the phenolic composition of Türkiye's multifloral honey, we seek to contribute to the body of knowledge surrounding honey's nutritional and medicinal properties while shedding light on the factors influencing its phenolic profile. Furthermore, the findings of this research may have implications for quality control measures within the honey industry, aiding in the authentication and standardization of Türkiye's multifloral honey products. Ultimately, this study endeavors to enhance our understanding of the phenolic constituents present in Türkiye's multifloral honey and their potential implications for human health and well-being.

Materials and Methods:

Sample collection

Honey samples taken from beekeepers in various provinces in different geographical regions of Türkiye were analyzed. The geographical origin of the total 99 honey samples that constitute the material of the study was presented in Table 1.

Analysis of phenolic composition by LC-MS/MS

These samples were carefully stored in sealed falcon tubes in a dark, room-temperature environment until they were ready for analysis. All chemicals utilized were of analytical grade and were procured from Merck in Darmstadt, Germany. The extraction method for LC-MS/MS analysis was adapted from Altun and Aydemir (2020), as outlined by Durmaz (2020). To prepare the samples, 25 grams of honey were thoroughly mixed with acidic water (pH 2, with HCl) in a 5:1 ratio and filtered through cotton to remove any solid particles. The resulting filtrate was passed through a glass column packed with Amberlit XAD-4 resin, which selectively retained phenolic compounds while allowing sugars and other polar substances to be washed away with an aqueous solution. After column washing with acidic water and purified water, the phenolic fraction was concentrated using methanol and reduced pressure. The concentrated residue was dissolved in water, filtered with diethyl ether, and then combined and concentrated using a rotary evaporator. For LC-MS/MS analysis, the desiccated residue was dissolved in methanol, filtered through a 0.45 µm membrane, and then subjected to analysis of phenolic substances using a UHPLC instrument coupled with a double MS device.

Table 1. The geographical origin of honey samples

Regions	Eastern Anatolia (n: 35)	Southeastern Anatolia (n:13)	Marmara (n:19)	Black Sea (n: 3)	Aegean (n:11)	Central Anatolia (n:7)	Mediterranean (n: 11)
Provinces	Ağrı (n:1)	Adıyaman (n:7)	Balıkesir (n: 12)	Artvin (n:1)	İzmir (n: 6)	Ankara (n: 1)	Adana (n: 4)
	Ardahan (n: 4)	Batman (n:1)	Bursa (n: 1)	Kastamonu (n:1)	Kütahya (n:1)	Afyon (n: 1)	Antalya (n: 1)
	Bitlis (n: 1)	Siirt (n:4)	Çanakkale (n: 3)	Tokat (n:1)	Muğla (n:4)	Konya (n: 1)	Hatay (n: 1)
	Bingöl (n: 4)	Şanlıurfa (n:1)	Edirne (n: 1)			Sivas (n: 4)	Kahramanmaraş (n: 2)
	Elazığ (n: 4)		Tekirdağ (n: 2)				Mersin (n: 3)
	Erzurum (n: 5)						
	Kars (n: 8)						
	Malatya (n: 2)						
	Tunceli (n: 2)						
	Van (n: 4)						

Results

Twenty-five phenolic compounds (catechin hydrate, acetohydroxamic acid, vanillic acid, resveratrol, fumaric acid, gallic acid, caffeic acid, phloridzin dihydrate, oleuropein, hydroxycinnamic acid, ellagic acid, myricetin, protocathetic acid, silymarin, 2-hydroxy 1.4 naphthoquinone, butein, naringenin, luteolin, kaempferol, curcumin, thymoquinone, alizarin, hydroxybenzoic acid, salicylic acid and quercetin) were analyzed. The composition of phenolic compounds in honey samples were summarized in Table 2. The highest phenolic component measured was hydroxycinnamic acid in honey samples taken from Eastern Anatolia (1036.05±177.61 ng/g), Central Anatolia (1009.43±127.99 ng/g), Marmara (1193.25±172.66 ng/g), Aegean (1478.83±159.99 ng/g), and Black Sea (2048.31±332.81 ng/g) regions while in honey samples from the Mediterranean (1143.82±1377.23 ng/g) and Southeastern Anatolia (1485.36±817.22 ng/g) regions was vanillic acid. Alizarin, myricetin, protocatechuic acid, thymoquinone, and 2-hydroxy-1, 4-naphthoquinone could not be detected among these phenolic compounds in honey samples.

Table 2. Lists the phenolic components of the honey samples (ng g^{-1}).

	REGIONS						
Phenolic Compounds	**Eastern Anatolia (n=35)**	**Southeastern Anatolia (n=13)**	**Central Anatolia (n=7)**	**Marmara (n=19)**	**Aegean (n=11)**	**Black Sea (n=3)**	**Mediterranean (n=11)**
Catechinhydrate	**61.22±18.71 (N.D.= 18)**	**30.17±5.58 (N.D.= 9)**	**36.96±5.07 (N.D.= 2)**	**50.50±4.67 (N.D.= 5)**	**58.1±76.73 (N.D.=2)**	**N.D.**	**66.92±24.72 (N.D.= 3)**
Acetohydroxamic acid	**4.74±1.17^{b} (N.D.= 28)**	**7.059±0.00^{a} (N.D.= 12)**	**5.60±0.00^{b} (N.D.= 7)**	**4.62±0.70^{b} (N.D.= 16)**	**N.D.**	**N.D.**	**N.D.**
Vanillic acid	**545.62±32.61**	**1485.36±817.22**	**745.23±106.31**	**1006.39±304.25**	**932.84±63.19**	**831.66±209.54**	**1143.82±415.25**
Resveratrol	**51.21±2.25^{c} (N.D.= 1)**	**62.15±5.55^{bc} (N.D.= 2)**	**78.06±8.20^{abc}**	**110.75±13.30^{a}**	**45.23±0.04^{c}**	**98.005±7.01^{ab} (N.D.= 1)**	**87.301±5.00^{ab} (N.D.= 1)**
Fumaric acid	**142.62±00.08^{c} (N.D.= 4)**	**112.40±26.27^{c} (N.D.= 6)**	**848.00±168.00^{ab} (N.D.= 2)**	**306.00±155.00^{b}**	**1189.00±290.00^{a} (N.D.= 1)**	**670.00±138.00^{b} (N.D.= 1)**	**291.53±77.83^{b}**
Gallic acid	**48.74±2.27^{c} (N.D.= 17)**	**58.91±5.8^{c} (N.D.= 10)**	**76.01±8.39^{b} (N.D.= 3)**	**100.49±5.52^{a} (N.D.= 2)**	**98.04±1.170^{a} (N.D.= 3)**	**95.45±4.84^{a} (N.D.= 1)**	**71.31±16.86^{b} (N.D.= 5)**
Caffeic acid	**438.76±57.54^{ab}**	**146.65±34.01^{b}**	**698.25±148.91^{a}**	**280.45±66.24^{b}**	**258.64±44.69^{b}**	**363.17±80.48^{b}**	**151.66±24.78^{b}**
Phloridzindihydrate	**110.63±10.42^{b}**	**99.34±40.09^{b}**	**129.64±20.41^{b} (N.D.= 1)**	**140.34±19.06^{b}**	**105.83±11.73^{b}**	**692.45±590.308^{a}**	**123.01±17.23^{b} (N.D.= 1)**
Oleuropein	**150.00±17.10^{b} (N.D.= 3)**	**118.10±12.3^{c} (N.D.= 4)**	**189.90±42.70^{a}**	**187.50±36.50^{a} (N.D.= 1)**	**154.10±26.8^{ab} (N.D.= 3)**	**161.50±13.5^{ab} (N.D.= 1)**	**151.06±34.06^{ab} (N.D.= 1)**
Hydoxycinamic acid	**1036.05±177.61^{bc} (N.D.= 1)**	**564.12±121.92^{c}**	**1009.43±127.99^{bc}**	**1193.25±172.66^{bc}**	**1478.83±159.99^{ab} (N.D.= 1)**	**2048.31±332.81^{a}**	**868.42±205.82^{bc} (N.D.= 2)**
Ellagic acid	**300.21±3.40^{e} (N.D.= 25)**	**397.00±131.00^{d} (N.D.= 10)**	**603.84±0.00^{b} (N.D.= 6)**	**630.56±36.56^{b} (N.D.= 6)**	**529.65±0.00^{c} (N.D.= 10)**	**851.00±356.00^{a}**	**597.36±135.62^{bc} (N.D.= 7)**

Myricetin	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**
Protcathetic acid	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**
Silymarin	**110.38±5.81^{c} (N.D.= 1)**	**124.58±13.73^{bc} (N.D.= 2)**	**146.81±14.55^{bc} (N.D.= 1)**	**157.55±12.10^{ab} (N.D.= 3)**	**148.78±6.15^{bc} (N.D.= 2)**	**193.22±57.17^{a}**	**158.66±28.75^{ab} (N.D.= 1)**
2-Hyroxy1,4nph	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**
Butein	**27.66±6.24^{b} (N.D.= 5)**	**22.53±12.63^{b} (N.D.= 9)**	**31.28±6.45^{b} (N.D.= 2)**	**35.23±13.18^{b} (N.D.= 14)**	**20.53±6.04^{b} (N.D.= 6)**	**89.3±59.85^{a} (N.D.= 1)**	**18.54±6.52^{b} (N.D.= 6)**
Naringenin	**160.30±28.72^{ab} (N.D.= 9)**	**197.78±125.23 ^{ab} (N.D.= 10)**	**200.62±38.58 ^{ab} (N.D.= 2)**	**257.76±149.06 ^{ab} (N.D.= 15)**	**90.99±34.91^{b} (N.D.= 9)**	**332.58±159.99^{a} (N.D.= 1)**	**148.78±0.00^{b} (N.D.= 10)**
Luteolin	**300.02±165.40 (N.D.= 29)**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**
Kaempferol	**263.60±33.10^{c} (N.D.= 20)**	**985.06± 0.00^{a} (N.D.= 12)**	**N.D.**	**339.83±0.70^{b} (N.D.= 13)**	**N.D.**	**701.00±350.00^{a} (N.D.= 1)**	**172.09±36.69^{c} (N.D.= 9)**
Curcumin	**46.89±0.75^{c} (N.D.= 23)**	**70.10±22.60^{b} (N.D.= 11)**	**76.00±10.06^{b} (N.D.= 4)**	**71.61±9.21^{b} (N.D.= 12)**	**91.62±0.28^{a} (N.D.= 7)**	**N.D.**	**69.621±8.92^{b} (N.D.=7)**
Thymoquinone	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**
Alizarin	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**	**N.D.**
Hydroxybenzoic acid	**65.43±9.60^{b}**	**43.87±8.89^{b}**	**124.52±76.27^{b}**	**504.10±89.07^{a}**	**651.98±96.56^{a}**	**122.38±53.63^{b}**	**405.43±83.81^{a}**
Salicylic acid	**56.71±9.91^{c} (N.D.= 1)**	**32.40±8.66^{c} (N.D.= 1)**	**105.15±73.01^{c}**	**481.24±84.21^{b}**	**708.45±79.90^{a} (N.D.= 1)**	**103.58±46.56^{c}**	**430.01±194.55^{b} (N.D.= 1)**
Quercetin	**110.76±34.06^{ab} (N.D.= 12)**	**74.88±7.18^{b} (N.D.= 5)**	**69.40±0.04^{c} (N.D.= 6)**	**95.09±9.41^{b} (N.D.= 11)**	**N.D.**	**183.99±0.00^{a} (N.D.= 2)**	**98.45±25.71^{b} (N.D.= 1)**

$^{a-e}$: Values with different superscripts in the same column are statistically different (P<0.05), n: Number of samples, N.D.= Not detection

Discussion

Honey is a natural food of animal origin that is assumed to have important contributions to health. The results of current studies indicate that the health benefits of honey can be attributed to bioactive components. The determination of phenolic compounds, which are among the bioactive components of honey, continues with sensitive analysis methods developed day by day. It is known that the phenolic content of honey is directly affected by the geographical features and flora where the honey is produced. Phenolic compounds are natural antioxidant substances found in plants and are responsible for nutritional value and the preservation of storage stability. Therefore, while the phenolic content of honey determines its authentic origin, it may also be a factor in choosing honey for apitherapy. This study tried to determine the phenolic content of honey obtained from various regions of Türkiye, which ranks among the world in honey production and has a unique flora.

Vanillic acid, caffeic acid, and hydroxybenzoic acid were detected in all honey samples. Luteolin was measured only in the Eastern Anatolia region honey (82.8%). Alizarin, myricetin, protocatechuic acid, thymoquinone, and 2-hydroxy-1,4-naphthoquinone were not detected in any honey sample. It has been identified that other phenolic contents of honey vary according to region.

Çayan et al. (2023), in their study on cotton honey obtained from Adana and Şanlıurfa provinces; stated that catechin hydrate, vanillic acid, hydroxybenzoic acid, quercetin, luteolin, and kaempferol were in different amounts in honey varieties. The results of the researchers and the findings of this study are quite different, except that luteolin and protocatechuic acid were not detected in the honey samples of the southeastern Anatolian provinces in both studies.

Saral (2023) analyzed 19 phenolic compounds in 8 chestnut honey collected from Artvin province in the Black Sea region, and in parallel with our study, luteolin could not be detected in the chestnut honey. In the present study, contrary to the results of Black Sea region honey, catechinhydrate was the most important flavonoid in chestnut honey samples.

When the results of the study conducted with 11 multi-floral honey samples from Northern Cyprus located in the Mediterranean were examined, similar to the results of this study, protocatechuic acid, luteolin, and myricetin (except one sample) were not detected in Mediterranean honey samples (Uçar et al. 2023). However, in our study gallic acid, caffeic acid, ellagic acid, resveratrol, quercetin, and curcumin were detected in Mediterranian region honey..

Conclusion

The results of the study once again emphasize the effect of geographical differences and the flora from which honey is derived on the phenolic content of honey and provide valuable information about the phenolic content of honey produced in various regions of Türkiye, which has a wide variety of flora and honey types.

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Bioactive properties of drone larva at different days

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Abstract: Apitherapy is the use of bee products for disease prevention and treatment support. One of these products is the drone larva apilarnil. Antiradical, antioxidant and total phenolic substance analyses were performed on male bee larvae and pupae as well as adult male bees emerging from pupae. The difference between the samples in terms of antiradical % inhibition in male bee larvae, pupae and adult stages was found to be statistically significant ($P<0.01$). However, the difference between the antioxidant and total phenolic substance amounts in drone larvae, pupae and adult stages was not found to be statistically significant ($P>0.05$). It is important to collect apilarnil from the hive when its bioactivity is at its highest. Therefore, such studies are needed.

Keywords: apitherapy, drone larvae, apilarnil, bioactivity

1. Introduction

The honeybee colony consists of three castes: queen (female), worker (sterile female) and drone (male). Drones represent the males of honeybees that hatch from unfertilized eggs, while worker and queen bees, the female individuals of honey bees, develop from fertilized eggs (Mărgăoan et al., 2017). In honeybees, worker bees reach adulthood in 21 days, drones in 24 days and queens in 16 days. The nutritional requirements of each caste are different. However, during the first three days after hatching, all larvae are fed with a protein-rich milky secretion called royal jelly, secreted from the salivary glands of worker bees aged 5–15 days. Brood in queen bee cells continue to be fed with royal jelly throughout their life, while brood in drone and worker cells are fed a mixture of dilute nectar and bee bread. Studies have shown that the weight of the larval food increases in different developmental stages of queen, worker and drone larvae. However, the amount of carbohydrates in the diet of drone and worker larvae first increased and then decreased and continued to decrease in the queen bee (Mandla and Kumar, 2016).

Drones and workers have different roles in the colony. This is reflected in many physiological, morphological and behavioural differences (Hrassnigg and Crailsheim, 2005). While workers perform very different tasks, the only function of drones is to produce sperm and mate with a queen. Drone production is regulated according to need and is not produced all the time. Drones are more costly to produce within the hive than workers due to their larger size (Seeley, 2002; Seeley and Mikheyev, 2003; Hrassnigg and Crailsheim, 2005).

Drones are defined as bees that develop from unfertilized eggs in comb cells by a form of reproduction known as “parthenogenesis”. The development of drones takes place in 24 days (Tozetto and Engels, 2013). During the first ten days, drones develop in an open cell (egg and larval stage). In the last 14 days of development, the cells of the comb are closed by worker bees (capping) and their development is completed there. Worker bees cover the cells where the queen bees are located and the cells where the worker bees are located in a convex manner. This difference in the covers allows the identification of male offspring in the comb. The prepupa and pupae do not feed during the time they remain closed in the cell and undergo metamorphosis (Figure 1). The process of spermatogenesis is completed (Czekońska and Tofilski, 2020). In the first days of development (day 1-3), regardless of the polymorphic form, the larva weighs approximately 0.11 mg (Tozetto and Engels, 2013). Depending on their polymorphic form, the body weight and length of various larvae begin to differ only from day 4 onwards. A seven-day-old queen larva weighs 270 mg, a worker larva weighs 80 mg, and a drone larva weighs 120 mg. On the day of sealing (day 11), drone larvae weigh 350 mg. They also contain more proteins, lipids, and sulfhydryl groups (Czekońska and Tofilski, 2020; Sidor and Dżugan, 2020).

2. Material and Methods

Samples were obtained from the apiary located in the Agricultural Research Unit of Erciyes University. Total phenolic substance Folin Ciocalteu, antioxidant activity ammonium molybdate and antiradical activity DPPH methods were used to test the larvae, pupae and adult samples of male bees (Silici, 2019).

3. Results

The difference between the samples in terms of antiradical % inhibition in male bee larvae, pupae and adult stages was found to be statistically significant ($P<0.01$). However, the difference between the antioxidant and total phenolic substance amounts in drone larvae, pupae and adult stages was not found to be statistically significant ($P>0.05$).

Table.1. Bioactivity of drone samples

Sample	Antiradical activity	Antioksidant activityAAE mg/L	Total Phenolic Content GAE mg/L
Larvae	8,99±1.81^{a}	158.77±19.52	793.10±133.76
Pupae	13.21±3.37^{ab}	162.78±18.05	777.28±123.11
Adult	15.61±0.01^{b}	184.84±0.01	615.99±0.01

4. Discussion

The total phenolic content of the apilarnil sample was determined as 774.43 GAE mg/L. The highest total phenolic content was seen on the fifth day of the larval period with 1004.72 GAE mg/L, while the lowest was seen on the ninth day of the pupal period with 501.43 GAE mg/L. In a study, Bolatovna et al. (2015) reported the content of flavonoid compounds in apilarnil as 0.014±0.007%. Silici (2019) determined the total phenolic content of apilarnil as 834.05 mg GAE/100 g. In the study conducted on the apilarnil sample collected from Ankara region, it was determined that the total phenolic content was 47.5 mg QE/100 g and the total flavonoid amount was 14.35 mg GAE/100 g (Kurtdede and Sevin, 2022). Total phenolic substances in drone brood homogenate samples collected from 7 different regions of Poland were reported as

0.78 GAE mg/g, 0.77 GAE mg/g, 0.79 GAE mg/g, 0.77 GAE mg/g, 0.75 GAE mg/g, 0.65 GAE mg/g and 0.75 GAE mg/g (Sawczuk et al., 2022). When the results of the present study were compared with the literature results, it had lower total phenolic content according to Silici (2019) but higher according to Bolatovna et al. (2015), Kurtdede and Sevin (2022) and Sawczuk et al. (2022).

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Honeybee products in neurodevelopmental disorders

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Abstract:
Neurodevelopmental disorders are disorders in the growth and development of the central nervous system that affect motor and sensory functions, mood, learning capacity and memory. Genetic diseases, brain malformations, metabolic, immunological and infectious diseases or traumatic, toxic, nutritional and environmental factors that are considered neurodevelopmental or that can lead to neurodevelopmental adverse outcomes when they occur during infancy and childhood are conditions that cause brain damage. Apitherapy is the use of honeybee products for the purpose of supporting the prevention and treatment of diseases. There are numerous scientific studies on the effects of products such as honey, propolis, royal jelly, pollen, perga and bee venom, which have many biological activities such as antioxidant, antimicrobial, immune stimulant, anti-inflammatory and anticarcinogenic, on neurodevelopmental diseases. The positive effect observed in these studies is generally attributed to the antioxidant activity of bee products.

Keywords: Neurodevelopmental disorders, royal jelly, propolis, bee venom, bee pollen

1. Introduction

Parkinson's disease is a chronic progressive neurodegenerative movement disorder, characterized by bradykinesia, rigidity, rest tremor and postural instability. In addition to these cardinal motor symptoms, secondary motor symptoms (e.g. flexion posture, hypomimia, dysarthria, loss of arm swing) and non-motor symptoms (e.g. cognitive impairments, autonomic disturbances, dementia, sleep disorders, pain, hyposmia) appear in Parkinson's disease. The neuropathologic features of Parkinson's disease are dopaminergic neuronal loss in the substantia nigra and the presence of cytoplasmic eosinophilic Lewy bodies. Genetic susceptibility and environmental factors share the responsibility in the pathogenesis of the disease. Although medical therapy may provide an effective control of motor symptoms, rehabilitation is an important treatment approach for improvement of quality of life and functional capacity of the patients. Individual rehabilitation program should be initiated in the early stages of the disease and treatment continuity should be targeted (Jankovic, 2008; Ramaker et al. 2002).

Animal studies indicate that whole propolis, CAPE, chrysin, whole RJ, and its lipid derivative might counteract oxidative stress, neuroinflammation, and mitochondrial dysfunction resulting in mitigation of neuronal damage and improvement of motor symptoms of Parkinson Disease. However, more studies are needed to examine the specific cellular and molecular mechanisms of whole propolis and RJ, as well as main compounds in RJ (MRJPs, peptides, phenols, bioactive substances) in Parkinson Disease. To identify the most effective compounds in propolis and RJ, it might be important to compare the effect of various active ingredients in these products either alone or in combination with other PD treatments. Mechanisms of action of propolis and royal jelly in Parkinson's disease are protecting neurons against Oxidative Stress, protecting neurons against neuroinflammation, inhibiting apoptosis of dopaminergic neurons, maintaining brain levels of dopamine, enhancement of the production of neurotrophins in the brain, restoration of normal brain structure. BV injection resulted in antinociceptive and anti-inflammatory effects on arthritis, neuralgia, and chronic inflammation in humans. The therapeutic action of BV may not only be attributable to bioactive BV compounds such as peptides (melittin, adolapin, and apamin), enzymes (phospholipase A2), and amines but also to the mechanical effect of peripheral stimulation. The protective effect of BV in animals with MPTP-induced dopaminergic cell loss and the inhibition of neuroinflammation by MAC-1 and iNOS activation (Cho et al. 2018).

Alzheimer's disease (AD) is characterized by neuronal and synaptic loss throughout the brain, involving the basal forebrain cholinergic system, amygdala, hippocampus and several cortical areas. Patients' memory and cognitive performance is progressively impaired; they develop dementia; and are likely to die prematurely. AD is a complex, multi-factorial disease involved in the gradual, progressive loss of memory and cognitive abilities that develop over the years

The current limitations of the conventional treatment modalities have prompted researchers to focus on honeybee products with multi-functionalities that can target the disease at different levels. Current therapies, such as treatment with acetylcholinesterase inhibitors to enhance cholinergic function, give only partial and temporary alleviation of symptoms (Baek et al. 2020)

Polyphenols especially flavonoids have been proven neuroprotectants through the inhibition of inflammatory mediators, activation of antioxidant enzymes, minimization of lipid peroxidation, modulation of gene expression and maintenance of healthy status of the neurons. AChE inhibitors are still the most used class of drugs for managing the symptoms of AD. Propolis showed potent AChE inhibitor activity in several studies (galangin, pinobanksin, chrysin). Studies reported on flavonoids screened against CDK5, identified as neuroprotective agents. There is inflammation associated in AD, there is increased expression of COX-2 found in the regions of brain. Therefore, COX-2 inhibitors are extensively explored for AD. For example Turkish propolis exhibited 64.5% inhibition. PC-12 cells possess characteristic neuroendocrine cells and are routinely used as suitable cell-line models to screen potential drug candidates against AD. Alzheimer's disease (AD) is the most common form of dementia characterized by aggregation of amyloid b (Ab) and neuronal loss. One of the risk factors for AD is high cholesterol levels, which are known to promote Ab deposition. RJ remarkably increased the number of neurons, enhanced antioxidant capacities, inhibited activated-capase-3 protein expression, and enhanced neuronal metabolicactivities by increasing N-acetyl aspartate (NAA) and glutamate and by reducing choline and myo-inositol in AD rabbits. It was demonstrated that RJ could reduce cholesterol levels, regulate Ab levels and enhance neuronal metabolic activities in AD rabbits, providing preclinical evidence that RJ treatment has the potential to protect neurons and prevent AD. Research denotes that queen bees, which feast on RJ throughout their entire lives, demonstrate up to 5-fold higher learning and memory abilities than worker bees, which feast on honey and pollens. nvestigations highlight the ability of RJ to enhance learning and memory retention, as well as to prevent and treat cognitive behavioral deficits. The improvement of cognitive function associated with RJ treatment results from the amelioration of oxidative damage and increase of antioxidant capacity. It significantly decreased the production of ROS, NOS, IL-6, IL-1β, and TNF-α and increased the mRNA expression of antioxidants such as heme oxygenase-1 (HO-1) and glutathione peroxidase (GSH-Px). RJ decreased Aβ synthesis and enhanced its clearance in AD rat models. RJ activated autophagy genes and protected against neuronal apoptosis. Preclinical trials show that RJ/eRJ, 10-HDA, RJ peptides, and MRJPs exhibit significant effects against AD pathology in animal models and in cell lines by interfering with amyloid synthesis and protein misfolding, enhancing amyloid clearance, and correcting pathologies that contribute to amyloidogenesis, such as inflammation and oxidative stress. AD is a severe neurodegenerative disorder characterized by the accumulation of two hallmark proteins, amyloid-β (Aβ) peptides and neurofibrillary tangles, that play key roles in neuroinflammation, including the production of pro-inflammatory cytokines and activation of microglial cells and complements. BV treatment can ameliorate Aβ pathology without adverse neuro-inflammation in Aβ-vaccinated 3xTg-AD mice. The neuroprotective and anti-inflammatory effects of bvPLA2 are mediated by IL-10 production and Treg cell modulation. BV could inhibit apoptosis and inflammatory responses in an AD cellular model. In addition, it prevented cell accumulation of βA, an important pathogenic mechanism in AD. Nuclear factor-kappa B (NF-κB), a member of the transcription factors, plays critical roles in various inflammatory and autoimmune diseases, including AD. Treatment with BV also downregulated signaling by NF-κB, which is considered to be an important factor in the regulation of neuroinflammatory and amyloidogenic responses, both *in vivo* and *in vitro*. BV can be used as a treatment to block the progression of AD in transgenic mice. This is due to the ability of BVPLA2 to reduce the accumulation of Aβ and improve cognitive functioning in mice brains. The same study similarly shows that bvPLA2 can increase glucose brain metabolism and reduce neuroinflammatory responses in the hippocampus, which can limit AD pathogenesis (Baek et al. 2018; Ali and Kunugi, 2020, Sankaran et al. 2024).

Epilepsy is the third most common neurological disorder characterized by recurrent spontaneous epileptic seizures, affecting approximately 1-2% of the population in the world. Globally, an estimated 2.4 million people are diagnosed with epilepsy each year. Seizure attacks are the result of excessive electrical discharges in a group of nerve cells in the brain.

Neurological and behavioral changes, which can vary from short-term attention deficit and severe muscle cramps to long-term convulsions, and impairment in cognitive performances such as learning and memory are observed. Additionally, due to recurrent seizures, neuropathological changes occur especially in the hippocampus (Hp). The central nervous system is the most susceptible organ to oxidative stress because it has the highest oxygen consumption, the highest concentration of unsaturated fatty acid and a weak antioxidant system compared to other organs. Oxidative stress leads to the oxidation of biological molecules such as lipids, proteins, nucleotides and carbohydrates contributing to a cascade of functional disturbances and neuronal damage and death in epilepsy disease. It has been reported that free radical generation induces seizure activity by inactivation of glutamine synthase, leading to an abnormal build-up of glutamate or by decreasing glutamate decarboxylase, thus causing decrease in GABA turnover. It should be noted that hyperactivation of glutamate receptors and oxidative stress cause excessive neuronal excitability and trigger epileptic seizures in patients with epilepsy. Royal jelly reduced MDA and TOS while increasing TAC. In addition, RJ reversed neuronal damage in the hippocampal CA1 and CA3 areas. RJ has anticonvulsant and neuroprotective effects in KA (kainic acid; hippocampal injury model) induced TLE (temporal lobe epilepsy) via its antioxidative properties. RJ inhibits the generation of free radicals, ROS, and MDA in the brain, particularly the cortex and hippocampus (Hashemi et al. 2023; Aly et al. 2023).

Autism as a neurodevelopmental disorder is characterized by severe social impairment and communication, repetitive behavior, and restricted interests. Several studies recorded abnormality of glutamatergic signaling pathways in autism. Glutamate receptors are mainly localized in brain areas that have been repeatedly implicated in autism, such as the cerebellum and hippocampus. Excitatory glutamate signaling through glutamate receptors (GluRs) plays a key role in cortical development which also indicate its involvement in the etiology of autistic features. Glutamate (Glu) is an amino acid that functions as an excitatory neurotransmitter in the central nervous system (CNS). Excitation causes depolarization of the postsynaptic neuron and promotes the propagation of action potentials. In contrast, GABA, the main inhibitory neurotransmitter, results in the hyperpolarization of the postsynaptic cell membrane. Bee pollen supplementation demonstrates protective potency presented as amelioration of most of the measured variables. Bee pollen demonstrated ameliorating effects on glutamate excitotoxicity and the impaired glutamine-glutamate-GABA circuit as two etiological mechanisms in PPA-induced neurotoxicity (Aabed et al. 2019; Alghamdi et al. 2022)

2. Conclusion

Consumption of antioxidant compounds can attenuate oxidative damage, improve cognitive performance in animals and slow the deterioration in memory and learning in middle- and older-age humans. There are numerous scientific studies on the effects of products such as honey, propolis, royal jelly, pollen, perga and bee venom, which have many biological activities such as antioxidant, antimicrobial, immune stimulant, anti-inflammatory and anticarcinogenic, on neurodevelopmental diseases. The positive effect observed in these studies is generally attributed to the antioxidant activity of bee products.

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Chestnut bee pollen: nutritional and biochemical properties

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Abstract:
In this study, the nutritional and biological active properties of the chestnut bee pollen were investigated. Total protein, Total fiber, and Total Sugars (Fructose, glucose, sucrose, maltose) were examined as nutritional properties. Total phenolic substance, total flavonoids, and phenolic compounds were examined by HPLC-PDA as biological active ingredients. Antioxidant capacity was analyzed according to DPPH radical scavenging assay. It was found that the crude protein amount of chestnut bee pollens varied between 24-25%, the total fiber amount varied between 14-15%, and the total sugar amount varied between 35-40%. It was determined that the amount of total polyphenol substance varied between 29 and 32 mg GAE/g, and the content of total flavonoids varied between 4.10 and 4,80 mg QUE/g. DPPH radical scavenging activity was found to vary between 0.78 and 0.84 mg/ml. When compared to result with other bee pollen samples, it was found that chestnut bee pollen contains approximately 4-5 times more polyphenols than other bee pollens and has a high antioxidant capacity accordingly. According to the 25 phenolic standards studied, p-OH Benzoic acid, t-cinnamic acid, p-Coumaric acid, Rutin, Chrysin and pinocembrin were detected as the major phenolic components in the samples. It was determined that chestnut pollen had a higher value than other pollens in terms of total protein and fiber. Conclusively, chestnut pollen exhibits substantial potential as a complementary medicinal agent.

Keywords: Bee pollen, antioxidant, phenolics

Introduction

Bee pollen is a natural substance collected by honeybees from the male reproductive cells of flowers. As bees forage for nectar, they inadvertently gather pollen on their bodies, forming granules that adhere to their legs. Once back at the hive, the bees mix the pollen with nectar and digestive enzymes, creating small pellets. Bee pollen serves as a vital source of nutrition for honeybee colonies, providing essential proteins, lipids, vitamins, minerals, and other organic compounds. It is recognized for its balanced composition, containing nutrients such as amino acids, fatty acids, minerals, B vitamins, carotenoids, polyphenols, and various sugars. Due to its rich nutritional profile, bee pollen is often consumed as a natural food supplement by humans, offering potential health benefits, including antioxidant, anti-inflammatory, and immune-strengthening properties (Kalaycıoğlu et al. 2017: Kolaylı et al. 2023; Denisow, & Denisow-Pietrzyk, 2016).

Bees engage in the collection and utilization of pollen from flowers, performing diverse functions vital for hive sustenance. This includes nutritional supplementation, larval nourishment, the production of bee bread, establishment of a food reserve, and fortification of the immune system. Pollen, rich in proteins, vitamins, and antioxidants, serves as a foundational component in the sustenance and vitality of the bee colony (Denisow, & Denisow-Pietrzyk, 2016; Khalifa, et al. 2021).

Studies show that bee pollen contains approximately 10-40% protein, 1-10% lipid, 13-50% carbohydrate and 1% mineral. Additionally, bee pollen is a mixture rich in B vitamins and polyphenols. The composition and biological active value of bee pollen varies depending on its botanical origin. In this study, the chemical composition and antioxidant properties of chestnut origin bee pollen were examined.

Materials and Methods

Three different area of dried chestnut bee pollen dried honeybee pollen obtained from experienced beekeepers from Black Sea Region. The samples were stored at +4 C.

Chemical analyses

The macronutrient composition, including moisture, raw protein, crude fiber, and carbohydrates, was analyzed following the procedures outlined by the AOAC (Cunnif, 1995). The estimation of crude protein content (N × 6.25) was conducted using the Kjeldahl method (Rabie et al. 1983). Quantification of dietary fiber content was performed utilizing the automatic enzymatic method with the Ankom TDF Fiber Analyzer following the AOAC 991.43 IDF/SDF method. Sugars of fructose, glucose, sucrose, and mannose sugars were determined by means of HPLC-RID analyses (Kolaylı et al. 2023).

Total phenolic content ((TPC) was measured using the classic Folin–Ciocalteu's assay in the ethanolic extract (Singleton et al. 1999). The total flavonoid content (TFC) of the bee pollen extracts was measured following spectrophotometric method (Fukumota and Mazza, 2000). Total antioxidant capacity of the samples was measured by Benzie IFF, Strain JJ (1996). Phenolic composition of the samples was measured by HPLC- PDA assay (Kara et al. 2022).

Results and Discussion

Chemical analysis values are summarized in Table 1. It was determined that the amount of fiber in the pollen was 15.84%. Compared to the data in the literature, it was determined that the total fiber amounts of bee pollen varied between 12% and 20% (Kolaylı et al. 2023; Serra et al. 1986).

The average total crude protein amount was determined to be 25% in chestnut bee pollen samples. A range of 10 to 35 g/100 g has been reported for the total protein content of bee pollen, which varies depending on its botanical source (Kalaycıoğlu et al. 2017; Kolaylı et al. 2023).

The average total sugar amount was determined to be 37% and varied between 35% and 38%.

Table 1. Chemical composition of bee pollen

	Total Fiber %	Total Protein %	Total Sugar %	Fructose %	Glucose %	Sucrose %	Maltose %
Samples 1	**15.58**	**26.50**	**35.10**	**21,20**	**10,70**	**0,27**	**0,15**
Samples 2	**16.10**	**24.07**	**39,38.**	**22,08**	**10,64**	**0,38**	**1,04**
Sample 3	**15,22**	**23.08**	**38.05**	**22,01**	**15,90**	**1,20**	**0,50**
Mean	**15.84±0.44**	**25,28±1,76**	**37.24±2,19**	**21,64±0,69**	**10,67±3,02**	**0,32±0,51**	**0,59±0,45**

Table 4. The mean and standard errors for phenolic contents and antioxidant status in the bee pollen samples

Pollens*	Total Phenolic Content (TPC) mgGAE/g	Total Flavonoid Content (TFC) (mgQUE/g)	Total Antioxidant Capacity (FRAP) µmolFeSO4.7H2O/g	DPPH radical scavenging activity SC50: mg/mL
BP1	7,00	1,94	52,63	1,40
	0,06^{fg}	0,05^{g}	0,39^{f}	0,01^{c}
BP2	11,17	4,31	61,10	0,80
	0,27^{c}	0,02^{bc}	0,67^{e}	0,01^{e}
BP3	9,03	2,33	68,32	1,84
	0,98^{d}	0,04^{e}	0,43^{c}	0,01^{b}
BP4	7,91	3,80	65,25	1,02

	0,06^{def}	0,06^{d}	0,51^{d}	0,01^{de}
BP5	8,37	4,31	43,83	1,41
	0,12^{de}	0,05^{bc}	0,34^{g}	0,01^{c}
BP6	29,62	4,24	389,67	0,80
	0,64^{b}	0,03^{c}	2,60^{a}	0,32^{e}
BP7	31,13	4,37	361,00	0,13
	0,52^{a}	0,06^{b}	1,53^{b}	0,01^{f}
BP8	6,91	2,17	26,30	3,03
	0,06^{fg}	0,05^{f}	0,25^{j}	0,04^{a}
BP9	6,08	4,60	30,68	1,30
	0,12^{g}	0,03^{a}	0,29^{i}	0,01^{cd}
BP10	7,52	1,73	38,67	1,82
	0,06^{ef}	0,02^{h}	0,12^{h}	0,01^{b}
BP11	8,40	1,58	36,39	1,73
	0,06^{de}	0,01^{i}	0,69^{h}	0,02^{b}
Mean	12,10	1,24	133,68	1,39
SD	9,13	1,18	127,46	0,75

*The values were given as mean and standard error. Different letters in the same column indicate different pollens in the same variable according to the Duncan Multiple Comparison Test ($p<0.01$)

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Presentation Type: Oral

Attitudes of Parents Who Prefer to Use Bee Products to Strengthen Children's Immune Systems

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Abstract:
Nutrition is important for the lives of individual's growth and development at the desired level of a sustainable quality of life. In addition, there is a direct relationship between the immune system and nutrition, and quality nutrition is needed to protect individuals from diseases and to achieve well-being during the disease. With the COVID-19 pandemic and viral infection outbreaks experienced worldwide, interest in bee products such as honey, bee pollen, and propolis by parents has increased. Honey is the main bee product that is produced by honey bees with its antioxidants such as organic acids, proteins, phenolic compounds, and flavonoids. It is also the most preferred natural product by parents because of its high nutritional value and sweet taste. Propolis is another popular bee product with its high antioxidant activity. It is not possible to use propolis directly and it should be extracted with a solvent (in general 70% ethanol-water solution). However, the use of propolis by the children is limited because of its hard taste and smell. Bee pollen is a whole food and it is also rich in amino acids and antioxidants. Considering infections spread rapidly, especially in environments where individuals are together, such as schools, children need to use food supplements and support their immune systems. Thus, in this study, the attitudes of parents who prefer to use bee products to strengthen their children's immune systems was investigated.

Keywords: Honey, propolis, bee pollen, antioxidant activity, immune system, children, parents

Introduction

Nutrition is one of the most important factors affecting human health. Especially for children who are experiencing rapid growth and development from a young age, children need to have adequate and balanced nutrition to complete their development in a healthy way and to gain resistance against diseases (Hunter, 2011; Özyürek, Bedge, and Özkan 2013). Suffering inadequate and unbalanced nutrition in childhood for various reasons slows down children's growth rate, disrupts the balance of body measurements, weakens the body's immune system, and can even cause death (Doğan, Ceylan, and Özgan, 2019). Therefore, immune system of children need to be strengthened through nutrition to protect them from diseases.

Since children are dependent on their parents for nutrition, parents' attitudes and opinions about nutrition gain great importance (Özyürek, Bedge and Özkan 2013). Parents should ensure that their children are fed with patience and without any pressure when they experience problems with their children's immune systems. At the same time, parents can use various nutrients as a complementary treatment method in line with expert recommendations, in addition to nutritional and medical treatments in case of illness of their children (Doğan, Ceylan, and Özgan, 2019).

Parents may prefer traditional application methods by using natural foods (Lanting et al., 2005) to strengthen children's immunity, protect them from diseases, and accelerate recovery in case of illness. Especially the fact that traditional application methods are economical, easily accessible, and natural products increases the preference of parents (Chang et al., 2011). Studies also indicate that traditional practices are preferred by parents due to their many conveniences and benefits (Aydın et al., 2015; Bülbül et al., 2014; Muslu and Öztürk, 2008).

Especially with the COVID-19 epidemic affecting the whole world, it is seen that families are turning to healthy nutrition and more traditional and complementary medicine methods to protect the health of themselves and their children and strengthen their immunity (Işık and Can, 2021; Tural Büyük et al., 2022; Uçar et al. ., 2020). While the rate of parents using traditional and complementary medicine methods to protect their health and strengthen immunity for their children before COVID-19 was 35.4%, it was observed that this rate was 87.1% during the Covid-19 period (Tural Büyük et al, 2022).

Different Bee products

In addition to their nutritional properties as food, bee products such as honey, pollen, bee bread, royal jelly and propolis are important to use in the treatment of diseases due to the their biological active substances (Dumitru et al., 2022). Due to the increasing epidemics caused by the COVID-19 pandemic, interest in products that will strengthen the immune system has increased. When the searches made using Google Trend are examined, it was seen that honey was the most searched bee product in the last year compared to propolis and pollen, which are other known important bee products (Figure 1 and Figure 2).

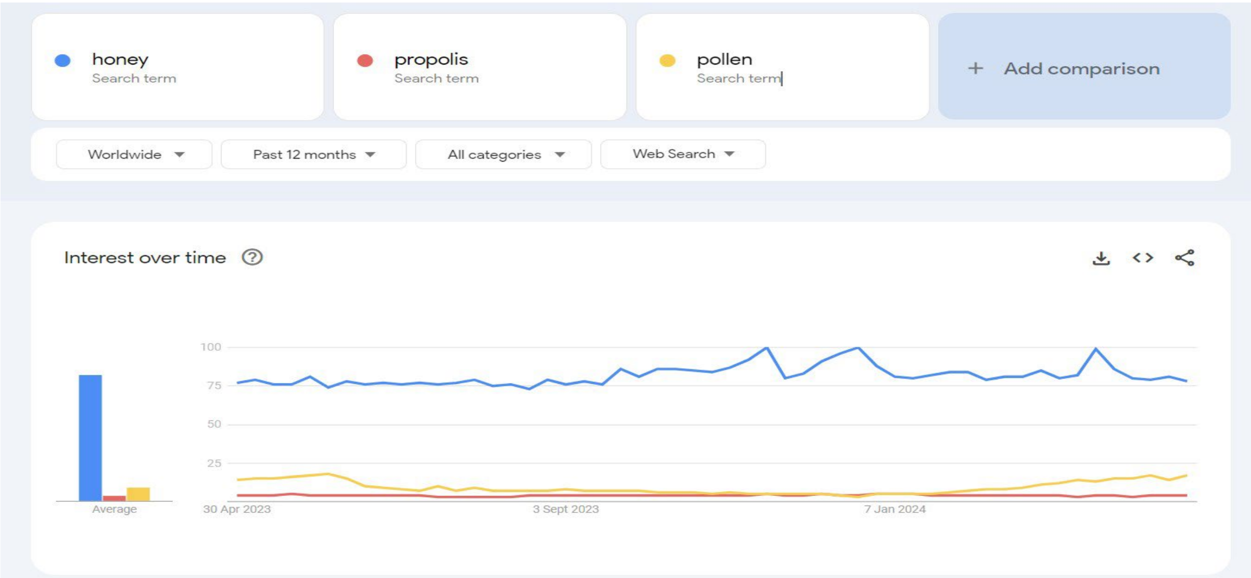

Figure 1. Comparison of honey, propolis, and pollen search on Google Trends in last 12 months over the world (https://trends.google.com/trends/explore?q=honey,propolis,pollen&hl=en)

Figure 2. Comparison of honey, propolis, and pollen search on Google Trends in last 12 months according to regions (https://trends.google.com/trends/explore?q=honey,propolis,pollen&hl=en)

On the other hand, although honey was the most searched product worldwide, it was seen that honey and propolis were searched at similar rates in Turkey (Figure 3 and Figure 4).

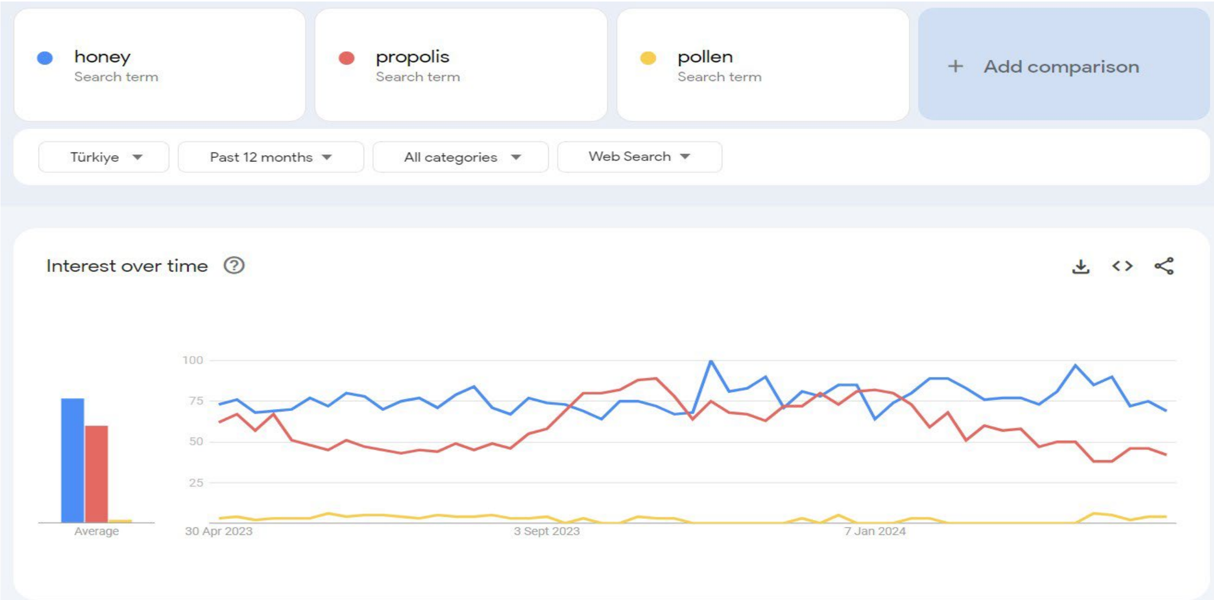

Figure 3. Comparison of honey, propolis, and pollen search on Google Trends in last 12 months in Türkiye (https://trends.google.com/trends/explore?geo=TR&q=honey,propolis,pollen&hl=en)

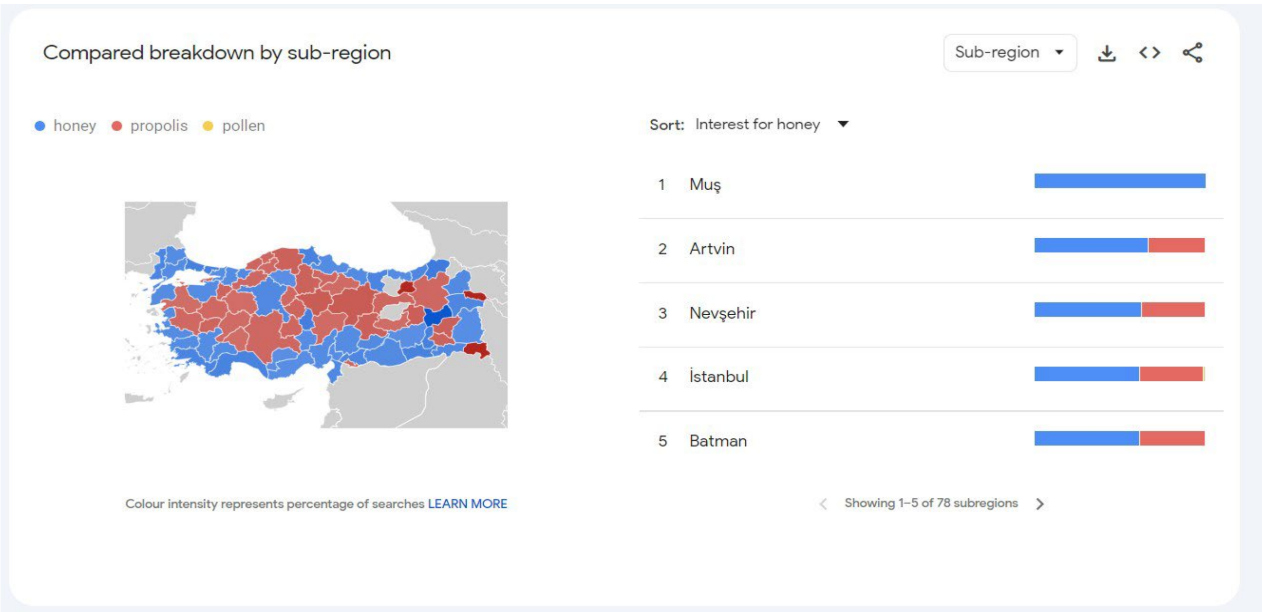

Figure 4. Comparison of honey, propolis, and pollen search on Google Trends in last 12 months according to city of Türkiye (https://trends.google.com/trends/explore?geo=TR&q=honey,propolis,pollen&hl=en)

Honey is the most known and consumed product that produced by honey bees. The collected nectar from the flowers of plants (flower honey) or the sweet secretions produced by some insects (secretory honey) is changed by bees and stored in the honeycomb cells (Bogdanov et al., 2008; Kris et al., 2011; Diafat et al., 2017). Although honey mainly contains sugar, it also contains protein, enzymes, amino acids, minerals, elements, vitamins, aroma and phenolic components. In this way, it is used as a supporter of the immune system, relieving physical and mental fatigue, energizing, revitalizing, and healing of asthma, stomach, circulation, respiratory, cancer, blood pressure and vascular diseases (Bogdanov et al., 2008; Kris et al., 2011; Diafat et al., 2017; Pasupuleti et al., 2017).

Pollen is collected by worker bees to meet the colony's protein needs for royal jelly production and brood rearing (Öztürk and Akçiçek, 2015; Pasupuleti et al., 2017) and is the main protein source for bees. The main components of bee pollen are proteins, amino acids, lipids, and sugars. Studies have reported that pollen prevents lipid peroxidation in vitro, scavenges many free radicals that have oxidant properties and are known to be carcinogenic, and is antimicrobial against bacteria (Silici, 2014). In addition to pollen's digestive, cell regenerating and appetite-increasing effects, it is also used as a supportive treatment for colds, osteoporosis, headaches, nervous and ulcer disorders (Karlıdağ and Keskin, 2020).

Propolis is a natural bee product, also called 'bee glue', which is obtained by mixing resinous substances collected by honey bees from parts of plants such as leaves, stems and buds, and plant nectar and pollen with wax and enzymes (Özkök et al., 2023). It consists of 45-55% resin, 25-35% vegetable waxes, 10% essential oils, 5% pollen and 5% organic compounds and mineral substances. It is stated in many studies that propolis has antimicrobial, antifungal and antioxidant properties (Krell, 1996; Kolayli et al., 2020; Cantero et al., 2023; Karimitabar et al., 2023).

Royal jelly is another bee product with its a very high nutritional value. It is white, homogeneous, mushy, creamy, slightly bitter, with an acidic taste, and a pungent odor. It is secreted from the hypopharyngeal and mandibular glands located in the head area of feeding worker bees (Özkök et al., 2023). The main components of royal jelly are water, proteins, sugars, lipids and minerals (Keskin et al., 2020). All essential amino acids are found in the structure of royal jelly. Royal jelly generally provides cell renewal in the body, gives vitality to tissues, strengthens health, energy and the immune system (Sabatini et al., 2009). The royal jelly has effects on many different diseases such as anorexia, body immune system deficiency due to chronic diseases, metabolic and nutritional disorders and digestive system disorders, asthma, bronchitis, nervousness, insomnia, and liver diseases (Sabatini et al., 2009; Doğaroğlu, 2015; Keskin et al., 2020).

Attitudes of Parents

Individuals prefer to use bee products, which have many benefits for their health, and some of the products that parents use as traditional and complementary medicine methods for their children are bee products such as honey, pollen, propolis, and royal jelly. In particular, honey constitutes the main bee product produced by honey bees with its antioxidants such as organic acids, proteins, phenolic compounds, and flavonoids. It is the natural product most preferred by parents due to its high nutritional value and sweet taste. Propolis is another popular bee product with high antioxidant activity. It is not possible to use propolis directly and it must be extracted with a solvent (usually 70% ethanol-water solution). However, its use in children is limited due to its strong taste and odor. Bee pollen is a complete food and is also rich in amino acids and antioxidants. With the COVID-19 epidemic and viral infection outbreaks, parents' interest in bee products such as honey, bee pollen, and propolis has increased. In a study conducted by Tural Büyük et al. (2022), it was determined that 40.2% of the parents participating in the study used honey and its products on their children during the COVID-19 period. In a study conducted by Doğan, Ceylan, and Özgan (2019), it was determined that among the traditional methods that parents do to strengthen their children's immune systems are giving them products such as honey and molasses and drinking drinks such as honey milk. On the other hand, in a study conducted by Alfaifi et al. (2023) it was reported that parents often rely on traditional medicine to treat their children's illnesses or maintain their health. However, the safety and efficacy of traditional medicine are often unclear, and there is a need to assess parents' knowledge, attitudes, and practices toward its use. The study was included 555 participants. Most of the participants strongly agreed that it is important to talk to a medical doctor or pharmacist before using traditional medicine (190, 34.20%). Most of the participants disagreed that traditional medicine is cheap and easily available (156, 28.10%) and not dangerous for children (175, 31.50%). Most of the participants answered neutrally that TM is safe because it is made from natural ingredients (175, 31.50%) (Alfaifi et al., 2023).

Conclusion

As a result, it is important to have an adequate and balanced diet to protect against viral infections and to have a healthy immune system. Research also shows that in cases where adequate nutrition cannot be provided, the use of nutritional supplements to meet some vitamin and mineral needs may have positive effects on protecting against viral infections (Bakan et al., 2020; Karaağaç and Bellikci Koyu, 2020). Considering that infections spread rapidly, especially in environments where individuals are together, such as schools, children should use food supplements and support their immune systems. Since some of the preparations produced with royal jelly, pollen and propolis etc. are thought to strengthen the immune system, these products attract great attention from parents in our country.

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Understanding the Factors Influencing the Reproductive Health of Honey Bee (*Apis mellifera* L.) Drones

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Abstract:
Understanding the factors influencing the reproductive health of honey bee drones is essential for ensuring the vitality and sustainability of honey bee populations. Honey bee drones play a critical role in colony reproduction by mating with virgin queens, thus contributing to genetic diversity within colonies. Various biological, environmental, and anthropogenic factors can influence drone reproductive success. Biological factors such as genetics, age, and physiology, as well as environmental factors like climate and habitat quality, can impact drone health and fertility. Additionally, anthropogenic factors including pesticide exposure, habitat loss, and beekeeping practices can pose significant challenges to drone reproductive health. Maintaining genetic diversity within honey bee colonies is crucial for mitigating the negative impacts of inbreeding and enhancing colony resilience. Collaboration between beekeepers, researchers, policymakers, and the broader community is essential for addressing environmental stressors and promoting pollinator-friendly practices. Advancements in research and technology provide valuable tools for monitoring and managing honey bee health, enabling targeted interventions to support drone reproductive health. By adopting a holistic approach that considers the complex interactions between genetic, environmental, and management factors, we can ensure a brighter future for honey bee populations and the essential ecosystem services they provide.

Keywords: honey bee, drone, reproductive quality, reproductive health, spermatozoa

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

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

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 Fisher and 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

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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