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Editor Ulaş ACARÖZ

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Editor's Note

The first 'International Congress on Bee Sciences' was organized online and free of charge. We are very happy and proud that various Bee science-related fields attended the congress. During this event, distinguished and respected scientists came together to exchange ideas, develop and implement new researches and joint projects. There were 25 invited speakers from 18 different countries. The scientific committee of the congress consisted of 211 scientists from more than 160 universities. Almost 500 participants participated in the congress. We would like to thank all participants and supporters. Hope to see you at our next congress.

Best wishes from Turkey

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CONTENTS

FULL

TEXTS

The new Animal Health Law of the EU and its implications of honey bees and bumblebees

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Abstract:
In order to ensure high standards of animal and public health in the European Union and the rational development of agriculture and animal farming, and to increase productivity, animal health rules should be laid down at Union level. The current Union legislation on animal health consists of a series of linked and interrelated basic acts that lay down rules on animal health applying to intra-Union trade, entry into the Union, health certificate, disease notification, eradication and surveillance, veterinary controls, and financial support in relation to different animal species, but an overarching legal framework, laying down harmonised principles across the sector, is missing. The regulation 2016/429 (Animal Health Law) proposes the adoption of a single and simplified regulatory framework for animal health seeking convergence with international standards while ensuring a firm commitment to high standards of animal health. The aim of this regulation is to implement the commitments and visions provided for in the Animal Health Strategy, including the 'One health' principle, and to consolidate the legal framework for a common Union animal health policy through a single, simplified and flexible regulatory framework for animal health. In this article, the new Animal Health Law of the EU and the correlated regulations available so far with implications for honey bees and bumblebees have been selected and condensed to improve their availability and usability for the operators of the sector.

Keywords: Bumblebee; Honey bee; Disease; Regulation; European Union;

1. Introduction

The Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 (EU 2016) on transmissible animal diseases that amends and repeals certain acts in the area of animal health is the new rule named Animal Health Law (AHL). This regulation was adopted by the European Parliament and the Council on 9 March 2016, entered into force on 21 April 2016 and was applied on 21 April 2021. Since then it was complemented by additional rules. The Commission has listened to the calls for the postponement of the date of entry into application of the AHL, but after undertaking an in depth analysis and assessed potential consequences, the conclusion was reached that postponing the date of application of the AHL would not be a good way forward. Instead, reinforced support to Member States and stakeholders for its smooth implementation and enforcement, including additional transitional measures, was guaranteed. Transitional measures have been foreseen for Animal health and veterinary certificates for entry into the EU until 20/10/2021 and for intra-EU movements until 17/10/2021.

This regulation lays down rules for the prevention and control of animal diseases which are transmissible to animals or to humans (Article 1). Those rules provide for: (a) the prioritisation and categorisation of diseases of Union concern and for the establishment of responsibilities for animal health (Part I: Articles 1 to 17); (b) the early detection, notification and reporting of diseases, surveillance, eradication programmes and disease-free status (Part II: Articles 18 to 42); (c) disease awareness, preparedness and control (Part III: Articles 43 to 83); (d) the registration and approval of establishments and transporters, movements and traceability of animals, germinal products and products of animal origin within the Union (Part IV: Articles 84 to 228; and Part VI: Articles 244 to 248 and 252 to 256); (e) the entry of animals, germinal products, and products of animal origin into the Union and the export of such consignments from the Union (Part V: Articles 229 to 243; and Part VI: Articles 244 to 246 and 252 to 256); (f) non-commercial movements of pet animals into a Member State from another Member State or from a third country or territory, (Part VI: Articles 244 to 256); (g) the emergency measures to be taken in the event of a disease emergency situation (Part VII: Articles 257 to 262).

These rules aim to ensure: (a) (i) improved animal health to support sustainable agricultural and aquaculture production in the Union; (ii) the effective functioning of the internal market; (iii) a reduction in the adverse effects on animal health, public health and the environment of certain diseases and of the measures taken to prevent and control diseases; (b) take into account: (i) the relationship between animal health and public health; the environment, including biodiversity and valuable genetic resources, as well as the impact of climate change; food and feed safety; animal welfare, including the sparing of any avoidable pain, distress or suffering; antimicrobial resistance; food security; (ii) the economic, social, cultural and environmental consequences arising from the application of disease control and prevention measures; (iii) relevant international standards.

Article 3 (Scope of Parts IV, V and VI). Title I of Part IV (Articles 84 to 171) shall apply to: (a) terrestrial animals, and animals which are not terrestrial animals but which may transmit diseases affecting terrestrial animals; (b) germinal products from terrestrial animals; (c) products of animal origin from terrestrial animals. For the purposes of this Regulation (Article 4 Definitions), the following definitions apply: (1) “animals” means vertebrate and invertebrate animals; (2) “terrestrial animals” means birds, terrestrial mammals, bees and bumblebees.

The responsibilities for animal health and biosecurity measures, the knowledge of animal health, the responsibilities of veterinarians, Member States' responsibilities, and delegation by a competent authority of official activities are defined in articles 11 to 14.

Responsibilities for animal health and biosecurity measures (Article 10). Operators shall (a) as regards kept animals and products under their responsibility, be responsible for: (i) the health of kept animals; (ii) prudent and responsible use of veterinary medicines, without prejudice to the role and responsibility of veterinarians, (iii) minimising the risk of the spread of diseases; (iv) good animal husbandry; (b) take such biosecurity measures regarding kept animals, and products under their responsibility, as are appropriate for: (i) the species and categories of kept animals and products; (ii) the type of production; and (iii) the risks involved, taking into account: geographical location and climatic conditions; and local circumstances and practices.

Animal professionals shall take action to minimise the risk of the spread of diseases in the context of their occupational relationship with animals and products.

Knowledge of animal health (Article 11). Operators and animal professionals shall have adequate knowledge of (a) animal diseases, including those that are transmissible to humans; (b) biosecurity principles; (c) the interaction between animal health, animal welfare and human health; (d) good practice of animal husbandry for the animal species under their care; (e) resistance to treatments, including antimicrobial resistance, and its implications. The content and the level of knowledge required shall depend on: (a) the species and categories of kept animals or products under the responsibility of the operators and animal professionals concerned and the nature of their occupational relationship with those animals or products; (b) the type of production; (c) the tasks performed. The above mentioned knowledge shall be acquired in one of the following ways: (a) professional experience or training; (b) existing programmes in agricultural or aquaculture sectors that are relevant for animal health; (c) formal education; (d) other experience or other training which results in the same level of knowledge as that covered by points (a), (b) or (c).

Responsibilities of veterinarians (Article 12). Within the scope of this Regulation veterinarians shall: (a) take all appropriate measures to prevent the introduction, development and spread of diseases; (b) take action to ensure the early detection of diseases by carrying out proper diagnosis and differential diagnosis to rule out or confirm a disease; (c) play an active role in: (i) raising animal health awareness, and awareness of the interaction between animal health, animal welfare and human health; (ii) disease prevention; (iii) the early detection of, and rapid response to, diseases. (iv) raising awareness of resistance to treatments, including antimicrobial resistance, and its implications; (d) cooperate with the competent authority, operators, animal professionals and pet keepers in the application of the disease prevention and control measures provided for in this Regulation.

Member States' responsibilities (Article 13). Each Member State shall ensure that competent authority for animal health has: (a) qualified personnel, facilities, equipment, financial resources and an effective organisation covering the whole territory of the Member State; (b) access to laboratories with the qualified personnel, facilities, equipment and financial resources needed to ensure the rapid and accurate diagnosis and differential diagnosis of listed diseases and emerging diseases; (c) sufficiently trained veterinarians involved in performing the activities referred to in article 12.

Delegation by a competent authority of official activities (Article 14). The competent authority may delegate one or more of the following activities to veterinarians other than official veterinarians: (a) practical application of measures under the eradication programmes; (b) supporting the competent authority in carrying out surveillance or in relation to surveillance programmes; (c) activities related to: (i) disease awareness, preparedness and control, concerning: - sampling activities and implementation of investigations and epidemiological enquiries in the event of the suspected presence of a disease; - carrying out activities relating to disease control measures in the event of an outbreak of disease; - carrying out emergency vaccination; (ii) registration, approval, traceability and movements; (iii) issuing and completing the identification documents for pet animals; (iv) the application and use of means of identification.

2. Materials and Methods

After the general concepts and definitions given above that characterize the animal health law, the regulations with relevance to honey bees and bumblebees are reviewed and commented on.

3. Results

Commission Delegated Regulation (EU) 2018/1629 of 25 July 2018

Commission Delegated Regulation (EU) 2018/1629 of 25 July 2018 (EU 2018a) amends the list of diseases set out in Annex II to Regulation (EU) 2016/429 of the European Parliament and of the Council (Animal Health Law).

In “ANNEX II LIST OF ANIMAL DISEASES”, honey bee diseases are listed as follows: infestation with *Varroa* spp. (Varroosis); infestation with *Aethina tumida* (Small hive beetle); American foulbrood; infestation with *Tropilaelaps* spp..

Commission Implementing Regulation (EU) 2018/1882

In addition, Commission Implementing Regulation (EU) 2018/1882 of 3 December 2018 (EU 2018b) applies certain disease prevention and control rules to categories (Table 1) of listed diseases and establishes a list of species and groups of species posing a considerable risk for the spread of those listed diseases (Table 2).

Table 1. The categorization given in Regulation (EU) 2016/429.

Category	Definitions
Category A disease	a listed disease that does not normally occur in the Union and for which immediate eradication measures must be taken as soon as it is detected, as referred to in article 9(1)(a) of Regulation (EU) 2016/429
Category B disease	a listed disease which must be controlled in all Member States with the goal of eradicating it throughout the Union, as referred to in article 9(1)(b) of Regulation (EU) 2016/429
Category C disease	a listed disease which is of relevance to some Member States and for which measures are needed to prevent it from spreading to parts of the Union that are officially disease-free or that have eradication programmes for the listed disease concerned, as referred to in article 9(1)(c) of Regulation (EU) 2016/429
Category D disease	a listed disease for which measures are needed to prevent it from spreading on account of its entry into the Union or movements between Member States, as referred to in article 9(1)(d) of Regulation (EU) 2016/429
Category E disease	a listed disease for which there is a need for surveillance within the Union, as referred to in article 9(1)(e) of Regulation (EU) 2016/429

This categorization is applied to honey bee and bumblebees listed diseases (Table 2).

Table 2. Categories of listed diseases and list of species and groups of species posing a considerable risk for the spread of those listed diseases.

Name of listed disease	Category of listed disease	Listed species	
		Species and group of species	Vector species
Infestation with *Varroa* spp. (Varroosis)	C+D+E	Apis	-
Infestation with *Aethina tumida* (Small hive beetle)	D+E	Apis, *Bombus* spp.	-
American foulbrood	D+E	Apis	-
Infestation with *Tropilaelaps* spp.	D+E	Apis	-

Commission Delegated Regulation (EU) 2020/688

Commission Delegated Regulation (EU) 2020/688 of 17 December 2019 (EU 2020a) supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council, as regards animal health requirements for movements within the Union of terrestrial animals and hatching eggs.

Prior to the application of Regulation (EU) 2016/429, Union rules for movement between Member States of certain kept terrestrial animals including primates, captive birds, honeybees and bumblebees, dogs, cats and ferrets were laid down in Directive 92/65/EEC. Those rules have proven to be effective in minimising the risk of spread of listed diseases between Member States. Accordingly, the main substance of those rules should be maintained in this regulation, but updated to take account of the practical experience gained in their application. In addition, this regulation should provide possibilities for derogations in cases where alternative risk-mitigation measures are applied.

Article 144(1)(c) of Regulation (EU) 2016/429 empowers the Commission to lay down the requirements for animal health certification for movements to other Member States of kept terrestrial animals other than ungulates, poultry and animals intended for confined establishments in cases where an animal health certificate is imperative in order to ensure that the movement in question complies with the animal health requirements provided for in articles 124 to 142 of Regulation (EU) 2016/429. This regulation should therefore establish requirements for animal health certification which would allow movements to other Member States of consignments of captive birds, honeybees, bumblebees (except bumblebees from approved environmentally isolated bumblebee production establishments), primates, dogs, cats, ferrets and other carnivores.

Article 5 provides requirements regarding containers in which kept terrestrial animals and hatching eggs are transported. In the case of queen honeybees transported under derogation provided for in article 49, operators, including transporters, shall ensure that containers or the entire consignment are covered with fine mesh of not more than 2 mm in pore size immediately after the visual examination for the health certification by the official veterinarian. In the case of bumblebees from environmentally isolated production establishments for bumblebees, operators, including transporters, shall ensure that they are isolated during the transport in separate epidemiological units with each colony in a closed container which was new or cleaned and disinfected before use.

Section 2 Honeybees and bumblebees

Article 48 Requirements regarding the movement of honeybees to other Member States.

Operators shall only move honeybees in any stage of their lifecycle, including honeybee brood, to other Member States when the following requirements are fulfilled: (a) the animals and the hives of origin do not show signs of American foulbrood, infestation with *Aethina tumida* (Small hive beetle) or infestation with *Tropilaelaps* spp.; (b) they come from an apiary situated in the centre of a circle of at least: (i) 3 km radius, where American foulbrood has not been reported during the last 30 days prior to departure and which is not restricted due to an outbreak of American foulbrood; (ii) 100 km radius, where infestation with *Aethina tumida* (Small hive beetle) has not been reported and which is not restricted due to a suspected case or the confirmed occurrence of infestation with *Aethina tumida* (Small hive beetle) unless a derogation is provided for in article 49; (iii) 100 km radius, where infestation with *Tropilaelaps* spp. has not been reported and which is not restricted due to a suspected case or confirmed occurrence of infestation with *Tropilaelaps* spp..

Article 49 provides derogation for the movement of queen honeybees to other Member States.

By way of derogation from article 48(b)(ii), operators may move queen honeybees where those animals fulfil the requirements of article 48(a), (b)(i) and (iii) and the following requirements: (a) in the apiary of origin infestation with *Aethina tumida* (Small hive beetle) has not been reported and that apiary is situated at a distance of at least 30 km from the limits of a protection zone of at least 20 km in radius established by the competent authority around a confirmed occurrence of infestation with *Aethina tumida* (Small hive beetle). This was previously established with Commission Implementing Decision 2017/2174/EU; (b) the apiary of origin is not located in a zone restricted by protective measures established by the Union due to the confirmed occurrence of infestation with *Aethina tumida*; (c) the apiary of origin is situated in an area where annual surveillance for the detection of infestation with *Aethina tumida* by the competent authority is ongoing to provide a confidence level of at least 95 % of detecting infestation with *Aethina tumida* if at least 2% of the apiaries were infested; (d) the apiary of origin is inspected every month during the production season by the competent authority with negative results to provide a confidence level of at least 95 % of detecting infestation with *A. tumida* if at least 2 % of the hives were infested; (e) they are caged individually with a maximum of 20 accompanying attendants.

Article 50 provides additional requirements as regards infestation with *Varroa* spp. for the movement of honeybees to other Member States.

Operators shall only move honeybees in any stage of their lifecycle, including honeybee brood, to another Member State or zone thereof with the status free from infestation with *Varroa* spp. when in compliance with the requirements set out in article 48 and provided that the following requirements are fulfilled: (a) they come from a Member State or zone thereof with the status free from infestation with *Varroa* spp.; (b) they are protected from infestation with *Varroa* spp. during transport.

Requirements for the movement of bumblebees to other Member States are set out in article 51.

Operators shall only move bumblebees to other Member States when the following requirements are fulfilled: (a) they do not show signs of infestation with *A. tumida* (Small hive beetle); (b) they come from an establishment situated in the centre of a circle around the establishment of at least 100 km radius, where infestation with *A. tumida* has not been reported and which is not restricted due to a suspected case or confirmed occurrence of infestation with *A. tumida*. These requirements shall not apply to bumblebees from environmentally isolated production establishments moved in accordance with article 52.

In article 52 derogation for the movement of bumblebees from environmentally isolated production establishments for bumblebees to other Member States is set out.

By way of derogation from article 51(b), operators may move bumblebees from environmentally isolated production establishments for bumblebees to other Member States when in compliance with article 51(a) and provided the following requirements are fulfilled: (a) they have been bred isolated in separate epidemiological units with each colony in a closed container which was new or cleaned and disinfected before use; (b) regular surveys on the epidemiological unit carried out in accordance with written standard operating procedures has not detected the infestation with *A. tumida* within the epidemiological unit.

Operators shall only move captive birds, honeybees, bumblebees except bumblebees from approved environmentally isolated production establishments, primates, dogs, cats, ferrets or other carnivores to another Member State if they are accompanied by an animal health certificate issued by the competent authority of the Member State of origin as set out in article 71 (Animal health certificate for certain kept terrestrial animals).

In article 84 details on content of animal health certificate for honeybees and bumblebees are set out: 1. The animal health certificate for honeybees, that is issued by the competent authority of the Member State of origin in accordance with article 71(1), shall contain the general information provided for in point 1 of Part 1 of Annex VIII and an attestation of compliance with the requirements provided for in article 48, and in articles 49 and 50 where applicable. 2. The animal health certificate for bumblebees, except bumblebees from approved environmentally isolated production establishments, that is issued by the competent authority of the Member State of origin in accordance with article 71(1), shall contain the general information provided for in point 1 of Part 1 of Annex VIII and an attestation of compliance with the requirements provided for in article 51.

The responsibility of the competent authority for animal health certification is set out in Section 4 and article 91. Before signing an animal health certificate, the official veterinarian shall carry out the following types of documentary, identity and physical checks and examinations in order to verify compliance with the requirements: (h) in relation to honeybees and bumblebees an identity check and either (i) a visual examination of the animals, their packaging and any accompanying feed or other material for the purpose of detection of occurrence of American foulbrood, *A. tumida* and *Tropilaelaps* spp. for honeybees or *A. tumida* for bumblebees; or (ii) in relation to queen honeybees to be certified under derogation provided for in article 49, a documentary check of the records of the monthly health inspection during the production season, a visual examination of their individual cages for the purpose of verification of the maximum number of attendants per cage and a visual examination of the animals, their packaging and any accompanying feed or other material for the purpose of detection of occurrence of American foulbrood, *A. tumida* and *Tropilaelaps* spp..

The official veterinarian shall carry out the documentary, identity and physical checks and examinations as provided above and issue the animal health certificate: (h) within the last 48 hours before departure from the establishment of origin, in relation to honeybees and bumblebees and within the last 24 hours before departure from the establishment of origin, in relation to queen honeybees to be certified under derogation.

Section 5 Detailed rules on notification of movements of kept terrestrial animals and hatching eggs to other Member States.

In the case of bumblebees from approved environmentally isolated production establishments being moved to another Member State, the operator of the establishment of origin shall notify the competent authority of the Member State of origin in advance of the departure of those bumblebees (Article 93).

As set out in article 96, the operators notifying the competent authority in their Member State of origin as provided for in article 152 of Regulation (EU) 2016/429, shall provide the competent authority with the information concerning each consignment of kept terrestrial animals to be moved to another Member State provided for in: (a) points 1(a) to (d) in Part 1 of Annex VIII concerning kept terrestrial animals except bumblebees from approved environmentally isolated production establishments to be moved to another Member State; (b) part 2 of Annex VIII concerning bumblebees from approved environmentally isolated production establishments.

As set out in article 97, the competent authority of the Member State of origin notifying the competent authority of the Member State of destination in accordance with article 153 (The notification shall be carried out prior to the movement in question and, whenever possible, through Traces) of Regulation (EU) 2016/429, shall provide the information concerning each consignment of kept terrestrial animals to be moved to another Member State provided for in: (a) points 1(a) to (d) in Part 1 of Annex VIII concerning kept terrestrial animals except bumblebees from approved environmentally isolated production establishments to be moved to another

Member State; (b) part 2 of Annex VIII concerning bumblebees from approved environmentally isolated production establishments.

ANNEX VIII, INFORMATION TO BE CONTAINED IN ANIMAL HEALTH CERTIFICATES AND NOTIFICATIONS

Part 2 Information in the notification of movements for certain terrestrial animals for which animal health certificate is not required.

The notification for moving bumblebees from approved environmentally isolated production establishments to another Member State must contain at least the following information: (a) the name and address of the consignor and the consignee; (b) the name, address and unique approval number of the establishment of dispatch; (c) the name and address of the establishment of destination, and (i) where the establishment of destination is an approved establishment, the unique approval number of that establishment; or (ii) where the establishment of destination is a registered establishment, the unique registration number of that establishment; (d) the species, category and quantity and size of colonies; (e) the date of dispatch.

Traces – TRAde Control and Expert System

In order to prepare for the entry into application of the new Official Controls Regulation - Regulation (EU) 2017/625 - on 14 December 2019 (EU 2017), and to further facilitate the administrative procedures for operators and competent authorities, the Commission developed a new version of TRACES, called TRACES-NT (TRACES New Technologies), enabling the entire process of production of documents to be performed electronically as of that date. According to that Regulation, TRACES had to be integrated as part of the Information Management System for Official Controls (IMSOC) aiming at supporting a more efficient management of official controls. The Commission adopted on 30 September 2019 the IMSOC Regulation - Implementing Regulation 2019/1715 (EU 2019a) - with the objective to lay down the rules for the functioning of the IMSOC and its system components (TRACES, iRASFF, ADIS, EUROPHYT). The new Official Controls Regulation has expanded the scope of TRACES to all the categories of animals and goods subject to official controls at the border control posts of the European Union. Hence, since 14 December 2019, the use of TRACES to issue the Common Health Entry Document (CHED) recording the outcome of official controls performed at EU border and decision taken on that basis has become mandatory for consignments of animals, animal products, plants and plant products, and food and feed of non-animal origin entering the Union. As a result of the new Official Controls Regulation and the IMSOC Regulation, the Commission had also to integrate into TRACES the functionalities of the EUROPHYT-Interceptions web-based system, developed some years ago by DG SANTE to help Member States notify interceptions of consignments of plants and plant products entering the Union or traded in the Union, that may present an imminent danger of introducing or spreading pests in the EU.

Commission Delegated Regulation (EU) 2020/689

Commission Delegated Regulation (EU) 2020/689 of 17 December 2019 (EU 2020b) supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for surveillance, eradication programmes, and disease-free status for certain listed and emerging diseases.

Article 2 Definitions, (31) ‘honeybees’ means animals of the *Apis mellifera* species.

PART III INFESTATION WITH *VARROA* SPP.

Varroa destructor mite is the most deleterious parasite of honey bees and has gained a worldwide distribution with the only exception of Australia considering the continents where weather conditions are compatible with beekeeping (Rosenkranz et al. 2010).

Section 1 Granting of the status to a Member State or zone as free from infestation with *Varroa* spp.

The status free from infestation with *Varroa* spp. may only be granted to the relevant honeybee population of a Member State or of a zone if: (a) a risk assessment has been conducted, identifying all potential factors for *Varroa* spp. occurrence and its potential presence in the past; (b) an ongoing awareness programme has been in place for at least one year to encourage reporting of all cases suggestive of *Varroa* spp.; (c) there has been no confirmed case of infestation with *Varroa* spp. either in kept or in wild honeybee colonies; (d) for at least one year, an annual surveillance has demonstrated the absence of infestations with *Varroa* spp. on a representative sample of kept honeybees of the Member State or zone thereof that allows at least for the detection, with a 95 % level of confidence, of the infestation with *Varroa* spp. at a target prevalence rate of 1 % of the apiaries and at a within-apiary target prevalence rate of 5 % of the beehives; (e) in the presence of a wild self-sustaining population of the species of the genus *Apis* there has been in place for at least one year an ongoing surveillance programme in the wild population which demonstrates no evidence of infestation with *Varroa* spp.; and (f) during the whole duration of the surveillance referred to in point (d) the competent authority makes appropriate arrangements for the survey and further handling of honeybees in any stage of their lifecycle, including honeybee brood, which are moved into that Member State or into that zone to prevent the infestation of its population from introduced honeybees of lesser health status.

Section 2 Maintenance of the status of a Member State or zone free from infestation with *Varroa* spp.

The status free from infestation with *Varroa* spp. granted to the relevant honeybee population of a Member State or of a zone may only be maintained if: (a) the competent authority maintains a surveillance that: (i) demonstrates the absence of infestations with *Varroa* spp. annually on a representative sample of kept honeybees of the free area; (ii) enables the early detection of infestation with *Varroa* spp. in apiaries and beehives; (iii) takes into consideration specifically target areas with higher likelihood of introduction of or infestation with *Varroa* spp., based on a risk assessment; (b) all the suspected cases have been investigated and no case of infestation with *Varroa* spp. has been confirmed, either in kept or in wild honeybee colonies; (c) either there is no wild self-sustaining population of the species of the genus *Apis* or there is an ongoing surveillance programme in the wild population which demonstrates no evidence of infestation with *Varroa* spp.; and (d) the honeybees in any stage of their lifecycle, including honeybee brood, are only moved into the free area when: (i) they come from a Member State or zone thereof or from a third country or territory with disease-free status regarding infestation with *Varroa* spp.; and (ii) they are protected from infestation with *Varroa* spp. during transport.

Commission Delegated Regulation (EU) 2019/2122

Commission Delegated Regulation (EU) 2019/2122 of 10 October 2019 (EU 2019b) supplementing Regulation (EU) 2017/625 of the European Parliament and of the Council as regards certain categories of animals and goods exempted from official controls at border control posts, specific controls on passengers' personal luggage and on small consignments of goods sent to natural persons which are not intended to be placed on the market and amending Commission Regulation (EU) No 142/2011.

Article 3 Animals intended for scientific purposes

Paragraph 1. Invertebrates intended for scientific purposes such as research, educational activities or research related to product development activities shall be exempted from official controls at border control posts other than controls carried out in accordance with article 15(2) of Regulation (EU) No 1143/2014 (EU 2014), provided that: (a) they comply with the animal health requirements set out in the rules referred to in point (d) of article 1(2) of Regulation (EU) 2017/625; (b) their entry into the Union is authorised in advance for that purpose by the competent authority of the Member State of destination; (c) when the activities related to the scientific purposes have been carried out, they and products derived from them, with the exception of the quantities used for the scientific purposes, shall be disposed of or re-dispatched to the third country of origin.

Paragraph 1 shall not apply to honey bees (*Apis mellifera*), bumblebees (*Bombus* spp.), molluscs belonging to the phylum Mollusca and crustaceans belonging to the subphylum Crustacea.

Commission Delegated Regulation (EU) 2020/692

Commission Delegated Regulation (EU) 2020/692 of 30 January 2020 (EU 2020c) supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for entry into the Union, and the movement and handling after entry of consignments of certain animals, germinal products and products of animal origin.

The infestation with the small hive beetle is one of the diseases of most concern for honey bees. It is largely exotic to the Union (see Decision 2021/597/EU concerning its occurrence in Calabria region, Italy) (EU 2021a) but has spread globally in recent decades, creating serious problems for the apiculture industry and potentially also affecting bumblebees (Neumann et al. 2016). Tropilaelaps mites (*Tropilaelaps* spp.) are potentially devastating pathogens of honeybees (Khongphinitbunjong et al. 2016). They are also exotic to the Union. Effective and safe treatments against these diseases are at present not available. If these diseases enter the Union by entering consignments, they would pose a risk to the sustainability of the apiculture sector and beyond, potentially affecting agriculture and the environment, which benefits from pollination services by managed and wild bees. American foulbrood (caused by the spore-forming bacterium *Paenibacillus larvae*) occasionally occurs in the EU but is controlled with regard to trade of honeybees, while certain areas in the Union have been recognised as free of Varroa mites and protected by additional trade guarantees to keep places of destination in the Union safe. Rules at EU level have been and remain essential to mitigate the risk of entry into the Union of the above pathogens as associated with consignments of honeybees and bumblebees. Therefore, such rules are laid down in this regulation.

Only queen honeybees without a brood and accompanied by a small number of attendants in single queen cages can be easily checked for infestation with small hive beetle or with Tropilaelaps mites, therefore the entry into the Union of honeybees should be limited to such consignments.

Colonies of bumblebees bred and reared in environmentally isolated establishments are often traded for the horticultural industry. Given the commonly used facilities, procedures and closed containers used for the shipped colonies, the entry into the Union of bumblebees (*Bombus* spp.) should be permitted only for colonies that are bred, reared and packaged solely under environmentally controlled conditions in establishments and which can be checked to ensure that they are free of the small hive beetle.

Article 1

Part II lays down the general animal health requirements for entry into the Union, as well as the movement and handling after the entry, and derogations from such requirements for certain terrestrial animals (Title 1). In addition, it lays down specific animal health requirements that are also applicable to the each of those species and categories of terrestrial animals, in particular: (c) honeybees (*Apis mellifera*) and bumblebees (*Bombus* spp.) (Title 4).

PART II

ANIMAL HEALTH REQUIREMENTS FOR ENTRY INTO THE UNION OF KEPT TERRESTRIAL ANIMALS AS REFERRED TO IN ARTICLES 3 (Obligations of the competent authorities of Member States) AND 5 (Obligations of operators).

TITLE 1 GENERAL ANIMAL HEALTH REQUIREMENTS FOR KEPT TERRESTRIAL ANIMALS

Article 11 The residency period required for kept terrestrial animals.

Consignments of kept terrestrial animals other than dogs, cats and ferrets, shall only be permitted to enter the Union subject to compliance with the following requirements: (a) the animals complied with the relevant residency period set out in the following tables of Annex III for a continuous period of time immediately prior to the date of dispatch to the Union: (i) Table 1 in the case of honeybees and bumblebees; (b) the animals: (i) remained continuously in the third country or territory of origin or zone thereof during the period indicated in the second column of Table 1 in Annex III; (ii) remained continuously in the establishment of origin, and no animals were introduced into that establishment during the period indicated in the third column of Table 1 in Annex III; (iii) had no contact with animals of a lower health status during the period indicated in the fourth column of Table 1 in Annex III.

Article 15 Derogation for the transhipment of terrestrial animals other than equine animals in non-listed third countries or territories in the event of a technical problem or another unforeseen incident.

1. By way of derogation from article 14(2), the competent authority shall authorise the entry into the Union of consignments of terrestrial animals, other than equine animals, which have been transhipped from the original means of transport of dispatch into another means of transport for onward travel in a third country or territory or zone thereof which is not a listed third country or territory or zone thereof for entry of the particular species and category of animals into the Union, only if the transhipment operation took place because of the occurrence of a technical problem or another unforeseen incident causing logistic problems during the transport of the animals to the Union by sea or by air, in order to complete the transport to the point of entry into Union.

2. The derogation provided for in paragraph 1 shall not apply to consignments of honeybees and bumblebees.

Article 17 General requirements regarding means of transport of terrestrial animals.

2. Paragraph 1 shall not apply to the transport of consignments of honeybees and bumblebees intended for entry into the Union.

TITLE 4 ANIMAL HEALTH REQUIREMENTS FOR HONEYBEES AND BUMBLEBEES (p. 46), CHAPTER 1 General animal health requirements for honeybees and bumblebees

Article 63 Authorised categories of bees

Only consignments of the following categories of bees shall be permitted to enter the Union: (a) queen honeybees; (b) bumblebees.

Article 64 Dispatch to the Union of honeybees and bumblebees

Consignments of queen honeybees and bumblebees shall only be permitted to enter the Union if they comply with the following requirements: (a) the packaging material and queen cages used to dispatch the honeybees and bumblebees into the Union must: (i) be new; (ii) not have been in contact with any bees and brood combs; (iii) have been subject to all precautions to prevent their contamination with pathogens causing diseases of honeybees or bumblebees; (b) the feedingstuff accompanying the honeybees and bumblebees must be free from pathogens causing their diseases; (c) the packaging material and accompanying products must have undergone a visual examination prior to dispatch to the Union to ensure that they do not pose an animal health risk and do not contain: (i) in the case of honeybees, *A. tumida* (Small hive beetle) and Tropilaelaps mite in any of their life stages; (ii) in the case of bumblebees, *A. tumida* (Small hive beetle), in any of their life stages.

CHAPTER 2 Specific animal health requirements for queen honeybees

Article 65 The apiary of origin of queen honeybees

Consignments of queen honeybees shall only be permitted to enter the Union if the honeybees of the consignment originate from an apiary which is situated in an area: (a) of at least a 100 km radius, including where appropriate the territory of a neighbouring third country: (i) where infestation with *A. tumida* (Small hive beetle) or infestation with *Tropilaelaps* spp. has not been reported; (ii) there are no restrictions in place due to a suspicion, case or outbreak of the diseases referred to in (i); (b) of at least 3 km radius, including where appropriate the territory of a neighbouring third country: (i) American foulbrood has not been reported for a period of at least 30 days prior to the date of loading for dispatch to the Union; (ii) there are no restrictions in place due to a suspicion or a confirmed case of American foulbrood during the period referred to in point (i); (iii) where there had been a previous confirmed case of American foulbrood before the period referred to in point (i), all hives were subsequently checked by the competent authority in the third country or territory of origin and all infected hives were treated and subsequently inspected with favourable results within a period of 30 days from the date of last recorded case of that disease.

Article 66 The hive of origin of queen honeybees

Consignments of queen honeybees shall only be permitted to enter the Union if the honeybees of the consignment originate from hives from which samples of the comb have been tested for American foulbrood with negative results within the period of 30 days prior to the date of loading for dispatch to the Union.

Article 67 The consignment of queen honeybees

Consignments of queen honeybees shall only be permitted to enter the Union if such consignments are in closed cages, each containing one single queen honeybee with a maximum of 20 accompanying attendants.

Article 68 Additional guarantees for queen honeybees destined to certain Member States or zone as regards the infestation with *Varroa* spp. (Varroosis)

Consignments of queen honeybees destined to a Member State or zone with disease-free status for infestation with *Varroa* spp. (Varroosis) shall only be permitted to enter the Union if such consignments comply with the following requirements: (a) the honeybees of the consignment must originate from a third country or territory or zone thereof free from infestation with infestation with *Varroa* spp. (Varroosis); (b) in the third country or territory of origin or zone thereof, infestation with *Varroa* spp. (Varroosis) has not been reported for a period of 30 days prior to the date of loading for dispatch to the Union; (c) every precaution has been taken to avoid contamination of the consignment with *Varroa* spp. during loading and dispatch to the Union.

CHAPTER 3 Specific animal health requirements for bumblebees

Article 69 The establishment of origin of bumblebees

Consignments of bumblebees shall only be permitted to enter the Union if the bumblebees of the consignment: (a) have been bred and kept in an environmentally isolated bumblebee production establishment which: (i) has facilities which ensure that the production of bumblebees is carried out inside of a flying insect-proof building; (ii) has facilities and equipment which ensure that the bumblebees are further isolated in separate epidemiological units and each colony in closed containers within the building throughout the whole production; (iii) the storage and handling of pollen within the facilities is isolated from the bumblebees throughout the whole production of bumblebees until it is fed to them; (iv) has standard operating procedures to prevent the entry of small hive beetle into the establishment and to regularly survey for the presence of small hive beetle within the establishment; (b) within the establishment referred to in point (a), the bumblebees must come from an epidemiological unit in which infestation with *A. tumida* (Small hive beetle) has not been detected.

Article 70 The consignment of bumblebees

Consignments of bumblebees shall only be permitted to enter the Union if such consignments have been dispatched to the Union in closed containers, each containing a colony of a maximum of 200 adult bumblebees, with or without a queen.

CHAPTER 4 Specific animal health requirements for handling after the entry of queen honeybees and bumblebees.

Article 71 Handling after the entry of queen honeybees and bumblebees

1. Following their entry into the Union, queen honeybees must not be introduced in local colonies unless they are transferred from the transport cage to new cages in accordance with paragraph 2 with the permission and, as appropriate, under the direct supervision of the competent authority.

2. Following the transfer in new cages as referred to in paragraph 1, the transport cages, attendants, and other material that accompanied the queen honeybees from the third country of origin must be submitted to an official laboratory for examination to rule out the presence of *A. tumida* (Small hive beetle), including eggs and larvae, and any signs of the Tropilaelaps mite.

3. Operators receiving bumblebees shall destroy the container and the packaging material that accompanied them from the third country or territory of origin but they may keep them in the container in which they entered into the Union until the end of the lifespan of the colony.

Article 72 Specific obligations for the competent authorities in the Member States

The competent authority of the Member State of the place of destination of consignments of honeybees or bumblebees shall: (a) supervise the transfer from the transport cage to the new cages referred to in article 71(1); (b) ensure the submission by the operator of the materials referred to in article 71(2); (c) ensure that the official laboratory referred to in article 71(2) have arrangements in place to destroy the cages, attendants and the material after the laboratory examination provided for in that provision.

Requirements as regards the residency periods for honeybees and bumblebees before their entry into the Union (ANNEX III) are given in table 3.

Table 3. Residency periods for honeybees and bumblebees before their entry into the Union.

Species and category of animals	Minimum residency period in the third country or territory of origin or zone thereof, as referred to article 11(b)(i)	Minimum residency period in the establishment of origin, as referred to in article 11(b)(ii)	Minimum period without contact with animals of a lower health status as referred to in article 11(b)(iii)
Honeybees and bumblebees	Since hatching	Since hatching	Since hatching

Commission Implementing Regulation (EU) 2021/403 of 24 March 2021 (EU 2021b) laying down rules for the application of Regulations (EU) 2016/429 and (EU) 2017/625 of the European Parliament and of the Council as regards model animal health certificates and model animal health/official certificates, for the entry into the Union and movements between Member States of consignments of certain categories of terrestrial animals and germinal products thereof, official certification regarding such certificates and repealing Decision 2010/470/EU.

Article 1. Subject matter and scope

This regulation lays down rules regarding animal health certificates provided for in Regulation (EU) 2016/429 and animal health/official certificates based on Regulation (EU) 2016/429 and on Regulation (EU) 2017/625 and as regards the issuance and replacement of those certificates required for the entry into the Union, movements within the Union and between Member States of certain consignments of terrestrial animals and germinal products thereof.

Article 2. Definitions

(20) 'honeybee' means an animal as defined in point (20) of article 2 of Delegated Regulation (EU) 2020/692 (= an animal of the *Apis mellifera* species); (21) 'bumblebee' means an animal as defined in point (21) of article 2 of Delegated Regulation (EU) 2020/692 (= an animal of the species belonging to the genus *Bombus*).

Article 12 Model animal health certificates for the movements between Member States of certain categories of bees.

The animal health certificates referred to in article 1(2)(a) to be used for movements between Member States of certain categories of bees shall correspond to one of the following models, depending on the species concerned: (a) HBEE-INTRA drawn up in accordance with the model set out in Chapter 55 of Annex I, for honeybees; (b) QUE-INTRA drawn up in accordance with the model set out in Chapter 56 of Annex I, for queen honeybees under derogation; (c) BBEE-INTRA drawn up in accordance with the model set out in Chapter 57 of Annex I, for bumblebees.

Article 18 Model animal health certificates for the entry into the Union of certain categories of bees.

The animal health certificates referred to in article 1(2)(b) to be used for the entry into the Union of certain categories of bees shall correspond to one of the following models, depending on the species concerned: (a) QUE drawn up in accordance with the model set out in Chapter 36 of Annex II, for queen honeybees; (b) BBEE drawn up in accordance with the model set out in Chapter 37 of Annex II, for bumblebees. ANNEX I contains the model animal health certificates and animal health/official certificates for the movement between Member States (Table 4).

Table 4. Model animal health certificates and animal health/official certificates for the movement between Member States.

Bees	
HBEE-INTRA	Chapter 55: Model animal health certificate for the movement between Member States of honeybees (p. 367-370)
QUE-INTRA	Chapter 56: Model animal health certificate for the movement between Member States of queen honeybees under derogation (p. 371-374)
BBEE-INTRA	Chapter 57: Model animal health certificate for the movement between Member States of bumblebees (p. 375-377)

ANNEX II contains the following model animal health certificates and animal health/official certificates and declarations for entry into the Union and transit through the Union (Table 5).

Table 5. Model animal health certificates and animal health/official certificates for the movement between Member States.

Bees	
QUE	Chapter 36: Model animal health certificate for entry into the Union of queen honeybees (p. 709-713)
BBEE	Chapter 37: Model animal health certificate for entry into the Union of bumblebees (p. 714-717)

Commission Implementing Regulation (EU) 2021/620
Commission Implementing Regulation (EU) 2021/620 of 15 April 2021 laying down rules for the application of Regulation (EU) 2016/429 of the European Parliament and of the Council as regards the approval of the disease-free and non-vaccination status of certain Member States or zones or compartments thereof as regards certain listed diseases and the approval of eradication programmes for those listed diseases (EU 2021c).

Article 10 Infestation with *Varroa* spp.
The Member States or zones thereof with disease-free status from infestation with *Varroa* spp. shall be listed in Annex IX (Table 6).

Table 6. Member States or zones thereof with disease-free status from infestation with *Varroa* spp.

Member State	Territory
Portugal	Island of Corvo Island of Graciosa Island of São Jorge Island of Santa Maria Island of São Miguel Island of Terceira

Finland	Åland islands

Commission Implementing Regulation (EU) 2021/404

Commission Implementing Regulation (EU) 2021/404 of 24 March 2021 (EU 2021d) laying down the lists of third countries, territories or zones thereof from which the entry into the Union of animals, germinal products and products of animal origin is permitted in accordance with Regulation (EU) 2016/429 of the European Parliament and the Council.

Article 3 lists third countries, territories or zones or compartments thereof from which the entry into the Union of animals, germinal products and products of animal origin shall be permitted: (f) Annex VII for queen honeybees and bumblebees.

ANNEX VII, QUEEN HONEYBEES AND BUMBLEBEES, PART 1

List of third countries, territories or zones thereof authorised for the entry into the Union of consignments of queen honeybees and bumblebees as referred to in point (1)(f) of article 3 (p. 45-46) are given in Table 7.

Table 7. List of third countries, territories or zones thereof authorised for the entry into the Union of consignments of queen honeybees and bumblebees.

ISO code and name of the third country or territory	Code of the zone as set out in Part 2	Categories permitted to enter the Union	Animal health certificates	Specific conditions as set out in Part 3	Animal health guarantees as set out in Part 4	Closing date	Opening date
1	2	3	4	5	6	7	8
AR Argentina	AR-0	Queen honeybees and bumblebees	QUE, BBEE				
AU Australia	AU-0	Queen honeybees and bumblebees	QUE, BBEE				
CA Canada	CA-0	Queen honeybees and bumblebees	QUE, BBEE				
CH Switzerland	CH-0	Subject to the Agreement referred to in point 7 of Annex I					
CL Chile	CL-0	Queen honeybees and bumblebees	QUE, BBEE				
CR Costa Rica	CR-0	Queen honeybees and bumblebees	QUE, BBEE				
IL Israel	IL-0	Queen honeybees and bumblebees	QUE, BBEE				

KE Kenya	KE-0	Queen honeybees and bumblebees	QUE, BBEE
MA Morocco	MA-0	Queen honeybees and bumblebees	QUE, BBEE
MK Republic of North Macedonia	MK-0	Queen honeybees and bumblebees	QUE, BBEE
MX Mexico	MX-0	Bumblebees	BBEE
NC New Caledonia	NC-0	Queen honeybees and bumblebees	QUE, BBEE
NZ New Zealand	NZ-0	Queen honeybees and bumblebees	QUE, BBEE
RS Serbia	RS-0	Queen honeybees and bumblebees	QUE, BBEE
RU Russia	RU-0	Queen honeybees and bumblebees	QUE, BBEE
TR Turkey	TR-0	Queen honeybees and bumblebees	QUE, BBEE
UA Ukraine	UA-0	Queen honeybees and bumblebees	QUE, BBEE
US United States	US-0	Bumblebees	BBEE
ZA South Africa	ZA-0	Queen honeybees and bumblebees	QUE, BBEE

(7) The animal health certification requirements for Switzerland are subject to the Agreement between the European Community and the Swiss Confederation on Trade in Agricultural Products, approved by Decision 2002/309/EC, Euratom of the Council, and of the Commission as regards the Agreement on Scientific and Technological Cooperation of 4 April 2002 on the conclusion of seven Agreements with the Swiss Federation (OJ L 114, 30.4.2002, p. 1).

PART 2 Descriptions of zones of third countries or territories referred to in column 2 of the table set out in Part 1: None

PART 3 Specific conditions referred to in column 5 of the table set out in Part 1: None

PART 4 Animal health guarantees referred to in column 6 of the table set out in Part 1:

VAR	The Union has recognised freedom from infestation with *Varroa* spp. (Varroosis) of the third country, territory or zone in accordance with article 10 of Delegated Regulation (EU) 2020/692

Commission Delegated Regulation (EU) 2019/2035

Commission Delegated Regulation (EU) 2019/2035 of 28 June 2019 (EU 2019b) supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for establishments keeping terrestrial animals and hatcheries, and the traceability of certain kept terrestrial animals and hatching eggs.
In most cases, bumblebees are bred in environmentally isolated establishments subject to high-level biosecurity measures and subject to regular controls by the competent authority and checked for the presence of diseases. When those establishments are recognised by and supervised by the competent authority, they are unlikely to be affected by the presence of small hive beetle, in contrast with open air colonies. This regulation should therefore provide for such establishments to be approved and supervised by the competent authority and lay down requirements for granting such approval.

PART I, Article 1. This regulation supplements the rules laid down in Regulation (EU) 2016/429 as regards:

4. Chapter 3 of Title I of Part II lays down the requirements for the approval of the following types of establishments: (d) environmentally isolated production establishments for bumblebees from which those animals are to be moved to another Member State.

Chapter 1 of Title III of Part II lays down the record-keeping obligations of operators, in addition to those provided for in article 102(1) of Regulation (EU) 2016/429, for the following types of registered or approved establishments: (v) honey bees.

Article 2. For the purposes of this regulation, the following definitions shall apply:

(11) ‘bumblebee’ means an animal of the species belonging to the genus *Bombus*; (13) ‘honeybees’ means animals of the *Apis mellifera* species.

CHAPTER 3 Approval of establishments keeping terrestrial animals

Article 9 Obligation on operators of certain type of establishments keeping terrestrial animals to apply to the competent authority for approval.

Operators of the following types of establishments shall apply to the competent authority for approval in accordance with article 96(1) of Regulation (EU) 2016/429 and shall not commence their activities until their establishment has been approved: (d) environmentally isolated production establishments for bumblebees from which those animals are moved to another Member State.

Article 13 Requirements for granting approval of environmentally isolated production establishments for bumblebees.

When granting approval for environmentally isolated production establishments for bumblebees from which bumblebees are to be moved to another Member State, the competent authority shall ensure that such establishments comply with the following requirements set out in Part 7 of Annex I hereto: (a) point 1, in relation to biosecurity and surveillance measures; (b) point 2, in relation to facilities and equipment.

PART 7 Requirements for granting approval of environmentally isolated production establishments of bumblebees referred to in article 13.

1. The requirements in relation to biosecurity and surveillance measures of environmentally isolated production establishments for bumblebees, as referred to in article 13, shall be the following: (a) the operator

must ensure, verify and record by internal controls that the entry into the establishment of small hive beetles is prevented and their presence within the establishment is detectable.

2. The requirements in relation to facilities and equipment of environmentally isolated production establishments for bumblebees, as referred to in article 13, shall be the following: (a) the production of bumblebees must be isolated from all the associated activities of the establishment and must be conducted in flying insect-proof facilities; (b) the bumblebees must be kept isolated within that building throughout the whole production; (c) the storage and handling of pollen within facilities must be isolated from the bumblebees throughout the whole production of bumblebees until it is fed to them.

Article 27 Record-keeping obligations of operators of establishments keeping honeybees.

Operators of registered establishments keeping honeybees shall record for each apiary the details of temporary transhumance, if any, of the kept beehives, comprising information covering at least the place of each transhumance, its date of start and finish, and the number of the beehives moved.

Commission Implementing Regulation (EU) 2020/2002

Commission Implementing Regulation (EU) 2020/2002 of 7 December 2020 (EU 2020d) laying down rules for the application of Regulation (EU) 2016/429 of the European Parliament and of the Council with regard to Union notification and Union reporting of listed diseases, to formats and procedures for submission and reporting of Union surveillance programmes and of eradication programmes and for application for recognition of disease-free status, and to the computerised information system.

Article 3 Union notification

1. Member States shall notify the Commission and the other Member States within 24 hours of confirmation of any primary outbreak in their territory of a listed disease referred to in: (a) points 1 and 2 of Annex I; (b) point 3 of Annex I, if the primary outbreak has been detected in the relevant targeted animal population in a disease-free Member State or zone; (c) points 4 and 5 of Annex I, if the primary outbreak has been detected in a disease-free Member State, zone or, where relevant, compartment.

2. Member States shall notify to the Commission at the latest on the first working day of each week covering the previous week, from 0.00 on Monday until 24.00 on Sunday, secondary outbreaks in their territory of a listed disease referred to in: (a) points 1 and 2 of Annex I; (b) point 3 of Annex I, if such secondary outbreaks have been detected in the relevant targeted animal population in a disease-free Member State or zone; (c) points 4 and 5 of Annex I, if such secondary outbreaks have been detected in a disease-free Member State, zone or, where relevant, compartment. If no information is received by the Commission, this is considered to mean that no secondary outbreaks have been confirmed during the period referred to in the first subparagraph.

3. The notifications referred to in paragraphs 1 and 2 shall contain the information specified in Annex II and be submitted electronically via the ADIS.

ANNEX I LISTED DISEASES SUBJECT TO UNION NOTIFICATION

1. Listed diseases of terrestrial animals subject to Union notification in accordance with point (a) of article 3(1) and point (a) of article 3(2): infestation with *A. tumida*, infestation with *Tropilaelaps* spp.

4. Listed diseases of terrestrial animals subject to Union notification in accordance with point (c) of article 3(1) and point (c) of article 3(2): infestation with *Varroa* spp.

ANNEX II

INFORMATION TO BE PROVIDED FOR UNION NOTIFICATION ON OUTBREAKS OF LISTED DISEASES IN ACCORDANCE WITH ARTICLE 3(3)

14. Animals involved in the outbreak: (b) Honeybees and bumblebees: (i) number of susceptible colonies; (ii) number of clinically or subclinically infected or infested colonies; (iii) number of dead colonies; (iv) number of destroyed colonies.

4. Discussion and Conclusions

The Regulation 2016/429, also known as Animal Health Law, proposes the adoption of a single and simplified regulatory framework for animal health pursuing convergence with international standards and guaranteeing a firm commitment to high standards of animal health. The aim of this regulation is to establish and consolidate the legal framework for a common Union animal health policy through a single, simplified and flexible regulatory framework for animal health. This regulation poses the basis for the first overarching legal framework, laying down harmonised principles across the sector of animal health. The regulations cited in this article cover intra-Union trade, entry into the Union, certification, disease notification, eradication and surveillance, disease-free status, and veterinary controls, concerning honey bees and bumblebees. The new Animal Health Law of the EU and the correlated regulations issued between 2018 and 2021 with implications for honey bees and bumblebees are extrapolated and condensed for an easy and practical consultation by competent authorities, veterinarians, beekeepers and operators of the sector aiming at facilitating their application and compliance.

5. Glossary

A **Decision:** is binding only on those to whom it is addressed without the need for national implementing measures.

A **Regulation** is a legal act of the European Union that becomes immediately enforceable as law in all member states simultaneously.

A **Directive**, at least in principle, need to be transposed into national law.

A **Delegated act**: the Commission adopts them on the basis of a delegation granted in the text of an EU law.

An **Implementing act**: primary responsibility for implementing EU law lies with EU countries. However, in areas where uniform conditions for implementation are needed (taxation, agriculture, the internal market, health and food safety, etc.), the Commission (or exceptionally the Council) adopts an implementing act.

6. References

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The Use of Biosensors in the Determination of Fenolic Compounds in Honey

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Abstract:

Oxidative damage caused by free radicals can lead to different levels of cell and tissue damage. Antioxidants, especially abundant in plant foods, contribute to the continuity of vital activities against oxidative damage in the body. This shows the importance of nutrition with natural products more clearly. The main sources that enable plants to have all these vital features for humans are phenolic compounds. We aimed to study a tyrosine kinase enzyme-based biosensor that will determine the amount of phenolic compounds in honey, since bees take propolis and flower essence containing abundant phenolic compounds while making honey. A biosensor based on tyrosine kinase enzyme was designed to determine the amount of phenolic compounds in honey. Tyrosine kinase enzyme is a group of enzymes that catalyzes the transfer of the gamma phosphate group of ATP to the phenolic hydroxyl group of tyrosine amino acids in target proteins. In this method, tyrosine kinase enzyme was immobilized to the graphite pencil tip electrode by bovine serum albumin (BSA) and gelatin. The principle of this method is based on the principle of electrobiochemical measurement of the potential change resulting from the ATP group transferring between the molecules with the help of a biosensor. During the nectar collection of bees, the amount of phenolic substances included in honey can range from 5 to 1300 mg/kg. The working range of the electrochemical biosensor used in the determination of phenolic substance was found to be 0.99 to 2710 mg/kg. When people buy honey, they want to make sure that the honey is real. Therefore, it is very important to find methods that are not laboratory dependent to show the quality of honey. Portable fenolic compounds measurement devices can be produced by developing biosensors. Biosensor method can be evaluated as an alternative method for diastase activity determination method.

Keywords: fenolic compounds, honey quality, biosensor

Introduction

Today, sensor technologies have entered every aspect of our lives. There is no one left without a parking sensor in their car. Especially with the Covid pandemic, sensors that measure temperature have become a part of our lives.

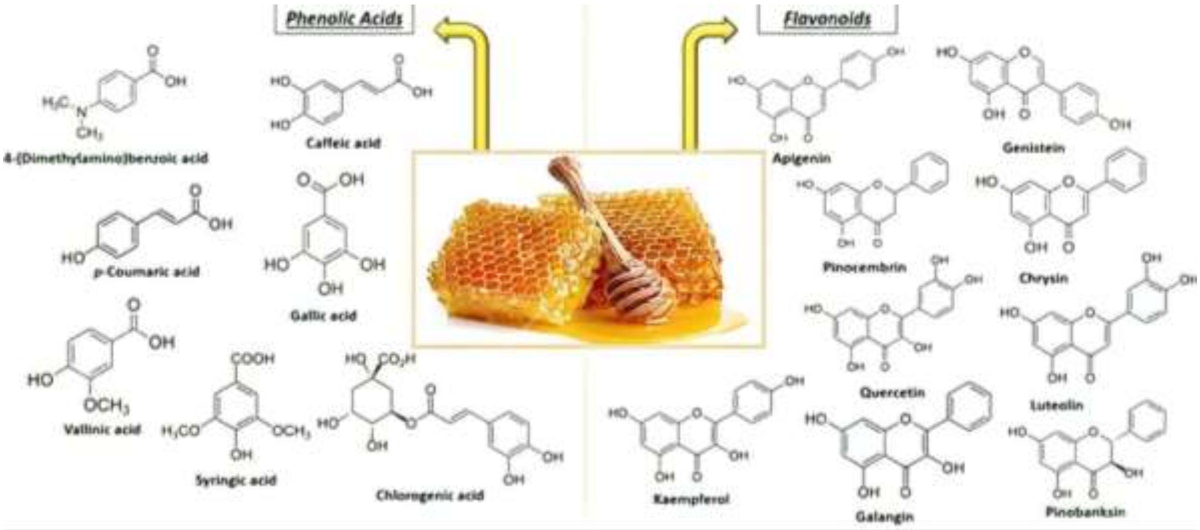
Biosensor technology is a system that combines physical measurement systems with the specificity of biological materials. Biosensors do this by mimicking the natural sensor systems in nature (Kokbas 2013).

Radar technology was developed with inspiration from bats' ability to navigate. The sonar device was also inspired by the whales' ability to find their way underwater. Just as a shark can detect only a drop of blood in a ton of water or The chameleon takes on the color of the object it touches, biosensors function in a similar way (Kokbas 2013).

Since the first periods of history, honey is a natural antioxidant substance known as a source of healing. Phenolic acids and flavonides in honey have high anti-inflammatory and antioxidant effects. Thanks to these properties, honey has taken an indispensable place for human health. The natural antioxidant content of honey shows a protective effect against cancer types. In the literature, it appears to have anticancer activity and antimicrobial capacity against tumors (Cianciosi 2018).

The therapeutic effect of honey is due to the presence of various antioxidant molecules possessed by phenolic compounds such as phenolic acids and flavonoid compounds (Petretto 2015).

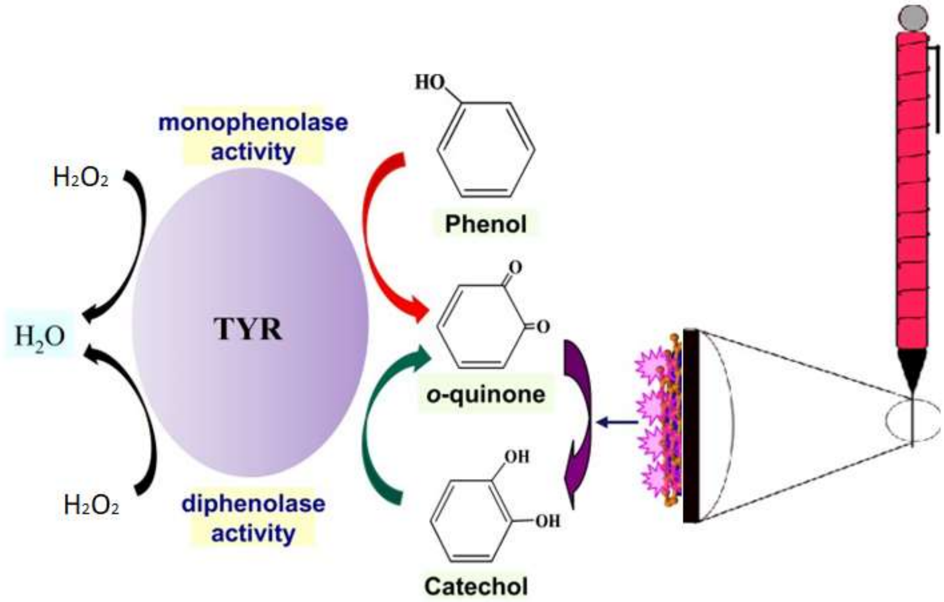
Phenolic compounds; Polyphenols are a heterogeneous class of chemical compounds that can be divided into flavonoids (isoflavones, chalcones, anthocyanidin, flavonols, flavanones, flavanols and flavones,) and non-flavonoids (phenolic acids). These compounds are often complex structures that are the product of plant metabolism. It differs from primary metabolites (simple carbohydrates, amino acids, and chlorophyll) from secondary metabolites. Secondary metabolites have important ecological functions but do not mediate differentiation, transport, respiration and assimilation processes. The phenolic compounds found in honey mainly originate from the flowers. Phenolic compounds commonly found in honey are shown in Figure 1 (Ranneh 2018).



Materials and Methods:

A biosensor based on tyrosine kinase enzyme was designed to determine the amount of phenolic compounds in honey. Tyrosine kinase enzyme is a group of enzymes that catalyzes the transfer of the gamma phosphate group of ATP to the phenolic hydroxyl group of tyrosine amino acids in target proteins.

In this method, tyrosine kinase enzyme was immobilized to the graphite pencil tip electrode by bovine serum albumin (BSA) and gelatin. The principle of this method is based on the principle of electrobiochemical measurement of the potential change resulting from the ATP group transferring between the molecules with the help of a biosensor. Working principle of the biosensor shown in figure 2.



We used the triple electrode system while doing the biosensor study. We measured the potential change with the help of the technique I mentioned in the previous slide. In order not to be affected by the external environment, the studies were carried out in a Faraday cage.

Results

Firstly, a cyclic voltammogram was taken for phenolic substance determination. Quantitative phenolic substance concentration determination was made with the help of the peak points determined in the cyclic voltammogram.

We use that the slope of the calibration curve changes with the change in the amount of enzyme used while preparing the bioactive layer. The optimum enzyme amount for the biosensor was determined as the amount of enzyme with the highest slope of the curve. As the slope of the curve increases, the detection power of the biosensor increases.

As another part of optimization studies, optimum pH value determination studies were carried out. As a result of these studies, it was found that the biosensor gave the best results around pH 5.

Studies were carried out to determine the optimum amount of BSA, one of the bioactive layer components. As a result of these studies, the amount of BSA that the biosensor gave the best results was found.

Discussion

Thanks to the natural antioxidants it contains, honey protects people against oxidative damage. Natural antioxidants in honey are taken from flowers by bees. The content varies according to the type of flower and the environment in which the flower is found.

By means of the designed sensor, the total amount of phenolic substances in honey can be determined. The optimum working conditions of the biosensor prepared for this purpose were determined.

Conclusion

When people buy honey, they want to make sure that the honey is real. Therefore, it is very important to find methods that are not laboratory dependent to show the quality of honey. Portable fenolic compounds measurement devices can be produced by developing biosensors. Biosensor method can be evaluated as an alternative method for diastase activity determination method.

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Artificial Intelligence and Digital Technologies Produced and Developed for the Health and Future of Honey Bees and Bee Colonies

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Abstract:
The number of honey bees in the world's ecosystems is decreasing. Those active in the beekeeping sector may fight the threat more effectively with the assistance of modern technology. Beekeepers are a vital component of a flourishing industry. They provide the hives for rent to farmers who recognize the importance of bees in the pollination process for their crops. Intelligent gadgets that provide beekeepers access to precise information on the condition of their hives are being developed by digital entrepreneurs. These technologies are being developed to decrease beekeeping losses and enhance the health of honey bees. There are now commercially available sensors powered by artificial intelligence and may provide beekeepers with early warnings if there are issues with their hives. This little, internet-connected sensor is tucked away within the beehive ceiling, where it monitors a variety of factors like temperature, humidity, sound, and movement. The information gleaned from the sensor is sent to the cloud, where it undergoes processing and analysis before being relayed to the beekeeper. Beekeepers will be able to control a much larger number of hives with the same number of workers and the same amount of money spent on supplies like feed and treat if they use the gadget. They will also significantly boost the quantity of honey production and pollination inside their businesses. The expansion of the pollination sector has led to an increase in demand for bees, which in turn has encouraged entrepreneurial businesses to focus more of their efforts on bee technologies. The field of computational intelligence known as swarm intelligence (SI) examines the collective behavior that arises inside self-organizing communities of individuals. This study highlights the significance of intelligent technologies for honey bees throughout the world, and technologies developed specifically for bees, particularly artificial intelligence, are investigated. After all, bees also play a significant role in the business world and marketing.

Keywords: Artificial intelligence, honey bees, swarm intelligence, bee technologies, marketing, fuzzy logic, artificial bee colony.

1. Introduction

The honey bee is a social insect, meaning it is an insect that lives in a community with other bees. Before we start thinking about how such an intricate organization as a honey bee society came to be, we must have a good understanding of what we mean when we say "insect society." This is because it is desired that we should be pretty clear about what we mean by an insect society (Butler, 1954). The honey bee swarm is a particularly fascinating example of a natural swarm because it dynamically divides up work and modifies itself in response to changes in its surroundings through collective intelligence. Honey bees have photographic memories, space-age sensory and navigation systems, possibly even insight skills, and a group decision-making process when selecting new nest sites, and they perform tasks such as tending to the queen and the brood, storing, retrieving, and distributing honey and pollen, communication, and foraging. Honey bees also can communicate with one another and forage for food. Researchers have been motivated to attempt to simulate the cognitive actions of bees due to these qualities. Following is an explanation of the colony's behavior, which comes before the presentation of the algorithms that are presented to employ intelligent behaviors and their applications (Karaboga and Akay, 2009).

The bees are a species of social insect that lives in colonies. Three different kinds of bees live together in a colony: workers, drones, and the queen. Queen bees can survive for several years. She is the only female in the colony that lays eggs, and she is the mother of all the other group members. Furthermore, drones are male bees, and as such, they are the "fathers" of the colony. Workers are responsible for the collection and storage of food, as well as the removal of trash and dead bees, as well as the ventilation and protection of the hive (Karaboga and Akay, 2009). Since ancient times, people in Egypt, Greece, and China have employed goods made by honey bees, such as honey, bee pollen, bee bread, propolis, royal jelly, beeswax, and bee venom, for medicinal purposes. Even though modern medicine has come a long way, there is still a place in clinical practice for bee products because they can act as anti-inflammatory, anti-oxidant, and neuroprotective agents (El-Seedi, 2020).

The pollination services provided by honey bees in the United States are considered among the most comprehensive marketplaces for pollination services elsewhere in the world. These marketplaces play crucial roles in organizing the activity of migratory beekeepers, who produce honey while also providing alternatives to the ecosystem pollination services that honey usually provides (Rucker et al., 2012). When marketing channel selections compared beekeeper characteristics, it was revealed that the elements crucial in choosing a marketing channel were the breeder's education status, income other than beekeeping, the status of receiving assistance, payment method, satisfaction with the marketing channel, method of calculating the price, the source of information, and credit utilization status (Kaygısız, 2022).

The research literature on honey bees has been combed through, and just a handful of papers on honey bee technology have been discovered. Kernbach et al. (2009) explained the re-embodiment of the biological aggregation behavior of honey bees using Jasmine micro-robots. In the context of the insect's sensor–actor system, the observed insect behavior is formalized as behavioral and motion-sensing meta-models. These models are used to explain the behavior. Using a method known as sensor virtualization, these meta-models are reimagined as a sensor–actor system comprised of microrobots. Jasmine micro-robot is a public open-hardware development with the purpose of building a simple and cost-effective micro-robotic platform as well as allowing information sharing within the swarm-robotics community (Jasmine, 2022).
Bassford and Painter (2016) described constructing a fuzzy logic approach that incorporates input from non-invasive beehive monitoring systems by merging data from specialized sensors and other diverse sources. In addition, they said that their methodology utilizes information from non-invasive beehive monitoring systems. A fuzzy logic system and an adaptive neuro-fuzzy inference system are being investigated as potential applications of fuzzy logic within the beehive setting. Jacob and Darney (2021) used artificial bee colony

optimization algorithm for enhancing routing in wireless networks. Their method consists of three steps: the first is the creation of a model that is specific to the wireless environment; the second is the improvement of routing through the careful selection of performance indicators, and the third step is performance optimization using the appropriate tools. The communication in wireless networks is improved thanks to an algorithm that optimizes artificial bee colonies and the evaluative aspects of such algorithms. The straightforward actions of the bee agents in this algorithm provide support for making simultaneous and decentralized routing decisions. The results of the MATLAB simulations make it easy to see the benefits of using this technique.

2. Artificial Bee Colony and Digital Technologies for Honey bees and Bee Colonies

Honey bees are essential to a wide variety of human pursuits, including agriculture, food production, medicine, and many other fields. Pollination is the most crucial role that bees play in the world. Pollinating insects like bees and other insects are on the verge of extinction, mainly because of intensive agriculture, the use of pesticides, the destruction of habitat, pollution, and climate change. The alarming decrease in the bee population poses a risk to agriculture and the general well-being of humans (Ammar et al., 2019). By utilizing the internet of things (IoT) and artificial intelligence, the Makers' Beehives project aims to create a cutting-edge beekeeping system that can autonomously analyze the health of honey bee colonies and the level of danger they face (Ammar et al., 2019). Murphy et al. (2015) used the technology known as Wireless Sensor Network (WSN) to monitor a beehive colony and collect critical information about activity and environment within a beehive as well as the region that surrounds the beehive. WSN is a new technology that is becoming increasingly popular and is essential to the innovative concept of the Internet of Things (IoT). To better understand how to monitor honey bees' health and behavior remotely, this effort used preexisting, commercially accessible WSN platforms and custom-designed systems in an innovative application to monitor honey bee health and activity.

It is common knowledge that honey bees have a beneficial impact on both the natural world and human life. As a result, the maintenance of honey bee populations is essential not only from an ecological standpoint but also for the socio-economic growth of rural communities. Intelligent sensor systems are currently being developed for real-time and long-term measurement of relevant parameters related to beehive conditions. These parameters include the weight of the hive, sounds emitted by the bees, temperature, humidity, and CO_2 levels inside the beehive, as well as the weather conditions inside and outside the beehive (Cecchi et al., 2020). Nearly a century ago, several technologies were combined to create electrical bee counters, which have been used for almost as long to monitor the foraging behavior of honey bee colonies. These counters should permit remote monitoring of the hives without interrupting the normal flying behavior of the bees while simultaneously collecting accurate scientific data. On the other hand, the market only offers a limited number of counters capable of performing this function (Odemer, 2022).

Karaboga (2005) has recently defined several new algorithms, one of which is called Artificial Bee Colony (ABC). In his work, specific intelligent behavior of a honey bee swarm called foraging behavior is considered. Additionally, a new artificial bee colony (ABC) algorithm that simulates this behavior of natural honey bees is described to resolve multidimensional and multimodal optimization issues. The simulation depicts the artificial bee colony as having three distinct types of bees: those who are employed, those that are bystanders, and those that are scouts. The fake bees used in the experiment make up the first half of the colony, while the bystanders make up the second half. There is just one worker bee assigned to each potential food source. To put it another way, the quantity of foraging bees is proportional to the number of potential food sources in the area surrounding the hive. The worker bee whose food supply is used up by the rest of the colony is promoted to the scout role.

ABC is a straightforward stochastic method that is simple to use, requires a small number of control parameters, and is easily adaptable and combinable with various metaheuristic algorithms. Because of the effectiveness of its implementation, several academics working in the fields of optimization and artificial intelligence have chosen to center their work on it as the primary focus of their investigation. Since 2005, several connected results have been published to improve the standard ABC's performance in the body of published work and to address the difficulties of new research problems that have been faced (Khader et al. 2013).

Researchers have created a variety of algorithms by mimicking the behavior of diverse swarms of animals and insects, such as ants, termites, bees, birds, and fishes. Swarm intelligence is a developing subfield within the study of optimization. The researchers have been particularly motivated to create new algorithms during the past ten years by the intelligence actions of bee swarms (Karaboga and Akay, 2009).

3. Conclusion

The rise of the pollination sector has led to an increase in demand for bees, which has prompted entrepreneurial firms to spend more of their efforts on bee technology. This research emphasizes the relevance of intelligent technologies for honey bees worldwide and investigates technologies that have been created expressly for bees, notably artificial intelligence. The artificial bee colony algorithm, which was inspired by Karaboga (2005) artificial bee colony search and published in the literature, was mentioned. Innovative apps that monitor honey bee health and activity were examined to understand better how to monitor honey bees' health and behavior remotely. It has been established that the literature contains a minimal number of technical research on honey bees. In this area, there is a need for more academic research, which might be supported by implementing digital technologies like artificial intelligence, machine learning and fuzzy logic. Consequently, investments must be made in the digital technologies available in this sector for bees, honey bees, bee colonies, hives, and beekeepers. In this manner, positive outcomes will be achieved for the future and health of bees, and the groundwork will be laid for developing new inventions in the field of economics and marketing.

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Natural Bee Products and Apitherapy for Cancer Prevention and Treatment

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Abstract

Cancer is a global health problem with a high incidence and mortality rate around the world, and therefore the global cancer burden is increasing significantly every year. About 12 million people are diagnosed with cancer each year, 7 million patients die from cancer, and 25 million people worldwide currently live with a cancer diagnosis. As a result of preventive medicine practices, early diagnosis of cancer, and treatment of cancer with appropriate treatment strategies, cancer survival has increased and cancer-related death rates have decreased globally. In the field of oncology, there are many treatment options from conventional medicine as well as complementary and alternative medicine (CAM) and more recently integrative medicine. One of the many complementary and alternative methods in oncology is apitherapy. Apitherapy is a field of CAM that focuses on treatment options using multifarious products obtained from the beehive, particularly honey, propolis, pollen, apilarnil, royal jelly and bee venom. With the support of scientific research on apitherapy, the interest in bees and bee products, known since ancient times and used as a source of healing by the public, is increasing day by day. Evidence concerning of cancer prevention potentials of bee products is mainly based on in-vitro studies and animal trials. A recent research on the anticancer properties of honey reveals that it consists of antioxidant components as well as phenolic acids and flavonoids that can be used to prevent cancer. Although there are some in vitro evidences that propolis may be effective in preventing cancer, especially with regard to colon cancer, studies are still ongoing to determine whether propolis is also clinically effective. The flavonoids found in the pollen, especially kaempferol, have been shown to have anticarcinogenic properties. In addition, clinical studies on propolis have revealed that propolis-containing drugs can reverse cervical dysplasia when administered intravaginally. As demonstrated by various studies, bee products may have different mechanisms of action that could be useful for cancer treatment. However, clinical trials are necessary before bee products can be recommended as supportive and complementary applications in the treatment of cancer. In this respect, the purpose of this review is to provide an overview of holistic apitherapy modalities based on cancer clinical findings, studies, trials and treatment concepts.

Keywords: Apitherapy, bee products, cancer, complementary therapy, oncology

1. Introduction

Cancer is a disease in which the cells grow uncontrollably and the cells spread to surrounding tissues. Cancer is a major health problem that effects the people globally. Along with heart disease, it is the second leading cause of death (Figure 1). Approximately 12 million people are diagnosed with cancer, 7 million patients die of cancer each year, and 25 million people are currently living with a cancer diagnosis around the world. As the average age of the population increases, so does the global burden of cancer. Most important in terms of how to improve the situation are efforts to prevent primary and secondary cancer, as well as to detect early, treat, and improve cancer survival. As a result of recent treatment approaches such as targeted therapy and immunotherapy, cancer mortality rates have declined worldwide, especially for cervix uteri, stomach, prostate, colon, and breast cancer (WHO, 2013; Gezici and Sekeroglu, 2019; Siegel et al., 2020).

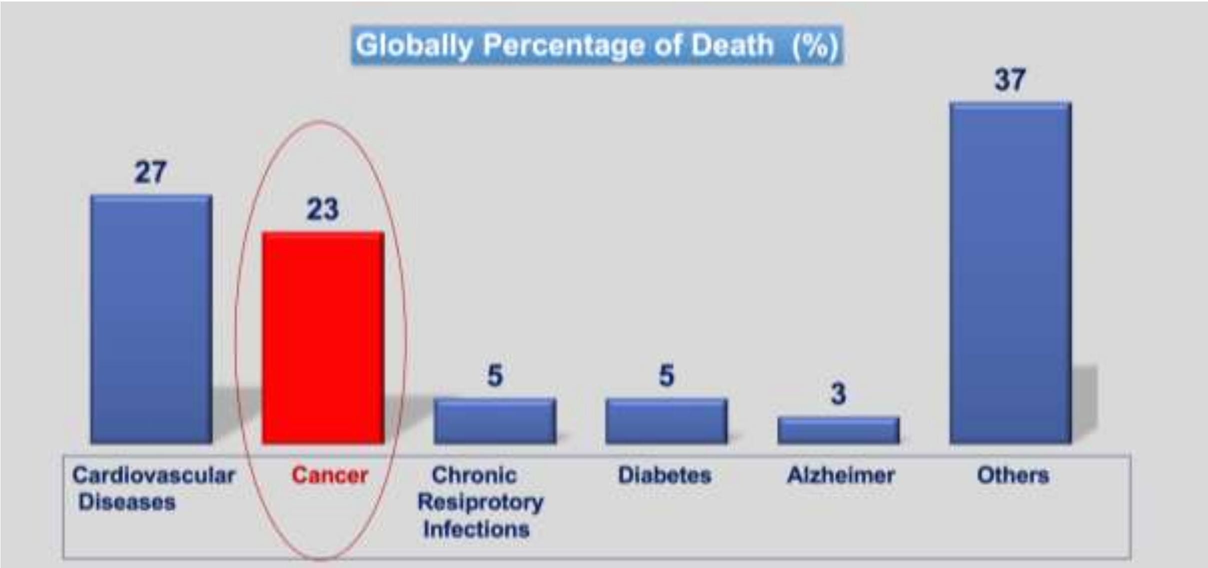

Figure 1. Global percentage of death from cancer (Siegel et al., 2020)

In recent years, cancer patients with higher education and income who have used complementary medicine before their disease are more likely to use complementary medicine for oncologic problems. Their reasons for using it are mainly to influence their cancer, improve their overall health, and treat complications of cancer or therapy. In general, the use of complementary medicine in oncology appears to have a negative impact on survival, which may be attributed to the fact that patients who chose complementary medicine were more likely to reject components of conventional treatment. However, it seems possible that complementary medicine improves outcomes by helping patients tolerate conventional medical care and complete recommended therapy (Hellner et al., 2008; Zhu and Wongsiri, 2008).

Even though, outright rejection of conventional medicine and use of alternative medicine for curable cancer is associated with a higher risk of death. Among the multitude of complementary and alternative methods in oncology, apitherapy is one that has been little discussed. Apitherapy encompasses a field of complementary and alternative medicine (CAM) that focuses on treatment options using various products from the beehive, specifically honey, pollen, propolis, royal jelly, and bee venom. Apitherapy (Apis is a Latin word meaning bee) is the practice of using bee products such as honey, pollen, propolis, royal jelly and bee venom to prevent or treat disease. It can also be described as "the science (and art) of using bee products to maintain health and to help individuals regain health when illness or accident intervenes." (Kuropatnicki et al., 2013; Trumbeckaite et al., 2015). In this review, it is aimed to evaluate the recommendations of the protagonists of holistic apitherapy in relation to various issues related to cancer based on preclinical and clinical findings, studies, trials and treatment concepts.

2. Cancer and Current Status

Cancer is a multifactorial disease that characterized by disorder of cell growth that results in invasion and destruction of surrounding healthy tissue by abnormal cells that have arisen from normal cells and whose nature is permanently altered. It can develop almost anywhere in human body that is reach up trillions of cells. The exact mechanism by which a normal cell is transformed into a tumor cell is still not clear and is considered to be very complex, involving several steps, although considerable advances in knowledge have been made in recent years regarding molecular mechanisms, growth regulatory factors, environmental influences, diet and nutritional habits, etc. (Weinberg, 1996; Martínez-Reyes and Chandel, 2021).

Cancer cells have some different characteristics, comparing the non-cancerous cells. The main characteristics of cancer cells are summarized below (Hanadan, 2022).

- They grow without signals, while normal cells grow only when they receive such signals.
- They ignore death signals that is necessary to stop dividing, as well as escapes the process known as programmed cell death or apoptosis.
- They invade nearby tissues and spread to other parts of the body, whilst normal cells do not migrate throughout the body.
- They cause blood vessels to grow toward the tumor, known as angiogenesis process. Thanks to new blood vessels, the cancer cells supply oxygen and nutrients from the other tissues and also remove waste products from the tumor.
- They escape the control of the immune system, when the immune system normally eliminates damaged or abnormal cells.
- They have chromosomal structural and numerical abnormalities, because of accumulating multiple changes in their chromosomes, including duplications and deletions of chromosome segments.
- They are dependent on different types of nutrients than normal cells, and they also make energy from nutrients in a different way, that is caused to rapidly grow in cancer cells

Cancer is still considered the most advanced and dreadful disease globally. It is a major social and economic problem worldwide, with an estimated almost 19 million new cases registered and about 10 million deaths from cancer in 2020, worldwide. Estimated new cancer cases and deaths from cancer depending on the cancer types are presented in the Figure 2(a) and (b), respectively (GLOBOCAN, 2020, Siegel et al, 2020)

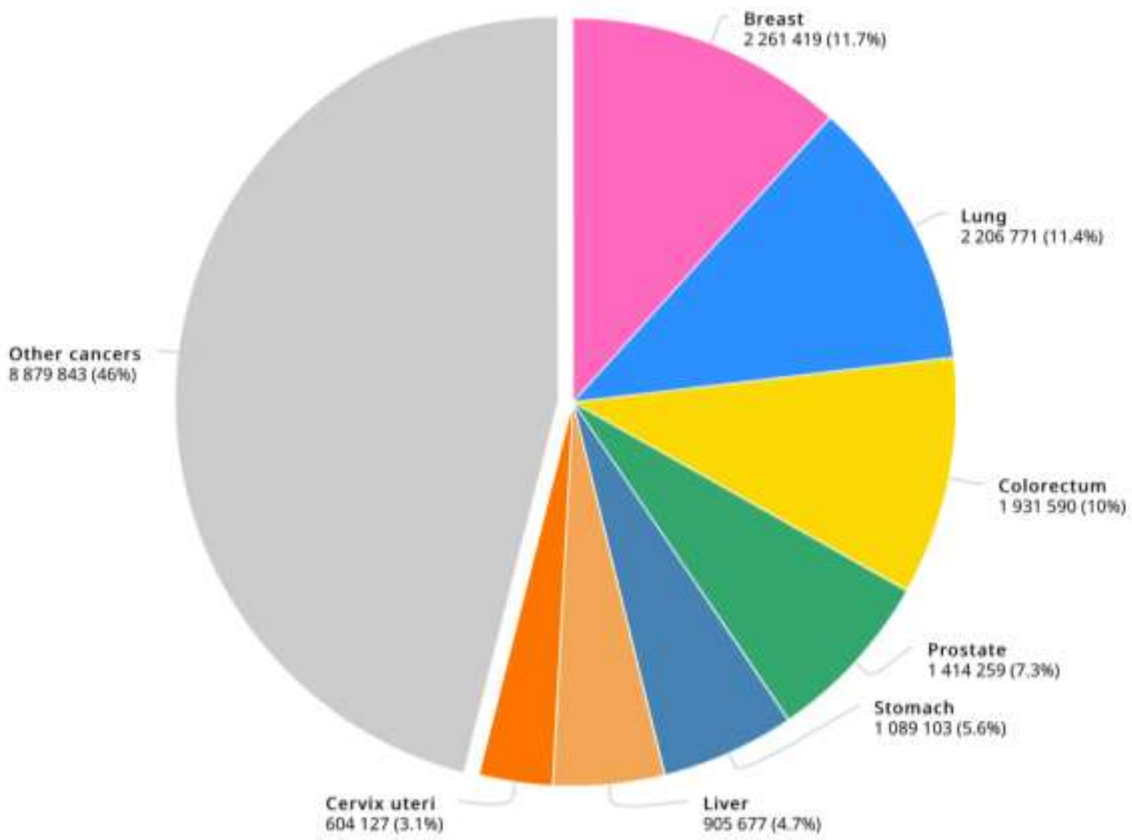

Figure 2. (a) Estimated number of new cancer cases in 2020

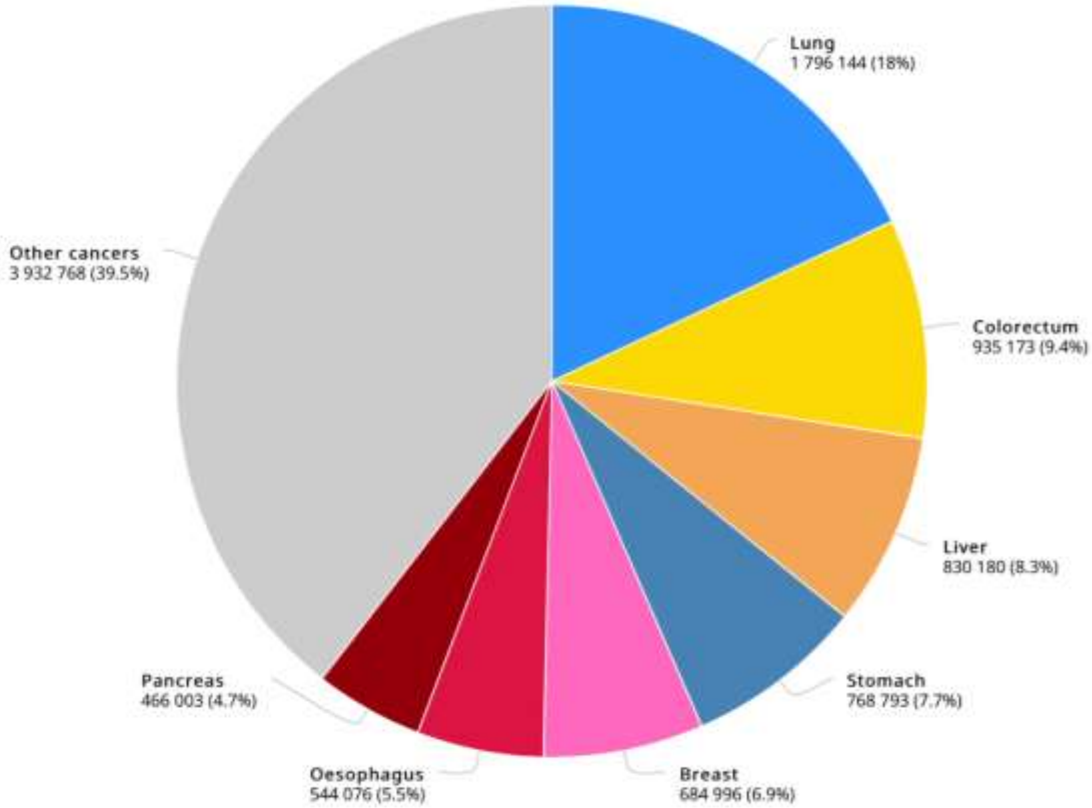

Figure 2. (b) Estimated number of deaths from cancer in 2020

Global burden of cancer using the GLOBOCAN 2020 estimates by country, world region, and Human Development Index (HDI) levels, as well as the predicted future burden by the year 2040 by HDI. The incidence of cancer is predicted almost 29.5 million by the year 2040 (GLOBOCAN, 2020; Sung et al., 2021). (Figure 3)

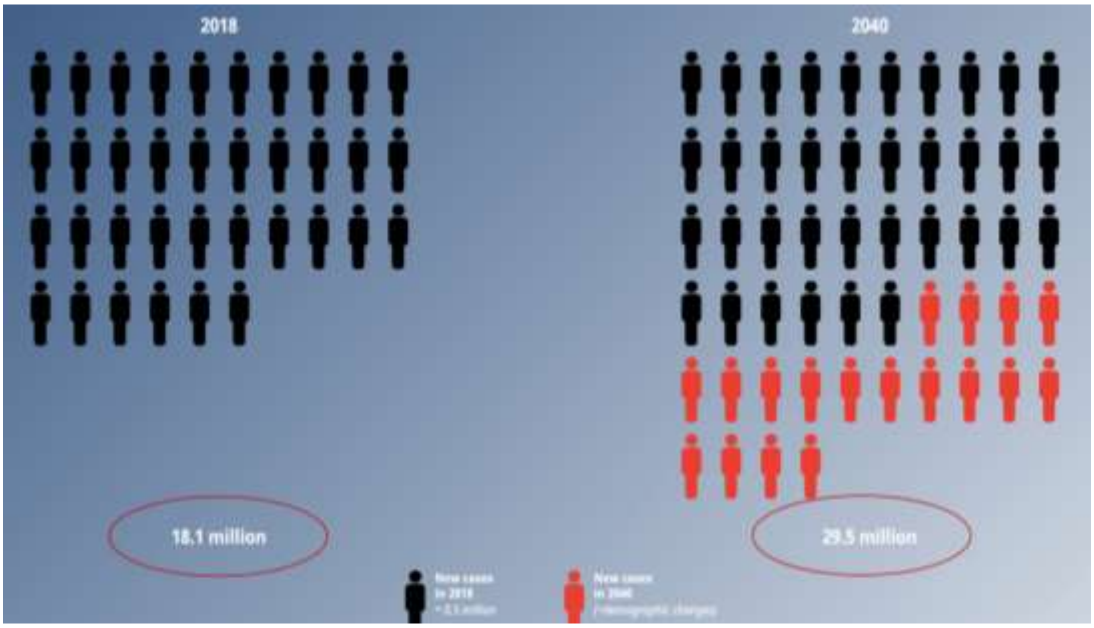

Figure 3. Incidence of cancer and future prediction for cancer incidence

The factors that determine certain types of cancer are age, gender, race, genetic predisposition, and environmental carcinogens. A number of chemicals have been clearly shown to cause cancer, such as tobacco smoke, benzene and some of its derivatives, and some dyes that have been shown in animal studies. Regardless of cause or location, it is a disease in which the process of cell proliferation and differentiation has shifted. In most cases, cells may proliferate excessively and form local tumours that may press on or invade adjacent structures. Such neoplasms with the characteristic of growing only locally are termed benign; neoplasms with the additional features of invasiveness and/or the ability to metastasize are classified as malignant. Notably, such tumour stem cells may thus exhibit a colonogenic or colony-forming capacity. The invasive and metabolic processes, as well as a number of metabolic abnormalities that result from the cancer, lead to disease and ultimately death of the patient unless the neoplasm can be eradicated by the treatment (Saha et al., 2011; Irigaray et al., 2015; Gezici et al., 2017).

3. Cancer Treatment Strategies

As a result of preventive medicine practices, early diagnosis of cancer, and treatment of cancer with appropriate treatment strategies, cancer survival has increased and cancer-related death rates have decreased globally. In the field of oncology, there are many treatment options from conventional medicine as well as complementary and alternative medicine (CAM) and more recently integrative medicine. In order to treat cancer, shrink the cancer, or stop the cancer from progressing, the most common cancer treatment strategies are chemotherapy, radiotherapy, immunotherapy, targeted therapy, surgery, bone marrow therapy, hormone therapy, and complementary therapy (Falzone et al., 2018; Münstedt and Männle, 2020). (Figure).

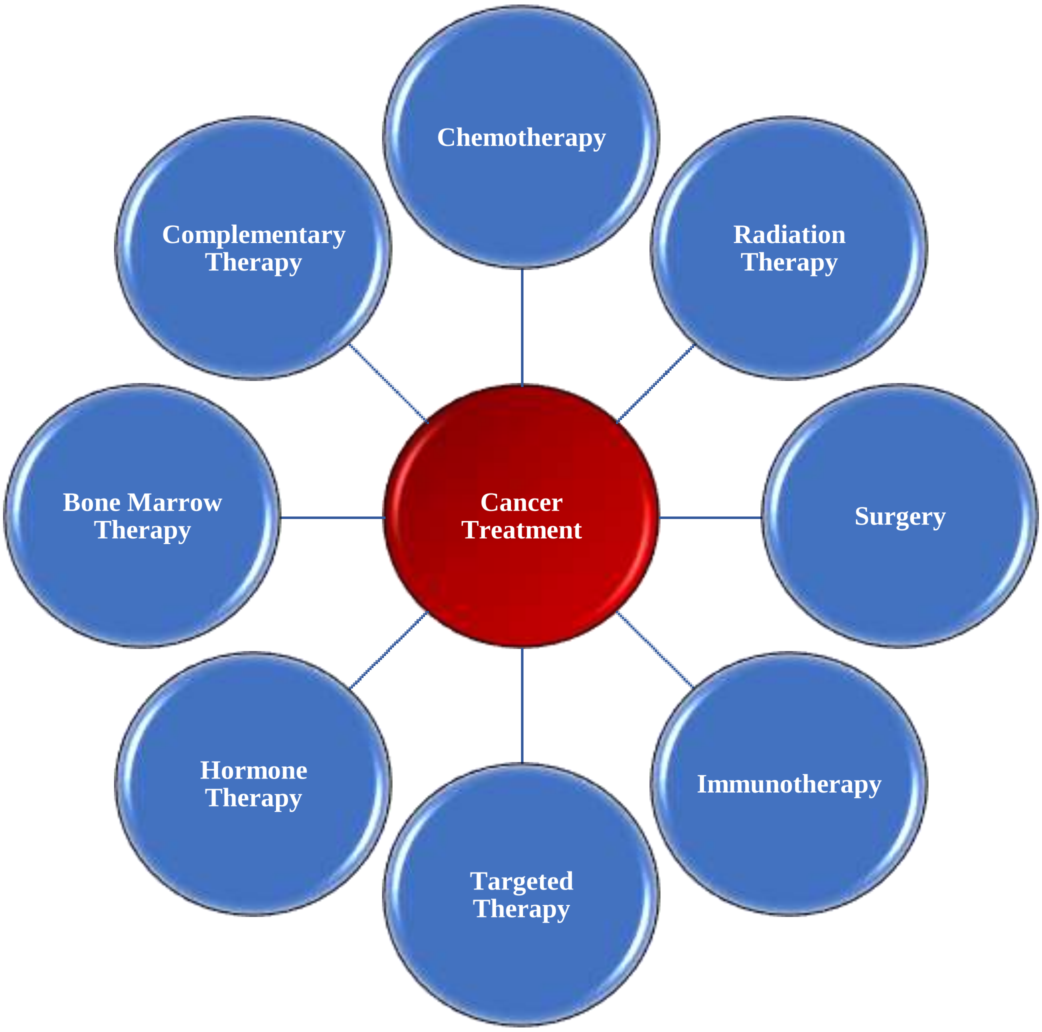

Figure 4. Types of cancer treatment strategies

In addition to conventional medicine, treatment options from complementary and alternative medicine (CAM), and more recently from integrative medicine, are very valuable in the field of oncology. Based on a recent literature data, almost 51% of cancer patients are benefit from complementary and alternative medicine. According to the Society for Integrative Oncology, some complementary medicine methods, including meditation, relaxation, yoga, massage, music therapy, acupressure, and acupuncture can be recommended for cancer patients Münstedt and Männle, 2020).

4. Apitherapy for Cancer Treatment

Aromatherapy, acupuncture, phytotherapy, hypnosis, leech application, homeopathy, chiropractic, cup application, larva application, mesotherapy, prolotherapy, osteopathy, ozone application, reflexology, and music therapy are Traditional and Complementary Medicine Practices. Apitherapy is one of the complementary and alternative treatment methods that can be used in oncology. It also could be used as cancer therapeutic agent or to complement conventional cancer treatments. Apitherapy is based on two distinct and divergent concepts: (1) The holistic concept of apitherapy, which states that all diseases can and should be treated with bee products, possibly in combination with other types of complementary and alternative medicine. This concept is mainly advocated by beekeepers and non-scientifically oriented people. (2) The scientific apitherapy method, which takes into account clinical findings, studies and trials as a basis for treatment concepts (Cherbuliez, 2013; Trumbeckaite et al., 2015; Münstedt and Männle, 2020).

In the history, apitherapy products were often used as natural remedies to maintain health in traditional medicine. The roots of apitherapy can be traced back to the more than 6000 years old medicine in ancient Egypt. Evidence from the third century BC found in Changsha in Hunan Province showed that bee products, particularly honey was a part of traditional Chinese medicine and used for medicinal purposes. More recently, bee products have been incorporated into modern medical practice, with a focus on disease and its prevention. Among complementary and alternative medicine methods (CAM), there are some dietary supplements whose efficacy in the prevention of some common diseases is relatively well established. Some results suggest that individuals who use alternative therapies, including herbal, mineral, and biological (including apitherapeutic) dietary supplements, are less likely overall to receive standard prevention than nonusers. In addition, supplement users tend to be more health conscious and appear to be a more health aware group (Trumbeckaite et al., 2015; Münstedt and Männle, 2020).

Apitherapy applications are performed by a certified apitherapy specialist in a Ministry of Health approved apitherapy unit or apitherapy application center. Apitherapy applications may not be suitable for all the patients in all cases. On the other hand, it should not be used on people who are allergic or hypersensitive. For application of bee venom on the skin, live bee stings or injections with extracts containing bee venom or ointments containing bee venom are used. For oral bee products (honey, propolis, royal jelly, pollen, apilarnil, etc.), chemical analysis should be carried out and these products should comply with the Turkish Food Codex Regulation and the instructions of the Turkish Standards Institute, and quality control should be carried out (Premratanachai and Chanchao, 2014; Sengul and Vatansev, 2021). The most common bee products for apitherapy are presented in the Figure 4.

Figure 5. Most common bee products for apitherapy

Multifarious products obtained from the beehive, particularly honey, propolis, pollen, apilarnil, royal jelly and bee venom are used for apitherapy (Figure 5). Among these products, bee venom (BV), mainly known as anti-inflammatory and analgesic agent, has been used to treat diverse disorders in the form of bee stings, apipuncture, injections, etc. The main component of BV, which constitutes approximately 50% of its dry matter, is melittin (MEL). MEL is 26 amino acids long peptide. Modern pharmacological studies showed that MEL exerts various antitumor effects by inhibiting tumor cell growth, promoting tumor cell apoptosis, and inhibiting angiogenesis and migration. Propolis is the resinous mixture that honey bees collect from different sources to use it as a sealant for unwanted open spaces in hives. Propolis as anti-inflammatory, antioxidant, anti-infective, and anticancer agents have been studied in vitro and in vivo. In addition, propolis and its preparations in various forms of application (oral sprays, tablets, capsules, etc.) are used in human medicine for the treatment of some diseases and disorders such as, colds, flu-like infections, wounds, sore throats and herpes simplex infections (Crişan et al., 1995; Kuropatnicki et al., 2013). Honey is a natural substance formed from nectar by honeybees. Honey constituents have been reported to exert anti-inflammatory, antioxidant, antiproliferative, antimetastatic, and anticancer effects. Additionally, it is used as a general ingredient or alone in folk medicine to relieve coughs, along with other bee products, for strengthening the immune system (Paul, 2012; Sugiyama et al., 2012; Münstedt and Männle, 2020).

5. Conclusion

Apitherapy applications have gained a great interest in recent years almost all over the world. Therefore, the effects of bee products and apitherapy in cancer cases highlighted in this review. Bee products, used for apitherapy applications, has been known since ancient times. These products have become one of the most popular products for healing, due to their rich bioactive content. Promising research results in clinical applications have also led to an increase in demand for the use of these products. Moreover, patients are avoiding side effects of the applications of synthetic drugs in the treatment of chronic diseases, especially cancer. To strengthen the use of apitherapy for cancer, it is necessary to ensure the production of high-quality, effective and reliable bee products, since human safety is the most important factor by using bee products.

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Seasonal changes in the circulation of viruses and mixed infection in the population of honey bees Apis mellifera L.

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Abstract: The article presents data on the circulation of seven species of viruses in the honey bee population in the summer (July) and autumn (September) periods in the apiary of the Central Federal District of Russia. It has been established that the SBV (sac brood virus) is the most common in the studied bee population - in the summer it can be detected in 100% of bee colonies. Two other viruses, wing deformity virus (DWV) and acute paralysis virus (ABPV), are also widespread, and circulate in approximately 50% of bee colonies in the summer. It is shown that in bee colonies in the autumn period, the percentage of families in which it is possible to detect at least one of the viruses decreases by an average of 2.5 times, the sac brood virus (SBV) is most susceptible to seasonal decline - 3.5 times. The wide distribution of mixed infections in bee colonies has been confirmed. The fact of clinical manifestations of sac brood disease was found in one of the 20 families studied, mixed infection was not observed in it, only the sac brood virus (SBV) was detected.

Keywords: seasonal circulation of viruses, honey bees, mixed infections

Introduction

Over the past two decades, scientists around the world have noted the high death of bees in their countries [3, 4, 5, 12, 18]. One of the probable causes of the death of bees, along with the anthropogenic transformation of biogenoses, may be the spread of infectious diseases, especially viral etiology. Almost 100% infection of Russian bee colonies with varroa mites causes a special interest of researchers in transmissible viral diseases, in which ticks act as effective carriers [11].

The results of the studies of the epizootic situation on viral infections in the European territory of Russia with varying degrees of damage to bee colonies by varroa mites [6]. revealed a very high load on bee colonies with black queen worm viruses (BQCV) - 100%, wing deformities (DWV) - 100%, sac brood (SBV) -77.3% and a lower load with Kashmir virus (KBV) - 37.5%, Israeli acute paralysis virus (IAPV) - 4.2%.

Viral diseases make up the majority of generally recognized especially dangerous bee diseases. Until the 60s and 70s of the last century, the virus of chronic paralysis and sac brood was ubiquitous. They periodically caused obvious signs of damage in some countries in 1-2% of bee colonies. However, most other viruses generally did not cause obvious clinical symptoms. However, the epizootic situation has changed dramatically with the widespread distribution of varroa ticks, which acted as activators of virus replication and their carriers.

Once in the body of a bee, viruses can be in it for a long time in a latent (latent) form and be transmitted from individual to individual without signs of disease. However, with various kinds of stress, the presence of pesticides in the nest, a decrease in the immunity of bee colonies for a number of reasons, a sharp change in climatic conditions, etc., they begin to multiply intensively and lead to the death of bees. The high level of infection of bee colonies with varroa mites and the possibility of activation of latent viral infections determine the relevance of research to the problem of circulation of a wide range of viruses in the body of bees and, above all: wing deformity virus (DWV), sac brood virus (SBV), black queen virus (BQCV), Kashmir virus (KBV), Israeli acute paralysis virus (IAPV), acute paralysis virus (ABPV) and chronic paralysis virus (CBPV).

A wide variety of viral infections [1, 15, 16], a high viral load on bee colonies [6, 20], the death of bees from viral diseases [7, 21], as well as the lack of medical methods to combat viral infections force scientists to study in more depth the issues associated with the spread and interaction of viruses in the honey bee population.

The purpose of this study was to determine the virus carrier in bee colonies in the apiary of the Central Federal District of Russia, taking into account the seasonal aspects of the circulation of viruses and the possibility of mix infections.

To achieve this goal, it was necessary to study the following issues:

1) To establish the level of virus carrier in bee colonies in the apiary in the summer and autumn periods.
2) To assess the pattern of seasonal variations in infection with 7 types of viruses - wing deformity virus (DWV), sac brood virus (SBV), black queen virus (BQCV), Kashmir virus (KBV), Israeli acute paralysis virus (IAPV), acute paralysis virus (ABPV) and chronic paralysis virus (CBPV).
3) Determine the spread of mixed infections in bee colonies.

Research materials and methods

The work was carried out in 2021 - 2022 at a separate apiary of the Ryazan region of the Central region of the European part of Russia. The main honey plants growing within the radius of productive summer of bees (2 km) are wild plants (shrubs, forest and meadow grasses). The main honey harvest is provided by the small-leaved linden tree *Tilia cordata* Mill.

The object of research was bees - mestizos of the Carpathian breed. Clinical examinations of families for the presence of infectious diseases were carried out in accordance with the current Instruction [2].

For the research, 20 bee colonies were selected (approximately the same strength and with about the same amount of honey and brood).

The technique of taking pathological material to determine the presence of viruses in the samples under study.

For laboratory diagnosis of viruses, a sample of live bees was taken in the amount of 30-50 pieces, placed in containers and labeled.

Live bees admitted to the laboratory were frozen for further molecular genetic analysis. Freezing was carried out at temperatures from -20C to -30C for 3 hours or more.

The presence of viruses in families was detected by RT-PCR with specific primers. The following viruses were identified: wing deformity virus (DWV), sac brood virus (SBV), black queen virus (BQCV), Kashmir virus (KBV), Israeli acute paralysis virus (IAPV) and acute paralysis virus (ABPV).

The analysis included the following works:

a) Preparation of samples for total RNA isolation;

b) isolation of total RNA.To isolate total RNA from each family, 30 bees were selected and ground in a mortar in the cold to a homogeneous state; Extract RNA reagent (Eurogen CJSC, Russia) was used for cell lysis. The allocation was carried out according to the manufacturer's protocol.
c) removal of genomic DNA from an RNA sample. To completely remove the DNA, RNA samples were incubated in the presence of DNase;
d) conducting an OT-PCR reverse transcription reaction. The synthesis of the first cDNA strand on the RNA template was carried out using the reverse transcriptase of the mouse leukemia virus (MMLV revertase). As a primer, a random decanucleotide primer was taken to produce cDNA.
e) assessment of the presence of viruses was carried out using classical PCR with specific primers (Table 1).

Table 1

Sequences of oligonucleotides (primers) used.

Name	Sequence, 5'→3'	Annealing temperature, °C
ABPV-F	AAGGATGAGAGAAGACCAATTG	53
ABPV-R	ATCTTGGGAATAAACATTAGTTCCT	53
DWV-F	CTGTATGTGGTGTGCCTGGT	53
DWV-R	TTCAAACAATCCGTGAATATAGTGT	53
SBV-F	ACCAACCGATTCCTCAGTAG	58
SBV-R	CCTTGGAACTCTGCTGTGGTA	58
BQCV-F	AAGGGTGTGGATTTCGTCAG	55
BQCV-R	GGCGTACCGATAAAGATGGA	55
KBV-F	TGAACGTCGACCTATTGAAAAA	52
KBV-R	TCGATTTTCCATCAAATGAGC	52
IAPV-F	CGAACTTGGTGACTTGAAGG	55
IAPV-R	GCATCAGTCGTCTTCCAGGT	55

Composition of the reaction mixture for PCR:
- 2 µl 10X reaction buffer;
- 0.5 µl Taq polymerase;
-0.4 µl 50X dNTP (10mmol);
- 1 µl F-primer (10 pmol);
- 1 µl R-primer (10 pmol);
- 14.1 µl H_2O;
- 1 µl DNA.

The reaction conditions were selected experimentally.

Stages and conditions of the reaction:
1) Initial denaturation (3 min, 94 °C).
2) 35-40 cycles, including:
- denaturation (10 s, 94 ° C);
- annealing of primers (30 s, the annealing temperature varies depending on the pair of primers);
- elongation (20 s, 72 °C);
3) Elongation of undersynthesized products (5 min, 72 ° C).
The reaction was carried out in the amplifier T100 thermal cycler of Bio-Rad (USA).

Subsequent detection of amplification products was carried out using gel electrophoresis in a 2% agarose gel.

To reduce the infection of bee colonies with the *Varroa destructor* mite, the drug "Vetfor" was used in the form of plates containing 80 mg of fluvalinate and 5 mg of ametraze per plate as an active ingredient. Treatment of bee colonies was carried out in accordance with the instructions for its use.

Research results and discussion.

As a result of the studies, a high level of virus carrier in the analyzed bee colonies was established - up to 100% in the summer. As can be seen from the data presented in Table 1, the SBV sac brood virus is most often found in bee colonies - in the summer months it was detected in 100% of families. Also, the group of widespread viruses can be attributed to the virus of acute paralysis ABPV and the virus of wing deformation DWV - they were found in the summer in about 50% of the studied families. The circulation of the remaining studied viruses is insignificant or absent even in the summer months. Data from other researchers also indicate an extremely wide distribution of the sac brood virus in honey bee populations of different geographical zones [8, 9, 16, 19, 20]. However, this may also be due to the fact that sac brood, unlike most other bee viruses, is reliably diagnosed by the characteristic and specific symptoms of infected larvae [10].

In the summer, as evidenced by our data, it is possible to detect the maximum possible number of viruses in honey bees. Therefore, to assess the overall level of virus carriage in beekeeping apiaries in various geographical regions, it is advisable to conduct research in the summer.

Table 2. Seasonal changes in the circulation of 7 types of viruses in bee colonies (20 families were studied in each period)

Viruses	Number of affected families in July		Number of affected families in September		Seasonal decrease
	n	%	n	%	time
acute paralysis virus (ABPV)	10	50	6	30	1,7
wing deformity virus (DWV)	9	45	4	20	2,3
black queen virus (BQCV)	0	0	0	0	-
chronic paralysis virus (CBPV)	1	5	0	0	-
sac brood virus (SBV)	20	100	6	30	3,3
Kashmir virus (KBV)	3	15	1	5	3,0
Israeli acute paralysis virus (IAPV)	0	0	0	0	-

When studying the virus carrier in bee colonies in the autumn period (September), it was found that the percentage of families in which it is possible to detect at least one of the viruses decreases by an average of 2.5 times. Most of all, the sac brood virus SBV is subject to a seasonal decrease in circulation - the number of families in which it is found in the autumn decreases by about 3.5 times. The metabolic rate of bees decreases in the fall, which may be the reason for the seasonal dynamics of the circulation of viruses, whose replication depends on the level of cellular metabolism. A more significant decrease (3.5 times) compared to the average (2.5 times) circulation of the sac brood virus SBV can also be explained by a sharp reduction in brood in the autumn period in bee colonies. In France, an analysis of samples from 360 apparently healthy bee colonies confirmed that the frequency of SBV-infected families increases from spring to summer and decreases in autumn in both adults and pupae [20]. According to Tentcheva and all (2004b), the incidence of infection of adult worker bees and pupae with the wing deformity virus increased from spring to autumn. Our studies showed the opposite picture, i.e. a decrease in the number of DWV-infected families from summer to autumn. Probably, this can be explained by the systematic control in our case over the size of the population of the varroa tick, which is the main carrier of this virus.

As a result of our research, a wide spread of mixed infections in bee colonies was detected. In the summer, almost half of the bee colonies, in addition to the sac brood virus, were characterized by the presence of one or even two other viruses circulating simultaneously in the body of bees. And in most cases, such additional viruses were viruses that dominate the bee population: wing deformity virus (DWV) and acute paralysis virus (ABPV). Only in a few bee colonies, along with the sac brood virus, was it possible to detect the Kashmir virus (KBV) and the chronic paralysis virus (CBPV), and only in the summer. In the autumn period, we found that, like the general virus carrier, the number of families with mixed viral infections decreases by about 2.5 times.

To date, there is clearly not enough knowledge about the nature of possible interactions of viruses and the effect of mixed viral infections on pathogenic processes in honey bees. Competition between viruses present in the same bee family or in the same worker bee may be one reason for the differences in the prevalence of the viruses. As stated earlier, ABPV does not multiply together with BQCV or SBV by experimental injection into pupae [9]. Another example is DWV, which multiplies to very high titers even in bees that show no symptoms of disease [14]. In our opinion, it is the low pathogenicity of viruses in bee colonies that explains the wide spread of mixed viral infections, otherwise mixed infections would lead to the predominant death of such families in the population. This assumption can also be indicated by the fact that we have discovered the clinical manifestations of sac brood disease in one family out of 20 studied, in this family it was possible to detect only the sac brood virus (SBV) and no mixed infection.

It is currently unknown whether mixed viral infections may lead to genetic recombination between coexisting viruses and whether such recombination may lead to the emergence of new viruses [13]. Although there is currently no indication that new recombinant viruses have suddenly appeared in bees. The lability of single-stranded RNA genomes and the establishment of new viral transmission pathways should prompt us to keep an eye on this.

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Comparative Study of the Prevalence of Nosemosis in Honey Bees in Bulgaria and Estonia

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Abstract:

Nosemosis is a disease of honey bees, affecting bee colonies worldwide, including in Bulgaria and Estonia. The present study aimed to determine the prevalence of *Nosema* spp. (nosemosis) in Bulgaria and Estonia in 2017, the degree of invasion, as well as to compare the prevalence of nosemosis in countries with colder and temperate climates. For each sample, 60 forage bees were collected from the flying boards of hives. The samples were placed in plastic tubes, cooled immediately for transportation, and frozen at −20 °C until laboratory analyses. Diagnostic methods used to proof spores and identification of *Nosema* spp. - *N. apis* and *N. ceranae*, including light microscopic examination (native and stained smears and counting spores in haemacytometer / flow cytometer) and multiplex PCR. During this period in Bulgaria 114 samples of bees from 82 apiaries located in different regions of the country were studied. The results showed 85 (74,6%) positive for nosemosis samples and 29 (25,4%) negative. Of the positive samples, 47.4% had an invasion rate of 2 to 10 million spores / bee, followed by those with up to 1 million spores / bee (17.5%) and the smallest number of samples showed an invasion rate of over 10 million / bee (9.6%). Among the 30 apiaries surveyed in Estonia the median number of spores per worker bee ranged from 1.6 to 14 million. *N. ceranae* has replaced *N. apis* in many countries. Estonia seems to be one of the few countries in the world where *N. apis* (43%) is still individually prevalent, while in Bulgaria in 98% of cases nosemosis caused by *N. ceranae* predominates.

Keywords: *Apis mellifera L.; Nosema* spp., Bulgaria, Estonia, pathogens

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1. Introduction

Honey bees (*Apis mellifera*) are pollinators with a significant worldwide economic value and are responsible for the pollination of many ecologically and agriculturally important crops (Gallay et. al., 2009). Colony losses have been linked to, environmental and migratory stress, pesticide exposure and poor nutrition; however, parasite and pathogen infections are likely the leading factors contributing to colony mortality. The spore-forming parasites *Nosema apis* and *Nosema ceranae* are considered to be one of the causes of increased honey bee mortality in recent years (Hristov et al., 2020; Laomettachit et al., 2021). *Nosema apis* was the historic species infecting A. mellifera honey bees (Matheson, 1996), but probably early in this century, *N. ceranae* became an invasive parasite of *A. mellifera*, transferring from Asian honey bees *A. cerana* (Higes et al., 2006;

Huang et al., 2007; Williams et al., 2008; Charbonneau et al., 2016). Nosemosis is a disease of honey bees, affecting bee colonies worldwide, including in Bulgaria and Estonia. *N. apis* was first identified in the western honey bee, *Apis mellifera*, over 100 years ago (Zander, 1909) The microsporidian intestinal parasite *Nosema ceranae* was first described from the Asian honey bee *Apis cerana* in 1996 (Fries et al., 1996). However, in some regions, usually with colder climates, *N. apis* is still prevalent. To achieve better disease control, it is important to determine the species distribution. Climate is considered to be one of the main factors in the spread of *Nosema* species Gisder et al., 2017; Papini et al., 2017; Ostroverkhova et al., 2020). In warmer climates, *N. ceranae* is more competitive than *N. apis*, in contrast, in cold climates, *N. ceranae* spores appear to be much more vulnerable than the *N. apis* spores (Fries I. 2010). The present study aimed to determine the prevalence of *Nosema* spp. (nosemosis) in Bulgaria and Estonia in 2017, the degree of invasion, as well as to compare the prevalence of nosemosis in countries with colder and temperate climates.

2. Materials and Methods

This study was conducted in Estonia and Bulgaria in 2017 year. For each sample, 60 forage bees were collected from the flying boards of hives. The samples were placed in plastic tubes, cooled immediately for transportation, and frozen at −20 °C until laboratory analyses. Diagnostic methods used to proof spores and identification of *Nosema* spp. - *N. apis* and *N. ceranae,* including light microscopic examination (native and stained smears and counting spores in haemacytometer / flow cytometer) and multiplex PCR (OIE Terrestrial Manual, (2013) - for the identification of *Nosema* spp.)

3. Results

During this period in Bulgaria 114 samples of bees from 82 apiaries located in different regions of the country were studied. The results showed 85 (74,6%) positive for nosemosis samples and 29 (25,4%) negative (Fig.1). Of the positive samples, 47.4% had an invasion rate of 2 to 10 million spores / bee, followed by those with up to 1 million spores / bee (17.5%) and the smallest number of samples showed an invasion rate of over 10 million / bee (9.6%). Among the 30 sampled apiaries in Estonia, 17% were positive for *N. ceranae*, 43% were positive for *N. apis*, and 7% were co-infected (Fig.2). In the remaining 33% of sampled apiaries, *Nosema* was no longer detected (Naudi et al., 2021). The median number of spores per worker bee ranged from 1.6 to 14 million.

Fig. 1. The level of infestation with spores of *Nosema* spp. in bee colonies in Bulgaria

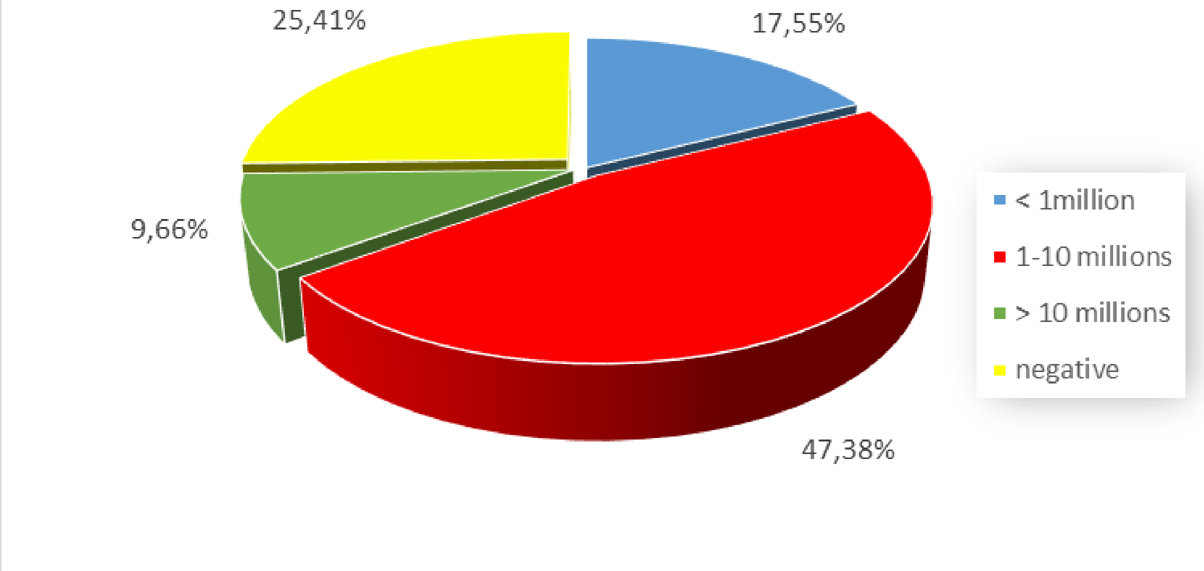
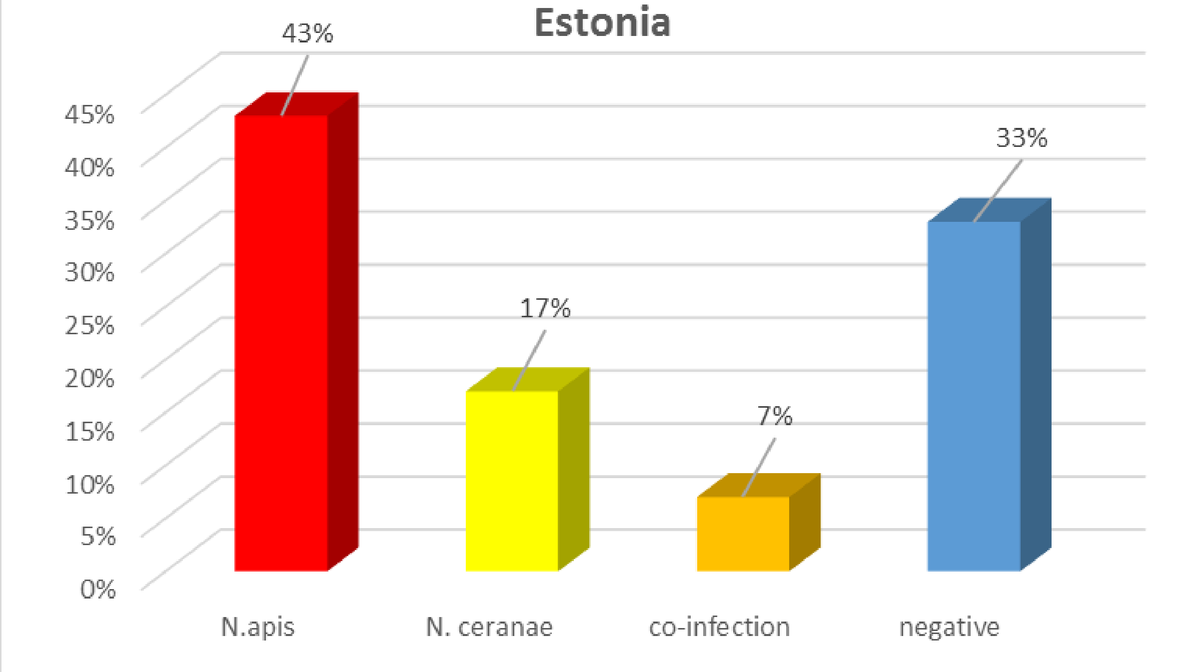


Fig.2. Prevalence of *N. apis* and *N. ceranae* in Estonia



4. Discussion

Epidemiological evidence indicates that *N. ceranae* may be replacing *N. apis* globally in A. mellifera populations, suggesting a potential competitive advantage of *N. ceranae* (Milbrath et al., 2015). Over the last few decades, the original pathogenic species, *N. apis*, has been displaced by this more aggressive pathogen, which is especially characteristic of countries with warmer climates, such as Bulgaria (Salkova et al., 2015;

Salkova et al., 2016). Although for a long time, these two species were thought to be host species specific. Despite generally similar descriptions, these two species affect honey bees differently. Some studies have shown that the geographic distribution of *N. apis* and *N. ceranae* may overlap and co-infections may occur (Milbrath et al., 2015) Nosemosis caused by *N. apis* (nosemosis type A) occurs mainly in spring, is milder and easier to diagnose due to clear clinical signs, whereas outbreaks of *N. ceranae* (nosemosis type C) are found during the entire period of active colony growth and often proceeds asymptomatically. Studies on the impact of Nosema on honeybee colonies need data concerning its prevalence, particularly in asymptomatic colonies (Papini et al., 2017). It has also been reported that *N. ceranae* is more virulent than *N. apis*, affects learning and homing behaviour, causes higher energy costs and immune suppression, and affects queen health (Huang, 2012). Our experiment confirms that *Nosema* spp. are virulent for their host *A. mellifera* and the successful spread of *N. ceranae* and its dominance over *N. apis* in some locations is probably more related to the competitive advantage of *N. ceranae* over *N. apis* in warmer regions of the world.

5. Conclusion

N. ceranae has replaced *N. apis* in many countries. Estonia seems to be one of the few countries in the world where *N. apis* (43%) is still individually prevalent, while in Bulgaria in 98% of cases nosemosis caused by *N. ceranae* predominates. As there is no effective cure for *Nosema*, beekeepers need to use uncontaminated equipment in apiaries. The internal temperature of a honey bee colony is always 32–35 °C, which is a favorable temperature for the pathogen. We conclude that the spread of *N. ceranae* may be lesser in regions with colder climates, but further research is needed.

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Determination Of The Effect of Honey Bee (*Apis mellifera* L.) Venom In The Treatment Of Epilepsy

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Abstract:
Insect poısons, have been used for many years in the treatment of various diseases. Honey bee (*Apis mellifera* L.) venom is also used in Traditional and Complementary Medicine in terms of its components, in the treatment of autoimmune and inflammatory diseases such as chronic pain, as well as diseases such as rheumatoid arthritis, osteoarthritis, neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Parkinson's, Alzheimer's, and multiple sclerosis (MS). In the presen study the effects of honey bee venom on epilepsy, which is also a neuronal disorder. According to the results of the literature review, it is thought that bee venom inhibits epilepsy pathways and has potential antiepileptic properties because it takes part in the regulation of voltage-gated ion channels such as sodium, potassium and calcium channels

Keywords: Honeybee venom, Epilepsy, Antiepileptic, Epileptogenesis, *Apis mellifera* L.

1. Introduction

Honey bees (Apis mellifera L.) are insects that live in colonies. Apart from providing pollination in plants in agriculture, the products they produce such as honey, beeswax, pollen, propolis, royal jelly, bee venom have been used by people since ancient times. Bee products other than honey bee venom are used in traditional and complementary medicine applications as well as being consumed as food thanks to the components they contain (Altıntaş & Neslihan, 2018).

The use of bee products in the treatment of diseases is called apitherapy. Apitherapy is a complementary medicine practice based on ancient medical records (Sig et al., 2019). Honey bee venom contains; Such as Hyaluronidase (1-2%), Adolapine (0.5-1%), Histamine (0.5-2%), Dopamine (0.2-1%) and Noradrenaline (0.1-0.5%) There are important components. As an indicator of quality and value of honey bee venom, four components are emphasized, considering both their effects on living things and their rate of poison content; Phospholipase A2 (12-20%), MCD peptide (2-3%), Apamin (2-3%) and Melittin (40-50%). Melittin, one of these components, is a component specific to honeybee venom (S. Karlıdağ, 2020).

Insect poısons were used in Chinese and Korean medicine by the ancient Greek and Egyptian civilizations. There is information that it has been used for the control and treatment of various diseases, including neurological diseases, BC 1000-3000 years (Pemberton, 1999). In addition, in religious texts such as the Bible and the Qur'an, it is mentioned that bee products can be used in the treatment of diseases(Ali, 2012). Some of the studies on honey bee venom are as follows; In a recent study by Kim et al. on the potential effects of honeybee venom components, animals treated with phospholipase A2 in a mouse model of MPTP induced Parkinson's showed better motor function than untreated controls (Kim et al., 2019). Another study by Minsook Ye et al., in this study, they observed that phospholipase A2, a component of honeybee venom, reduced the accumulation of Alzheimer's-causing Aβ (Amyloid Beta), thereby increasing the ability to improve cognitive function in mouse brains (Ye et al., 2016).

In 2011, Yang and colleagues examined the therapeutic effect of the honeybee venom component Melittin, in a transgenic mouse model for ALS, also a neurodegenerative disease. In this model, they found that animals treated with Melittin had a decrease in the number of activated microglia and the expression of the proinflammatory factor TNF a, inhibiting increased neuroinflammation, which is responsible for neuronal death in this disease.(Yang et al., 2011).

In recent years, it has been seen that honey bee venom, like antiepileptic drugs, inhibits epilepsy pathways and plays a role in the regulation of voltage-gated ion channels such as sodium, potassium and calcium channels. An example of this is a study by Abd al-Hameed and his colleagues. They found that honey bee venom inhibited the high expression of proinflamuar cytokines, while honey bee venom, with acupuncture treatment, was effective in preventing some of the harmful consequences of epileptogenesis associated with glutamate and DOPA, which are found in high levels in the hippocampus (Abd El-Hameed et al., 2021). Silva et al. stated in one study that honey bee venom corrects brain neurochemistry (Silva et al., 2015).

Epilepsy; It is a dysfunction that occurs in all or a part of the brain, which causes sudden involuntary contractions, emotional changes or unconsciousness as a result of repetitive abnormal discharges of neurons as a result of the electrical current between neurons in the brain being greatly impaired (Akdağ et al., 2016). Although the causes of epilepsy are not completely clear, there are many mechanisms of action. Some of those; Structures and events such as ion channels, synapses, receptors, inflammatory cytokines, oxidative stress, glycogen and glucocorticoid metabolisms, glial cells, neurotransmitters, gene mutations, apoptosis, mitochondrial dysfunction play a role in the occurrence of epilepsy (He et al., 2021).

Neurodegenerative diseases can be transmitted genetically or they can occur suddenly or later. Today, increasing life expectancy and intense stress cause neurodegenerative diseases to become widespread worldwide. Epilepsy, which is a neurodegenerative disease, is a disorder that negatively affects both the patient and his family and close environment. Anti-epileptic drugs approved for treatments act on voltage-gated ion channels in nerve cells, such as sodium, potassium, and calcium channels, to control the electrical firing of the neuron. Most of the drugs used for epilepsy do not completely eliminate epileptic seizures and cause many side effects such as mental, emotional and cognitive. However, in recent studies, it has been seen that honeybee venom, which is a natural product and has fewer side effects, inhibits epilepsy pathways like antiepileptic drugs and plays a role in the regulation of sodium, potassium, calcium channels and voltage-gated ion channels. It provides new alternatives for the control of neurodegenerative diseases thanks to wasp and honey bee venoms and biological active components they contain. These poisons and their components are sources of neuroprotectors or neuromodulators(Silva et al., 2015). There are differences between the venom components of these two bees. The most important of these is that Melittin and Apamin are specific to honeybee venom and these components are not found in wasp venom (Silva et al., 2015). Table 1 contains honeybee venom components and percentages (Hider, 1988).

Table 1. Components in Honey Bee Venom and Their Percentages;

Molecule Class	Apitoxin Component	Percentages in Poison, % of venom	Molecular weight
Small Proteins and Peptides	Melittin	%40-50	12000 (as tetramer)
	Apamin	%1-3	2000
	Mast Cell Degranulation Peptide	%1-2	2500
	Adolapin	%0.5-1	
	Tertiapin	%0.1	2500
	Prokamin A, B	%1-2	600
	Sekapin	%0.5-2	3000
	Minimin	%2	
	Pamin	%1-3	
	Small peptides less than 5 amino acids	%13-15	<600
	Phospholipaz A2	%10-12	20000
	Phospholipaz B	%1	
	Hyaluronidase	%1-2	41000
	Phosphatase	%1	
	α– Glukozidaz	%0.6	

Biogenic Amines	Histamine	%0.5-2	150
	Dopamine	%0.2-1	150
	Noradrenaline	%0.1-0.5	150
	α -aminobutyric acid	0.5	150
Phospholipids		%5	700
α-Amino Acids		%1	150
Sugars	Glucose	%2	180
	Fructose		
Volatile Compounds (pheromones)		%4-8	200
Minerals	P, Ca, Mg	%3-4	

Components of Honey Bee Venom Affecting the Central Nervous System and Neurons and Their Effects on Some Neurodegenerative Diseases;

1. **Melittin**

Melittin is an amphipathic (both hydrophilic and hydrophobic) molecule consisting of 26 amino acids (Koyama et al., 2000). Melittin is the molecule with the highest percentage of honey bee venom.

The Effect Of Melittin On S100B Protein, Which Is One Of The Causes Of Epilepsy.

Studies have shown that overexpression of S100B (a calcium-binding protein) is associated with calcium ion imbalance and neurogenerative diseases. In a study by Verma and Singh on the S100B protein, it was mentioned that Melittin forms a complex by binding with the S100B protein. and these bonds, apart from the van der Walls interactions, have also stated that the existence of some hydrogen bonds that provide the stability of the complex has been revealed in the studies. Thus, it shows that Melittin has the ability to interfere with the functionality of the s100B protein responsible for epilepsy (Verma & Singh, 2013).

2. **Apamin**

Apamin, on the other hand, is a peptide consisting of 18 amino acids and two disulfide bridges, and is the smallest neurotoxin of bee venom. The rate of presence in the dry venom of apamin is between 1-3%. It is one of the smallest neurotoxins in honeybee venom. Thanks to this feature, they have the ability to cross the brain blood barrier and affect the functioning of the central nervous system (Wehbe et al., 2019). The mechanism of action of apamin is shown in figure 1 (Hider, 1988).

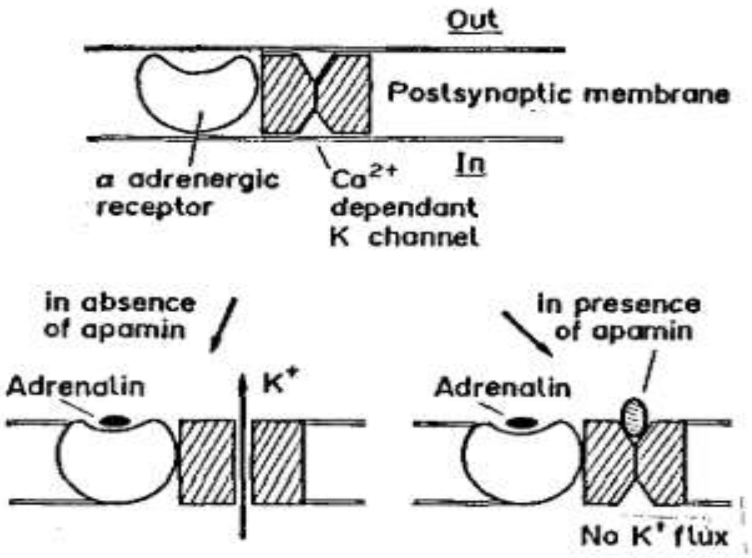

Figure 1; Apamin selectively blocks some classes of K^+ channels. When blocked, the normal effect of adrenaline, that is, opening the channels, is prevented.

The Effect Of Apamin On The Regular Functioning Of The Impaired Ca And K Channels That Cause Epilepsy.

Ca ion is a widely used ion in muscle contraction, enzymatic reactions and activation of cells, and this ion must be present at certain levels. However, many researchers have stated that the entry of excessive Ca ions into the cells causes neuronal death. The entry of Ca ions into cells is considered as the first step in the onset of epilepsy events. As a result of the increase in the concentration of Ca ion in the neuron, it increases the release of glutamate, a neurotransmitter. The increase in glutamate also causes more Ca ions to enter the cell (Bagirici & Bostanci, 2001). The SC ducts (with small conductors) associated with epilepsy are predominantly located in the nervous system and are inhibited by apamine, one of the components of bee venom and the smallest neurotoxin.(Mustafa Emre, 2020). K^+ channels activated by calcium ion concentration have assumed functions such as regulating calcium intake, regulating ignition frequency, and ending ignition (Kubo et al., 2005).

Brain-Blood Barrier and Apamin: Apamin, being one of the smallest components in honey bee venom, has the ability to cross the brain blood barrier. It is well known for its ability to block Ca^{+2}-activated K^+ channels, which are intensely present in the central nervous system and associated with epilepsy, and it is considered the most widely used blocker for this type of channels (Moreno & Giralt, 2015),(Mustafa Emre, 2020).

K^+ channels, activated by low-permeability Ca^{+2}, control the firing frequency of neurons, especially at AMPA and NMDA glutamatergic synapses, and are responsible for the hyperpolarization that follows action potentials (Lam et al., 2013).

3. **Phospholipaz A²**

Phospholipase A2, an enzyme, is a single polypeptide chain of 128 amino acids containing four disulfide bridges. Honeybee venom phospholipase A2 belongs to group III sPLA2 enzymes and can act as a ligand for specific receptors. Honeybee venom phospholipase A2 accounts for 12-15% of the dry weight of honeybee venom and is extremely alkaline. The honey bee venom phospholipase A2 is a hydrolytic enzyme that can specifically break down the sn-2 ester bond of phospholipids at the water/lipid interface (Samel et al., 2013). Interestingly, its activity can be improved with melittin. This has been shown to occur during the erythrocyte lysis process, proving the existence of a synergistic effect between both the honey bee venom phospholipase A2 and melittin (Leandro et al., 2015). In addition, new experimental data have shown protective immune responses of the honey bee venom phospholipase A2 against a wide range of diseases, such as Alzheimer's disease and Parkinson's disease (Ye et al., 2016).

A recent study by Kim et al. on the potential cytoprotective effects of honeybee venom components suggests that phospholipase A2, but not melittin, protects against neuroinflammatory processes in an MPTP-induced mouse model of Parkinson's. One day after MPTP-treated mice, phospholipase A2 and melittin were administered (0.5 mg/kg by subcutaneous injection) for six consecutive days, and interestingly, phospholipase A2-treated animals showed better motor function than untreated controls, whereas melittin No significant effects were observed in mice receiving the injection. In addition, phospholipase A2 treatment appeared to inhibit the loss of dopaminergic neurons in the substantia nigra in MPTP-treated mice (Kim et al., 2019).

Alzheimer's disease is a neurodegenerative disease that occurs with the loss of neurons and synapses in some parts of the central nervous system and progresses. Alzheimer's disease has many causes. The most common of these is genetic predisposition. Two basic pathological findings have been identified in Alzheimer's, these are neurofibrillary tangles and amyloid plaques (Adali et al., 2020). In the study of Minsook Ye et al., phospholipase A2, a component of honeybee venom, played a role in reducing the accumulation of Aβ (Amyloid Beta) and thus increased the ability to improve cognitive function in mouse brains. In addition, studies have shown that honeybee venom phospholipase A2 increases glucose metabolism in the brain (Ye et al., 2016).

2. Conclusion

According to the results of the literature review, Honey bee venom inhibits epilepsy pathways and has potential antiepileptic properties because it takes part in the regulation of voltage-gated ion channels such as sodium, potassium and calcium channels. Our future goal is to investigat the effect of the bee venom and apamin against to epilepsy diseases

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Biochemical and Physiochemical Properties of Turkish Forest Honeys

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Abstract:

Honey is one of the most natural and sweetest products produced from nectar and secretions collected by honeybees from nature. Due to its geographical location, Turkey is very rich in terms of honey plants and is a habitat for a wide variety of flower and secretion honeys. It has a large variety of honeys such as blossom honeys, dew honeys, forest honeys and industrial honeys etc. Forest honey is the honey produced from trees and is divided into two classes as flower and secretory honeys. Chestnut, acacia, linden, rhododendron, naringin, blackthorn and heather honeys are blossomed forest honeys. Pine, oak, cedar, spruce, and fir honeys are forest honeys with secretory honey properties.

Keywords: Forest honey, Turkiye, oak, pine, dew

1. Introduction

Honey is a natural sweet product that has been used for nutritional and medicinal purposes throughout the course of history. It exhibits a wide range of biological active features such as antioxidant, antimicrobial, antitumoral, anti-inflammatory, and anti-depressant activities (Can et al., 2015; Al-Nahari et al., 2015). The composition and biological active value of honey vary according to the flora of the region where it is produced and the production method. The dry weight of honey is 95% sugar, and the rest reveals its biological value. Most of the bioactive components in honey are polyphenols and dark colored honeys always have high polyphenol values (Kolaylı et al. 2018).

Turkiye has rich floral sources as well as plant diversity deriving from its geographical location and is the world's third largest honey producer. It produces a wide variety of unifloral and heterofloral blossom and dew honeys. The honeys of forest honey are also divided into two parts among themselves, as blossom or flower honeys and secretory or dew honeys. Blossom honey is the honey that honeybees collect only from flower nectars, and chestnut (*Chestnut sativa* L), acacia (Acacia ssp.) linden(*Tilia* ssp), naringenin(Citrus spp.), strawberry(Arbutus ssp.) tree honey and ayıfındığı (*Styrax officinal*) honeys are the most produced forest blossom honey in Turkey(Gül et al. 2018). And also, dew honeys are the honeys produced from the secretions secreted from the non-flowering parts of the trees in various ways, pine (Pinus spp.) honey, oak(Quercus ssp.) honey, cedar (*Cedrus libani*) honey are the most important forest-secretory honeys(Kolaylı et al. 2018; Can et al. 2015; Gül et al. 2018). The most important indicator of whether a honey is secretory or floral honey is the optical rotation value, this value is always negative in flower honeys and positive in secretory honeys(Kaygusuz et al. 2016; Can et al. 2015; Kolaylı et al. 2018). The other indicator is pollen analysis values, and there is a relatively low number of pollen in dew honeys. Due to its low pollen value, slag honey does not crystallize easily. The most important feature that distinguishes forest honeys from flower and other honeys is that the conductivity value of these honeys is always high.

2. Materials and Methods

Honey Samples

Since this study is a compilation study, it was created by scanning the literature. For this reason, literature was used as the material method.

3. Results

In the evaluation of the data obtained because of the literature review, the physicochemical properties of chestnut, linden, pine and oak honeys, which are major forest honeys produced in Turkey, were given in Table I. The results are showed that the optical rotation values were found to be negative in flower honeys and positive in secretory honeys. Among the forest honey, linden, strawberry trees and rhododendron honeys were found light colored honey, but oak and chestnut honeys were darker colored honeys.

Table I. Some physicochemical properties of forest honey

	Optic Rotation $[\alpha]_{20}$	Conductivity (mS/cm^2)	Color (Hunter L)	Total polyphenols (mgGAE/g)	References
Chestnut	-1,50±0,86	1,20±0,55	35,60±4,50	76,30±7,20	Can et al. (2015)
Chestnut	-2,72±1,66	1,54±0,30	52,93±4,60	70,80±22,38	Yildiz et al. (2022)
Chestnut	-	-	-	112,20±20	Kaygusuz et al. 2016
Chestnut	-	-	-	239,00	Küçük et al. (2007)
Thyme	-1,28±0,55	0,74±0,24	68,80±4,30	32,30±2,04	Can et al. (2015)
Thyme	-	--	-		Sıcak, (2021)
Strawberry	-	-	-	-	Tuberoso et al. (2010).
Strawberry	--		-	31,10	Lovaković, et al. (2018).
Strawberry	-	--	-	91-96	Ulloa et al. (2015).
Acacia		-	-		Gul et al. (2018)
Pine	-	-	-	52,40±3,00	Kaygusuz et al. 2016
Citrus	-	-	--		Gül et al. (2018)
Rhododendron spp.(n=3)	-2,47±0,86	0,64±0,12	78,63±0,78	49,53±8,10	Yildiz et al. (2022)
Rhododendron spp.(n=3)	--	-	-		Şahin et al. (2015)
Pine (n=6)	0,78±0,03	1,13±0,17	66,67±9,85	42,30±4,50	Can et al. (2015)
Pine (n=6)	-	-	-	-	Tananaki et al. (2007).
Cedrus	-	-	-	68,30±	Kaygusuz et al. 2016
Oak (n=6)	+0,78±0,03	0,95±0,50	40,08±4,50	66,35±5,28	Kolaylı et al. 2018
Oak				44-75	Kara et al. (2019)
Oak				134	Sahin, (2016).
Oak					Ozkok, & Sorkun, (2018).

4. Discussion

The value of honey is increasing day to day due to both its nutritional and medical benefits. Since the interest in honey with high medicinal value in the world is increasing every day and forest honey is emerging once again. The only known optical rotation values in the world to distinguish between floral and secretory honeys are the data of this study confirming this. There is not exactly a distinguishing indicator used to distinguish forest honeys from flower honeys, but higher conductivity values and polyphenol contents of forest honeys are noteworthy. however, forest honeys are divided into two sub-classes as dark-colored honeys and light-colored honeys. For example, rhododendron, linden and citrus honeys and arbutus honeys are in the light-colored forest honey class.

Chestnut honey is one of the most studied honeys in the literature with its medicinal honey properties, and it is reported that this honey is superior to the honeys that do not crystallize easily with its high polyphenol and mineral substance properties (Kaygusuz et al.2016; Sarıkaya et al. 2009; Küçük et al. 2007). Turkey is one of the countries where chestnut honey is produced the most in the world, and chestnut honey is produced mostly in the Black Sea region and the Marmara region. The color values of this honey vary depending on the monofloral feature (Gül et al. 2018). It is also reported that chestnut honey is rich in minerals (Küçük et al. 20007).

Rhododendron honey is produced mostly in Turkey. R. ponticum species, which grows as a shrub plant throughout the Black Sea region, is a purple-flowered plant with high honey yield and has the quality of mad honey, sometimes called toxic honey (Can et al. 2018; Gül et al. 2015; Şahin et al. 2015).

Dew honey is produced with two different ways. First way is collected from some sugars or liquids leaking from trees and leaves in the form of sweating depending on climate conditions such as oak (*Kernes guercus*), spruce, fir honey (Kolaylı et al. 2018). Second way is excretions of sucking insects, such as aphids, mealy bugs, whiteflies, soft scale insect such as *Thelaxes dryophila*. *Marchalina hellenica* is mostly habitat in the Aegean and Mediterranean regions and live on certain pine tree species such as *P. brutia* (Karabagias, et al. 2014). The most pine honey (nearly 90%) is produced in Aegean and Mediterranean coast of Turkey, nearly 90% of the world's pine honey is produced in Aegean and Mediterranean coast of Turkey is suitable climatic conditions and relative humidity for living of the aphits. In Turkey, mostly pine honey production is carried out around Muğla province, and lesser than in Aegean and Mediterranean coast. Nearly, ten thousand beekeepers and forty thousand tons of the pine honey are produced annually in this region. Oak (Quercus ssp.) honey is the second type of dew honey is produced in high amount by sweating leaves under stress conditions such as sudden temperature changes, or nocturnal and diurnal temperature changes(Kolaylı et al. 2018).

5. Conclusion

As a result, it was determined that Turkey has a rich diversity of forest honeys in terms of flora, but a geography rich in secretory honeys. In addition, the high conductivity values of forest honey show that flowers are richer in terms of minerals.

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Newly discovered probiotic product: perga

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Abstract:
Bee bread is a bee product that is not easily harvested because it is not easy to harvest and the honeycomb is destroyed during harvest. “Bee bread (Perga) is obtained as a result of lactic acid fermentation, which is formed by covering with a thin layer of honey and bee wax after the pollen collected from plants and mixed with digestive enzymes are stored in the honeycomb cells. Honeybees use perga especially for feeding larvae and young bees producing royal jelly. The nutritional value of bee bread is higher than that of bee pollen, and it is more digestible due to its higher free amino acid content and the presence of easily assimilable sugars. This event is provided by the degradation of the pollen wall, which restricts the digestion and bioavailability of pollen, by fermentation in bee bread, and the part containing nutrients comes out. In addition, despite the biochemical transformation that occurs during the fermentation of bee bread, phenolic compounds are not affected and remain unchanged. Studies on the chemical content of bee bread generally show that it consists of water, proteins, free amino acids, carbohydrates, fatty acids and other bioactive molecules. It is also shown to have components such as phenolic compounds, alpha-tocopherol and coenzyme Q10. The composition of bee products may vary depending on the plant source from which the pollen is collected, climate and seasonal conditions. This feature makes its standardization difficult. The biological effect of bee bread is probably associated with the presence of antioxidants such as phenolic compounds, especially flavonoids. After a period full of processed and additives/preservatives in the food industry, people's return to natural foods is accepted all over the world. The expectations of consumers who do not want chemicals in the food they consume are increasing. Consumer demand is the introduction of functional foods, which are characterized by health-oriented properties, are effective in increasing the overall efficiency of an organism and even prevent the development of certain diseases.

Keywords: bee bread, perga, bee products, bioactivity

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1. Introduction

1.1. Bee collected pollen and bee bread (Perga)

Bee collected pollen (BP) and bee bread (BB) have a high nutritional value and include bioactive compounds, which have a positive effect on human health, and therefore are regarded as “functional foods”. They are rich in proteins, unsaturated fatty acids (omega fatty acids), simple sugars, essential amino acids. These properties strengthen immun system and help the body to fight microorganisms, which will keep the body healthy (8). Bee pollen and bee bread are in the structure of many biochemical compunds found throughout functional foods such as phytochemicals (phenolic acids, polyphenols, terpens), bioactive peptides, prebiotics and probiotics, fibre, vitamin and minerals and organic acids.

Today, the idea that some foods will help protect human health in the food industry has been the subject of many researchers. It is known that nutrition plays an important role in the prevention and treatment of diseases. Today, even the basic concepts of nutrition can change. The classical concept of "adequate nutrition", that is, the understanding of consuming basic nutrients (carbohydrate, protein, fat, vitamin-minerals) has left its place to the concept of "optimal nutrition". Terms such as nutraceuticals, therapeutic foods, functional foods, super foods, functional foods have come to the fore in the sense that foods increase general well-being and reduce the risk of developing certain diseases. In fact, with these expressions, it is meant that some of the components in the food or a single component have a physiological or psychological positive effect, and this positive effect reduces the risk of a certain disease and ensures the maintenance of health and well-being. A functional food can be natural or effective by eliminating or replacing one/more ingredients. Some components (omega-3, vitamins, probiotics, prebiotics, symbiotics, fiber, phytochemicals, bioactive peptides, etc.) can add functionality to food.

1.2. Functional Foods

Beyond meeting the basic nutritional needs of the body, they are food or food components that are effective in preventing diseases and leading a healthier life by providing additional benefits on human physiology and metabolic functions. Functional foods can be an unprocessed natural food or a food enriched with a functional nutrient or genetically modified. For example, eggs with omega 3 fatty acids. In addition, functional food can be obtained by adding phenolic substances, antioxidants, dietary fibers, oligosaccharides, probiotics, prebiotics, vitamins, polyunsaturated fatty acids, sulfur-containing compounds, plant sterols and phyto-estrogens to foods. Functional food includes foodstuffs that reduce the risk of disease and have a beneficial effect on health.

1.3. Probiotics

Probiotics are live microorganisms that settle in the intestinal tract of their host, improve the microbial balance and perform beneficial activities. Probiotics are effective by producing inhibitory substances (bacteriocins, organic acids, etc.) against harmful microorganisms, by competing for nutrients and making it difficult for them to adhere, and by breaking down their toxins that can turn into carcinogenic and mutagenic compounds. The best-known microorganisms with probiotic properties are; Lactobacillus spp., Bifidobacterium spp., *Streptococus thermophilus* and *Sacharomyces boulardi*. Substances that can reach the colon without being digested and selectively support the development of probiotics are called prebiotics. Substances such as inulin, fructooligosaccharides and beta-glucan can be given as examples. Some fruits and vegetables (mussels, onions), grains (wheat, oats) and legumes (soy) also contain prebiotics.

Colonization of toxin-producing bacteria in the intestine plays an important role in the intestinal problems of autistic patients. Probiotics (live microorganisms) can balance intestinal bacteria, while prebiotics can inhibit the growth of pathogenic microorganisms by feeding beneficial bacteria. Thus, both probiotics and prebiotics can preserve healthy bacteria in the gut. Aabed et al. (2019) determined the effect of probiotics and prebiotics on balancing the intestinal flora in a rodent model of autism associated with an altered intestinal structure due to the use of clindamycin, and investigated their effects on oxidative stress markers in the brain. To create an autistic model, rats were administered 250 mg of propionic acid/kgbw/day for 3 days. Animals treated with clindamycin 30 mg/kg BW for 3 days, bee pollen and propolis (default prebiotics, 250 mg/kg/day) for 28 days, and *Lactobacillus paracaseii* and Protexin® (probiotic) for 28 days and finally treated by anorectal transplant with faeces from normal animals for 5 days. Significant changes in oxidative stress markers, primarily glutathione and vitamin C, were measured in the brains of animals in the group treated with propionic acid and treated with clindamycin. While all probiotic/prebiotic treatments showed curative effects, Lactobacilli had the strongest effect. Stool transplants did not show the expected effect. It has been concluded that pro- and prebiotic supplements can be effective in stimulating healthy digestive system function in autistic patients, and propolis and pollen have an important role in this regard.

Probiotics are defined as food additives that provide positive effects on host health when consumed within certain limits. Lactic acid bacteria are the group of microorganisms most studied for the effects of probiotics on human health. Scientific research suggests the use of probiotics as an inexpensive and safe way to maintain a healthy diet for humans and animals and to protect them from infections. Lactobacillus acidophilus, *Lactobacillus paracasei* and Bifidobacterium species isolated from the intestinal tract of humans and animals are commonly used bacteria for this purpose. The use of probiotic preparations prepared using these bacteria in industrial food production processes is becoming increasingly common. However, consumers prefer to use natural additives without using preservatives.

The properties required for a bacterial strain to be defined as an effective probiotic are determined on the basis of both its behavior in the food system in which it will be used and the positive effects it will have on the health of the target consumer. In this regard, the strain's human origin and survival in the gastrointestinal tract, resistance to gastric acid and bile salt at physiological concentration, as well as its ability to adhere to small intestinal epithelial cells are among the micro-organism behaviors that should be known. The adhesion properties of probiotic microorganisms to the small intestinal mucosa constitute the first stage of colonization in the gastrointestinal tract. Thanks to this colonization, the duration of action of the probiotic microorganism increases and immune system modulation can be achieved.

Due to their increasing value in industrial use, studies are concentrated on the identification of strains with superior probiotic properties.

They are live microbial food supplements that provide beneficial effects on the health of the host by regulating the microbial balance of the intestinal system. The definition of probiotic includes all microbial preparations added to food, feed or food additives that support and strengthen human and animal health. The most important group of probiotic microorganisms is lactic acid bacteria. Among them, Bifidobacterium and Lactobacillus species are the most widely used probiotic microorganisms.

The effects of probiotics on health have been proven by clinical trials to lead a healthy life, to increase body resistance, to use probiotics in the fight against intestinal irregularity and diseases. Effects of probiotics;

a) In lactose intolerance; Lactose intolerance (inability to digest lactose) results from a decrease in the lactase enzyme as a result of deterioration of the intestinal mucosa caused by advancing age, digestive system diseases or some treatments such as antibiotic use. Due to the lactose remaining undigested in patients, the osmotic balance is disturbed and fluid and electrolyte accumulation occurs in the intestine, and hydrogen, methane and carbon dioxide gases are released as a result of the fermentation of lactose by the bacteria in the flora. This causes excessive gas, bloating, nausea and diarrhea. It has been determined that as a result of the disintegration of probiotic bacteria in the small intestine by the effect of bile salts, bacterial lactase is released and metabolizes lactose, reducing the activity of fecal bacterial enzymes such as beta-glucuronidase, nitroreductase and azonitroreductase.

b) Diarrhea (diarrhoea); It occurs as a result of increased peristaltic movements in the intestine, decreased absorption and/or increased secretion. It is caused by infections caused by Escherichia coli, Salmonella, Clostridium difficile and rotaviruses. There are clinical studies showing the most positive effects of the prophylactic use of Lactobacillus GG on pediatric diarrhea. The most common (5-25% of patients) complication seen in patients receiving antibiotic therapy is antibiotic-induced diarrhea. In the study examining the effect of Lactobacillus GG on the prevention of AID in adults, asymptomatic *Helicobacter pylori*-infected patients resulted in a decrease in diarrhea and vomiting complaints as a result of antibiotic treatment with or without probiotics. Diarrhea is the main complication seen in cancer patients undergoing radiotherapy. In a study of 190 patients who received radiotherapy, it was determined that the probiotic product significantly reduced the severity of acute diarrhea compared to the placebo group.

c) *Helicobacter pylori* infections; *H. pylori* is a cause of chronic gastritis, peptic ulcer and gastric cancer. In studies on the effect of probiotics on *H. pylori* infections, *Lb. salivarius* inhibits and decreases the colonization of *H. pylori* and stimulates IL-8 secretion. In the study, in which patients with *H pylori* who received triple antibiotic therapy were fed yoghurt containing probiotic Lactobacillus and Bifidobacterium strains, it was determined that the group that received probiotics was more successful in the treatment of infection.

d) Crohn's disease; It is defined as transmural inflammation affecting the gastrointestinal tract. In a study investigating the effect of preventing recurrence of postoperative Crohn's disease lesions, a significant decrease was found in the recurrence rate of lesions in patients treated with antibiotic-probiotic combination compared to the placebo group.

e) Ulcerative colitis (inflammatory bowel disease) In a study conducted on this

subject, it was observed that symptoms increased in only three of 11 patients in the probiotic-administered patient group and in nine of 10 patients in the placebo group.

f) Pouchitis; It is a disease defined by inflammation occurring in the ileal region after

ileal-anal anastomosis, which is a factor that triggers the irregularities in the intestinal flora. In a study, it was determined that the use of probiotics induced changes in the sac microflora.

g) Irritable bowel syndrome; It is a functional disease characterized by abdominal pain

or complaints related to irregular defecation. It was determined that the intestinal microflora of these patients was different from healthy individuals and that the foods were highly fermented in these patients. In the study, it was determined that the probiotic product had a therapeutic effect in 50% of the patients.

h) Cancer; Bacteria in the intestinal system are important in the inactivation of carcinogens, preventing their spread and conversion to cancer-causing substances. In some in vitro studies, it has been determined that the risk of cancer is reduced by preventing the possible mutagenic and genotoxic effects of probiotic bacteria. Most of the studies on the effects of probiotics on cancer are on colorectal cancer. As the mechanisms of inhibition of colon cancer by lactic acid bacteria; Strengthening the host's immune response, deterioration of the structures of potential carcinogenic compounds, qualitative and/or quantitative changes in intestinal flora, production of antimutagenic and antitumorogenic compounds in the colon, alteration of metabolic activities in intestinal microflora (inhibition of conversion of precarcinogens to cancer), physicochemical change in the colon (improved intestinal permeability, prevention of toxin absorption or delay, strengthened intestinal barrier mechanisms, and positive effects on host physiology.

i) Other effects; Cholesterol-lowering properties of probiotics (by lowering the pH due to acid production by lactic acid bacteria, causing precipitation of cholesterol with deconjugated bile salts), increasing calcium absorption, positive effects in patients with atopic dermatitis and food allergy (by stimulating natural barrier mechanisms), preventing urogenital Candida albians infections or It has also been reported that it reduces the recurrence rate and its effect on bacterial vaginosis (the development of anaerobic pathogens is caused by the decrease or absence of lactobacilli in the vagina), diarrhea in children, respiratory and tooth decay infections, atopic dermatitis in infants and necrotizing enterocolitis in newborns.

1.4. Probiotic properties of bee bread (Perga)

Gilliam et al. (1979) isolated 113 yeasts from almond blossom pollen, pollen collected from traps, and samples of 1,3 and 6 weeks old bee bread. Torulopsis magnoiae was detected as the most common isolate in all samples except flower pollen. For this reason, it was thought that it was added by bees. The number of isolates and species decreased with time and storage. Most of the yeast species from flower pollen and trap pollen in general were not found in bee bread. In addition, yeast isolated from flower pollen and pollen traps fermented more sugar and assimilated more carbohydrate compounds than bee bread. The lactic acid fermentation caused by bacteria and yeasts in the transformation of pollen into bee bread causes biochemical changes as a result of the activity of microorganisms.

Essentially, microbial changes in the fermentation process were described by Chevtchik in the 1950s. The researcher, who divided the fermentation process into 4 phases in the microbial activity that took place for 7 days from the appearance of anaerobic bacteria and lactic acid bacteria in the transformation from pollen to bee bread, was characterized by the development of a heterogeneous group of microorganisms, including yeasts, in the first phase completed in 12 hours. In the second phase, growth factors produced by putrifying bacteria and yeasts are utilized by decreasing the pH of the pollen. Then phase 3 is characterized by the disappearance of streptococci and the growth of lactobacilli (which produce more acid than Streptococci). Then, the 4th Phase, which started at the end of the 7th day, was characterized by the disappearance of some yeasts due to the abundant lactic acid produced by the lactic acid bacteria.

Pollen has a pH of about 4. Pain and Maugenet (1966) reported that 3 microbial genera (Lactobacillus, Pseudomonas and Saccaharomyces) are important in the modification of pollen during storage. While lactobacilli cause lactic acid fermentation, which stabilizes pollen thanks to increased acidity, the role of Pseudomonas and Saccharomyces has not been fully defined. Pain and Maugenet (1966) reported that pesudomonas probably contributes to the anaerobiosis required by lactobacilli and degradation of pollen walls. Because they developed as soon as they were placed in the comb cell, but were not seen after 2-3 days. Later, lactic acid is produced by lactobacilli. Although lactic acid fermentation is completed after 15 days, the responsible organisms remain as a stable population for months. Initially few yeasts increase after fermentation and are substituted in stored pollen for a longer period of time than other microorganisms. These studies have shown that when pollen is sterilized by gamma irrigation and only lactic acid bacteria (LAB) fermentation, pollen becomes poor in nutritional value for them.

Gilliam et al. (1989) isolated 148 molds from corbicular pollen collected from traps attached to A. mellifera colonies in almond orchards, hand-picked flower pollen (Prunus dulcis; almond) and bee bread samples stored in comb cells at 1, 3 and 6 weeks of age. Most of the molds identified belonged to Penicillia (32%), Mucorales (21%) and Aspergilli (17%) genera. In general, it was determined that the number of isolates decreased in pollen collected and stored by bees. Each pollen sample differed according to mold flora and dominant microorganzima species. Molds of *Aurebasidium pullulans, Penicillium corylophilum, Penicillium crustosum* and *Rhizopus nigricans* have been identified in collected and stored pollen. Mucor sp. While it was the dominant mold in floral pollen, it was not found in corbicular pollen and bee bread. The 19 enzyme tests performed showed that most molds produced caprylate esterase, lipase, leucine aminopeptidase, acid phosphatase, phosphosmidase, beta glucosidase and N-acetyl beta glucominidase. For this reason, it has been determined that these enzymes, which are involved in lipid protein and carbohydrate metabolism, are produced by pollen molds.

Egorova et al. (1971) found bacteria called Lactobacterium pollinisi in bee bread samples collected in spring and summer, both in pure and mixed cultures. The pure culture was subcultured every 30 days, while the yeasts survived for 6 months without subculture. This showed that yeasts provided growth factors to lactic acid producing bacteria. *Torulopsis magnoliae* was observed in all samples except flower pollen, while *Candida guilliermondi var guilliermondii* is a common isolate and was found only in pollen taken from flowers and traps. While *Cryptococcus albidus* was isolated from flower pollen, most other isolates were isolated from 3 and 6 week old bee bread, and the number of species isolated from flower pollen decreased with time and storage.

Mathialagan et al. (2018) isolation, characterization and identification of LAB species from honey stomach, bee pollen and bee bread and royal jelly of different bee species (*Apis cerana indica, Apis mellifera, Apis florea, Apis dorsata, Tetragonula iridipennis*). 42 LAB isolates were obtained from the tested samples. Enterococci (23.8%), micrococci (18.8%), streptococci (13.8%), pediococci (13.8%), lactobacilli strains (10.0%), lactococci (10.0%) and leukonostoks (10.0%) were found. Bacterial populations ranged from 16.40-35.87 x 105 CFU/ml in honey stomach, 10.43-31.30 x 105 CFU/ml in honey, and 5.14-14.35 x 105 CFU/ml in bee pollen. 13.05 - 25.15 x 105 CFU/ml in bee bread and 10.23 - 20.15 x 105 CFU/ml in royal jelly.

Cadez et al. (2015) isolated five obligate osmophilic yeast strains that could not survive in high water activity from honey and bee bread produced in Hungary. Although 32 yeast strains with incomplete characterization were isolated from bee bread in the study, it was reported that the dominant genus was Zygosaccharomyces. As a result, 6 strains of Z. rouxii, 5 strains of Z. mellis and 3 strains of Z. siamensis were identified.

Songkun et al. (2001) Tea (*Camelia sinensis)* flower pollen and 207 bacterial colonies belonging to 4 genera and 12 species (Lactobacillus jensenii, Lactob. fructosus, Lactob. Sanfrancisco. Lactob. viridescens, Lactob. salivarius, Lactob. minor, Lactob. casei subsp. rhamnosus, Lactob. Plantarum, Listeria innocua, Liste. Grayi, Bacillus subtilis and Lactococcus plantarum). Songkun et al. (2002) found the pH values of tea pollen, corbicular pollen and bee bread to be 5.63, 5.05 and 4.06, respectively, in their research published the following year. Lactobacillus jensenii, Lactobacillus fructosus and Lactococcus plantarum were identified as the main organic acid producing bacteria in bee bread and they reported that Listeria had little effect on the pH of bee bread. Songkun et al. (2002) in the same year, in another study, hydrolyzed amino acid content was determined in tea corbicular (carried in pollen basket) pollen and 5,10 and 15 days old bee bread samples. While the average hydrolyzed amino acid content detected in bee bread was 23.36%, this value was 0.26%

higher than the corbicular pollen content. Total amino acid content in pollen and bee bread was 1037.46 and 801.75 mg/100g. The concentrations of vitamins A, B1, B2, C, D, E (mg/100g) in bee bread and pollen were determined as 0.19, 0.15, 1.15, 0.31, 0.0156, 10.2 and 0.79, 0.09, 2.74, 1.20, 0.0158, 6.6, respectively. Palmitic, stearic, linoleic, linolenic acid contents (%) in bee pollen and bee bread were found to be 1.3, 0.2, 0.16, 2.5 and 1.1, 1.0, 0.3, 1.0. Amylase and hydrogen peroxidase enzyme activity in one-day-old bee bread is higher than that in corbicular pollen, while superoxide dismutase activity is the opposite. Hydrogen peroxidase and superoxide dismutase enzyme activity in bee bread decreased with increasing storage time, while amylase activity increased with increasing storage time.

2. Results and Disussion

Bee bread (perga) is a unique bee product not only for bees but also for humans. Since it is not easy to obtain, the price of honey is higher. Bee bread basically contains pollen, honey and salivary gland secretions of bees. It is filled in honeycomb cells and fixed with honey and beeswax. It undergoes lactic acid fermentation in the comb cell and this fermented pollen is called “bee bread/perga”. Bee bread is characterized by better digestibility than pollen and higher nutritional value according to its chemical composition. Most importantly, since the components of bee bread are fermented, they are more easily assimilated in the organism and better digested by both the bee and human body. Bee bread contains quite a lot of peptides and amino acids. It is an excellent product that can fill the nutrient deficiency in the human organism. It strengthens the organism and ensures its healthy functioning. Because it contains all of the essential amino acids, the bee product has a better composition than many products preferred for animal proteins.

3. Conclusion

For centuries, people have unknowingly used lactic acid bacteria for fermentation in different areas such as bread, silage, cheese, fermented milk, meat products, fish, legumes. When the bees that bring pollen from the plants encounter the trap barrier at the entrance to the hive, the pollen loads they carry on their legs are scraped off in the perforated traps and fall into the pollen tray. Thus, bee pollen can be obtained easily. However, it is difficult to obtain bee bread in large quantities for technical reasons. They are difficult to remove as they are strongly compressed in the honeycomb eyes. The tools developed for this purpose are based on the principle of grinding bee bread together with a honeycomb. Bee bread is produced by artificial fermentation in a laboratory environment, but its differences with the natural one are clear and studies on this subject continue.

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Functional effect of royal jelly and apilarnil on reproductive health

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Abstract:
Royal jelly is a product secreted from the mandibular and hypopharyngeal glands of young worker bees (5-15 days old) to ensure the nutrition and development of the queen bee. In fact, during the first three days of their life, the young of all three castes (drones, worker bees and queen bees) are fed royal jelly. After the first 3 days, drone and worker bee larvae are fed with a mixture of honey and pollen. The queen bee continues to be fed with royal jelly. It is reported that royal jelly is a strong supporter of healthy aging and longevity. Because it is known to increase the general health and productivity of queen bees, which can live up to five years and lay 3000 eggs per day, compared to infertile worker bees, which live an average of 45 days. As an antioxidant, royal jelly has been shown to reduce oxidative stress damage in reproductive organs.

Apilarnil is a bee product with biologically active properties. It is obtained by filtration and pulverization of drone larva homogenate harvested at the 7-day larval stage before the comb cells are closed. Since apilarnil contains male-specific hormones, it is reported to have male sex-specific enhancing effects. For example, it is recommended as a natural anabolism stimulator in men as it increases muscular body weight. In some cultures, honeybee larvae have been used to treat impotence. While its success in infertility treatment is not attributed to its high protein content, the presence of sex hormones has been demonstrated in later studies.

It is reported that eighty million people worldwide are affected by the inability to have children. Approximately 15% of couples in the reproductive period have infertility problems. While it is estimated that 25-40% of infertility in couples is due to male factors such as oligospermia and/or asthenospermia, it is stated in more recent publications that the male factor ratio is around 50% and one out of every 20 men will be affected by subfertility.

For this reason, it is thought that royal jelly and apilarnil are potential bee products that can support infertility treatment due to their beneficial biological activities.

Keywords: royal jelly, apilarnil, infertility, reproductive health

1. Introduction

Apitherapy, which has been in existence since the beginning of humanity and whose importance has emerged with increasing studies in recent years, draws attention with the prevention of many diseases and support for treatment. Natural products such as honey, pollen, perga, propolis, royal jelly and apilarnil are friendly to all cells in the body and have high antioxidant capacity. There are many compounds such as protein, carbohydrates, vitamins, coenzymes, polyphenols, aroma compounds, phytosterols, terpene and terpenoids, aliphatic compounds, and fatty acids in these products, which honeybees process by collecting nectar and pollen from plants in nature. Since plants have unlimited capacity to produce aromatic and aliphatic compounds, there are plenty of polyphenolic substances in the structure of honey, pollen, propolis and other bee products produced with raw materials collected from plants by honeybees (Silici, 2020).

Royal jelly is a product secreted from the mandibular and hypopharyngeal glands of young worker bees (5-15 days old) to ensure the nutrition and development of the queen bee. In fact, during the first three days of their life, the offspring of all three castes (drones, worker bees and queen bees) are fed royal jelly. After the first 3 days, drone and worker bee larvae are fed with a mixture of honey and pollen. The queen bee continues to be fed with royal jelly. It is reported that royal jelly is a strong supporter of healthy aging and longevity. Because it is known to increase the general health and productivity of queen bees, which can live up to five years and lay 3000 eggs per day, compared to infertile worker bees, which live an average of 45 days. Although both are fertilized eggs, the difference in nutrition causes significant differences in morphology, lifespan and behavior between queen and worker bees. Royal jelly is an optimal food as it contains large number of bioactive substances, thus causing the development and differentiation of the queen bee. However, worker bees that cannot be fed with this product have a shorter life and cannot be fertilized. This is one of the unique differences in honeybee life. The differentiation brought about by the parthenogenesis (development of a living thing from an unfertilized egg) and the formation of drones despite being fertilized, and the nutritional system that provides changes specific to their life and duties, is an adaptation not seen in any other living group. Studies on this subject have shown that when worker bees are fed with royal jelly under experimental conditions, their body size increase and they gain queen bee-specific features such as ovary development. In these experimental conditions, it was determined that ovarian development in worker bees fed with royal jelly was achieved by increasing the levels of tyrosine, dopamine and tyramine in the brain, and by activating the growth-regulating juvenile hormone and epidermal growth factor receptors.

The main components of royal jelly are 60-70% water, 9-18% proteins, 7-18% sugars 3-8% lipids, minerals (Fe, Na, Ca, K, Zn, Mg, Mn and Cu), amino acids (Val, Leu, Ile, Thr, Met, Phe, Lys and Trp), vitamins (A, B complex, C and E), enzymes, hormones, polyphenols, nucleotides and small heterocyclic compounds. The pH value of fresh royal jelly is generally between 3.6-4.2. Royal jelly is a viscous, creamy, acidic, white-yellow colored bee product with a pungent odour, sour-sweet taste. Royal jelly is relatively water soluble and has a density of 1.1 g/mL. Changes in chemical compositions; different feeding (without sugars and/or protein supplements), production methods, environmental conditions, flora, bee breed, storage and processing conditions (Lercker et al. 1992; Nascimento et al. 2015)

It has been shown that royal jelly has numerous functional properties such as antibacterial activity, anti-inflammatory properties, vasodilator (vasodilator) and hypotensive (blood pressure lowering) activities, disinfectant effect, antioxidant effects, anti-hypercholesterolaemic activity and antitumor effect (Chen et al. 2016; Jalali et al. 2015; Kanbur et al. 2009; Kashima et al., 2014; Karaca et al. 2015; Miyata and Sakai, 2018).

While infertility can affect approximately 15% of married couples, half of this is due to male infertility. In married couples receiving chemotherapy, the post-treatment infertility rate may increase to 15-30%. Male infertility is defined as a change in one of the parameters such as sperm motility, morphology and number in at least one of the two ejaculates taken from the male at intervals of 1-4 weeks. One of the negative effects of chemotherapeutic drugs in the reproductive system is male infertility and even sterility. In addition, the undesirable effects of chemotherapeutics in the reproductive system include defects in spermatogenesis, ejaculation disorders, sperm quality parameters, dysfunctions in the hypothalamus-pituitary-gonad axis and sexual dysfunctions. Although Leydig and Sertoli cells in the testis are partially resistant to chemotherapeutics, the germinal epithelium is highly sensitive. However, if the stem cells in the germinal epithelium remain intact, depending on the dose and duration of administration of the drug used, spermatogenesis may return after treatment (Sigman and Jarow, 2007).

In studies conducted with human cancer and cancer-induced experimental animals, hormone applications, sperm and testicular tissue cryopreservation, testicular biopsy and in vitro spermatogenesis, testicular transplantation and various substances with antioxidant activity, support and complementary medicine applications can reduce the side effects on the male reproductive system before or during chemotherapy. It has also been reported to be effective in preventing However, many in vitro and in vivo studies have been and continue to be conducted in order to detect the negative effects of different chemotherapeutics on the male reproductive system in healthy animals (human/experimental animals) and to reduce/prevent these effects. As a matter of fact, in most of these studies, the side effects of chemotherapeutics in the male reproductive system with the mechanism of DNA damage and the protective roles of substances with antioxidant properties are emphasized.

Spermatogenesis is the process in which approximately one thousand mature spermatozoa are formed per second from spermatogonia, and spermatocytogenesis is the processes involving cell division and differentiation in the stages of meiosis and spermiogenesis. As a result of spermatocytogenesis, spermatocytes develop from spermatogonia, spermatids from spermatocytes by meiosis, and mature sperms from spermatids by spermiogenesis. According to the development order in spermatogonia; There are stem, proliferative and differentiating cells. At this point, although the side-effect mechanism of chemotherapeutics in the male reproductive system is not clearly known, chromatin and DNA damage that occurs directly and/or related to oxidative stress in spermatogenic cells and problems in steroidogensis are held responsible for these effects. Since chemotherapeutics have primary side effects on healthy cells capable of rapid proliferation and differentiation, cytotoxic chemotherapy directly causes gonadal damage, while the degree of damage may vary depending on factors such as the dose of the drug, the mode of administration and the age of the patient. If radiotherapy is given together with chemotherapy, spermatogenesis is significantly affected even at low doses. Root spermatogonia may be more resistant to direct effects because they do not show proliferation and differentiation, whereas spermatogonia, spermatocytes and spermatids that show proliferation and differentiation are more sensitive to direct effects, and fertility potential decreases 4-6 weeks after the start of treatment. Although sperm DNA has a denser structure than the DNA of normal somatic cells, it can be directly or indirectly affected by negative factors. Chemotherapeutics have direct side effects by causing DNA and chromatin damage such as chromosomal aneuploidies, disruptions in spermatozoal decondensation time, breaks in spindle fibers, negative changes in the chromatin structure of proliferative and differentiated spermatogenic cells, DNA methylation profile, simple and core matrix proteins in the head of sperm, and negative changes in the structure of protamine and histones. they can create (Sigman and Jarow, 2007).

Most of the drugs used in cancer treatment cause gonadotoxic damages in the male reproductive system either directly or indirectly by causing oxidative stress in testicles and semen. There are many studies on the use of antioxidants to prevent/reduce the side effects of drugs used in cancer treatment on the male reproductive system. For example, omega-3 and vitamin C have been found to be effective in reducing sperm count caused by azathioprine, testicular atrophy, increase in morphological structure disorder, and DNA and chromosome damage. It has been reported that methotrexate can reduce testicular and spermatozoal toxicity, folic acid can reduce the folate mechanism, and caffeine, taurine and chlorogenic acid can reduce the oxidant-antioxidant balance. There are studies that show that herbal extracts such as grapefruit, green tea, goji berry, prune prevent all or a significant part of testicular, spermatozoal and mutagenic damage caused by doxorubicin, thanks to their strong antioxidant and antimutagenic effects. Apart from these, it has been reported that chemicals such as flavonoids ellagic acid, a carotenoid lycopene, beta-amino acid taurine, with their free radical scavenging effects, can prevent the negative effects in sperm quality parameters (motility, density, abnormality) caused by dexorubucine, damage to steroidogenesis, and increases in the number of apoptotic germ cells. In order to prevent the side effects of cisplatin, which is one of the chemotherapeutics with the most side effects, many antioxidants such as melatonin, resveratrol, lycopene, ascorbic acid, ellagic acid, curcumin, royal jelly, apilarnil, vitamin C, ginger, structural, functional and genetic disorders in testicles and sperm and testosterone (Lewis et al. 1997; Showel et al. 2014; Silici et al. 2009)

Delkhoshe-Kasmaie et al (2014) evaluated the protective effects of royal jelly on the damage caused by Taxol (TXL) of the testis. While the sperm viability reduced by Taxol was 27.5% in the control group, 80.5% ± 10.6% of the sperm were found alive in the animal group that received 150 mg/kg royal jelly, and it was recovered with royal jelly application. TXL-exposed and TXL plus royal jelly administered animals showed a marked upregulation of the transcription factor E2f1 mRNA. Their data showed that the histopathological and biochemical changes induced by TXL could be preserved by the application of royal jelly. The protective effects of royal jelly have been attributed to its antioxidant capacity and its ability to regulate E2f1 expression. Ahmed et al. (2014) investigated the potential protective effects of royal jelly against azathioprine-induced toxicity in the rat. In addition, royal jelly provided significant protection against azathioprine-induced liver injury by reducing the increased activities of serum hepatic enzymes. In addition, royal jelly blocked azathioprine-induced lipid peroxidation by reducing malondialdehyde formation. As a result, it has been reported that royal jelly has the ability to alleviate azathioprine-induced toxicity. El-Alfy et al. (2013) determined the protective role of royal jelly on mice after endoxan treatment. Amirshahi et al. (2014) reported that bleomycin (BL) is routinely used in the treatment of human cancers and adversely affects sperm parameters. They showed that royal jelly with antioxidant properties has positive effects on MDA and other parameters. Abdel-Hafez et al. (2017) evaluated the possible protective role of royal jelly in ameliorating the toxic effects of cyclophosphamide (CP) overdose in rat prostate tissue. Prostate samples were used in biochemical, histological and immunohistochemical studies. Mean area fractions of eNOS and Bax expression in all groups were measured and statistical analysis was performed. The results showed obvious biological changes in the CP-treated group, in the form of a marked increase in prostatic malondialdehyde (MDA) and C-reactive protein (CRP). There was a significant decrease in glutathione peroxidase (GPx) in prostate tissue compared to the control group. In addition, histological changes showed marked acinar and stromal prostatic degeneration. Most prostate acini showed less PAS reaction and more eNOS and Bax expression compared to the control group. Co-administration of royal jelly with CP provided a noticeable improvement in these biochemical and histological changes. In conclusion, royal jelly provided biochemical and histopathological improvement in CP-induced prostatic tissue toxicity. These findings suggested that this improvement was associated with a reduction in tissue oxidative damage and apoptosis.

Apilarnil is a bee product with biologically active properties. It is obtained by filtration and pulverization of drone larva homogenate harvested at the 7-day larval stage before the comb cells are closed. In Romania, apilarnil was first produced by Nicolae V Illesiu in 1980, at the time of the highest development in the chemical synthesis of medicinal drugs. The name APILARNIL is abbreviated from the name of the Romanian scientist; API; bee LAR, larva and NIL (Nicolae Iliesiu).

Apilarnil is a homogeneous and milky substance, yellowish gray in color, with a sour taste. It is ripened easily and stored in the deep freeze in its raw form. It contains water (65-75%), proteins (9-12%), carbohydrates (6-12%), fatty acids and lipids (3.5-8%), K, Na, Ca, Mg minerals (1-1.5%), amino acids (threonine, leucine, isoleucine, methionine) as well as sex hormones such as testosterone, prolactin, progesterone and estradiol. Although its physicochemical properties are similar to royal jelly, it contains some differences. It is also reported to have male sex-specific strengthening effects as it contains male-specific hormones. For example, it is recommended as a natural anabolism stimulator in men as it increases muscular body weight. Its basic component is amino acids, and it is among the unique products that contain all essential amino acids that cannot be synthesized by human or animal organisms.

In some cultures, honeybee larvae have been used to treat impotence. While its success in infertility treatment is not attributed to its high protein content, the presence of sex hormones has been revealed in later studies. Fresh drone homogenate was found to contain 0.31 nmol/100g testosterone, 51.3 nmol/100g progesterone, 410 nmol/100g prolactin and 677.6 nmol/100g estradiol Budnikova et al. (2009) revealed the dynamics of sex hormones from larva to pupa, while five-day-old larvae contained 8.2 nmol/l testosterone and 2745 nmol/l, 15-17-day-old pupae contained 15.6 nmol/l testosterone and 343.5 nmol/l estradiol. Drone larva was found to have more pronounced gonadotropic activity than royal jelly. Valer'evna et al. (2018) investigated the effects of drone brood homogenate on testosterone and porcine cortisol levels and their productive parameters. They reported that the inclusion of drone brood homogenate in the pig's diet affected the growth rate of the pigs as evidenced by the increase in indicators such as hormonal status, live weight, average daily gain, percentage of slaughter yield of the animals (Balkanska et al. 2014; Barnutiu 2013; Finke 2005)

Shoinbayeva et al. (2017) conducted their research to examine the physico-chemical properties of apistimul preparation based on sodium chloride and drone homogenate lyophilized by vacuum freeze drying and to examine the effect of this preparation on the productivity and reproductive function of rams. As a result of studies, they determined the physico-chemical composition of the preparation and determined the presence of biologically active substances and hormones - estradiol, testosterone, progesterone, cortisol and prolactin. They stated that the administration of the drug to animals increases the amount of hemoglobin and erythrocytes in the blood of experimental animals, improves the qualitative and quantitative characteristics of the ejaculate, and has a stimulating effect on the reproductive function of rams.

Altan et al. (2013) aimed to determine the possibility of stimulating sexual development at an early age in male and female broiler chickens by the application of apilarnil, a natural bee product, in the pre-pubertal period. In the study, apilarnil was given orally to animals between 28 and 55 days of age. They evaluated the effects of low (2.5 g/broiler) and high (7.5 g/broiler) doses of apilarnil on growth performance, testicular weight, secondary sexual characteristics, blood lipids, testosterone, and frightening behavior. They stated that the application of apilarnil did not cause a positive effect on the growth performance of male and female broilers and that apilarnil did not have an anabolic effect. They reported that Apilarnil administration suppressed blood glucose and cholesterol. Broilers that received Apilarnil remained still for a shorter period of time in the immobility test and showed cage avoidance behavior, indicating a lower level of fear. They reported that the increase in testicular weight, testosterone concentration, and comb growth in men taking Apilarnil meant that it stimulated sexual maturation at an early age. However, they noted that no similar secondary sexual stimulation was observed in women.

Bolatova et al. (2015) collected nine and ten-day-old drone broods, homogenized them and stabilized them in 70-degree alcohol. Chemical analyzes of the resulting extract revealed the presence of a number of biologically active substances, including sex hormones. They reported that injecting the extract into young pigs increased the weight of the seminal glands by 20.1-21.9% and the epididymis by 21.8--25.8%, while also reducing the sexual dysfunction of pigs (83.3%). It was reported that giving the extract to the pig increased ejaculate volume by 54.3%, germ cell density by 27.1%, survival time by 51.2% and mobility by 14.2%. They determined that it reduced the number of damaged acrosomes in the spermatozoon by 2.1 times and increased the sowing yield by 76.4%. Low androgenic activity is called androgen deficiency syndrome. Androgen deficiency syndrome causes a decrease in the development of the penis and testicles at an early age and prevents puberty. In young people, gynecomastia causes weakness in facial, body or pubic hair and voice development, while in adults, it causes problems such as mood changes, decreased muscle strength, increase in body fat, decreased libido, difficulty in erection, low sperm volume and gynecomastia. Erdem (2018) stated that Apilarnil can increase androgen in patients with androgen deficiency syndrome.

2. Results and Discussion

The quality of apilarnil is determined by the proteins, carbohydrates, fats, polyphenols, amino acids, vitamins and mineral substances in its structure, and its protein profile changes depending on the type of pollen consumed by the bees. The rich polyphenols in its structure ensure that it has a high antioxidant capacity and shows higher antioxidant properties than royal jelly. It has been stated that apilarnil, which triggers the release of androgenic hormones, but whose mechanism of action is not fully known, gives successful results in sexual performance (impotence), erection difficulty, premature ejaculation, and sperm deficiency problems, especially in men, and is sold as "natural viagra" in Far Eastern countries. As a natural anabolic stimulant in men, it increases muscle weight in the body and promotes muscle contraction in athletes engaged in body sports with natural methods.

3. Conclusion

It is reported that eighty million people worldwide are affected by the inability to have children. Approximately 15% of couples in the reproductive period have infertility problems. While it is estimated that 25-40% of infertility in couples is due to male factors such as oligospermia and/or asthenospermia, it is stated in more recent publications that the male factor ratio is around 50% and one out of every 20 men will be affected by subfertility.

For this reason, it is thought that royal jelly and apilarnil are potential bee products that can support infertility treatment due to their beneficial biological activities.

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Determination of Total Phenol, Total Flavonoid Amounts and Antioxidant Activity of Commercial Turkish Propolis Extracts#

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Abstract:
Propolis is a resinous substance that is produced by bees via bee enzymes, from the exudates of buds and plants. Propolis contains phenolic and flavonoid compounds, so it is a wellknown antioxidant source. This study was carried out to determine the total phenol, total flavonoid and antioxidant activities of commercial propolis, extracted in ethanol, sold in the markets. In this study, 4 different commercially available propolis extracted in alcohol (Turkish propolis) were used. To determine the amount of total phenol, Folin-Ciocalteu method was used while aluminium chloride colorimetric method was used to determine the total flavonoids. Antioxidant activity of the propolis extracts were examined by CUPRAC spectrophotometric method. The total phenol and total flavanoid amounts were determined between 5893.33-27138.19 mg/GAE/L and 14.58-118.39 mg/QE/L, respectively. The antioxidant activity was found between 4.82-66.91 µg/ml when the CUPRAC method was used to evaluate the propolis extracted with ethyl alcohol. In conclusion, solvent type, total phenol, total flavonoid contents and antioxidant activity should be taken into account when purchasing propolis from the market.

Keywords: Antioxidant avtivity, Commercial propolis extracts, Total Flavanoid, Total Phenol

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1. Introduction

Propolis is the general name of a complex lipophilic, resinous mixture collected from the buds and exudates of various plants by honey bees (Apismellifera L) (De-Groot 2013). Propolis is widely used in traditional medicine due to its antibacterial, antiseptic, anti-inflammatory, antifungal, antiviral and antioxidant properties (Shruthi and Suma 2012). Flavonoids, phenolic acids, tannins, stilbenes, curcuminoids, coumarins and kinins are phenolic compounds that contribute to the biological effects of propolis. Propolis is a well-known natural source of antioxidants and phenolic and flavonoid compounds play important roles in this capacity (Memedov et al. 2017). An ideal solvent for crude propolis has not been fully found. Since ethanol is more polar than oils and more nonpolar than water, it has the ability to dissolve both nonpolar and polar compounds (Kolaylı et al. 2021). Although it is used in some glycol derivatives and vegetable oils in the preparation of crude propolis extracts, it is known that the best solvent is 70% ethanol (Oroian et al. 2020). Propolis, which has a very complex structure, is mostly extracted in ethanol, and a large part of the dry weight of this extract consists of phenolic compounds and their esters (Memedov et al. 2017).

There are propolis products on the market that contain different solvents and are produced with different production technologies and packaging types (disposable, dropper, spray, etc.) (Sağdıç et al. 2020) .This study was carried out to determine the total phenol (TPC) and total flavonoid (TFC) amounts and antioxidant activities of commercial propolis extracted in alcohol sold in the markets.

4. Materials and Methods

In this study, 4 commercial propolis samples made in Turkey, dissolved in ethanol and purchased from the markets were used. The information declared on the labels of the products and the packaging forms are given in Table 1.

Table 1. Codes and properties of commercial propolis samples used in the study

Sample	Label Statement	Packaging shape
P1-EEP	Liquid propolis extract (declared prepared with distilled water, ethanol (food grade)	dropper bottle
P2-EEP	Organic propolis (declared glycol free) It was prepared with propolis, water and ethanol.	Dropper bottle
P3-EEP	Liquid propolis extract	dropper bottle
P4-EEP	Propolis extract drops (Prepared with distilled water, ethanol and propolis)	dropper bottle

Total phenol and total 103lavonoid amounts were determined by using Folin-Ciocalteu (Slinkard and Singleton 1977) and aluminium chloride (Moreno et al. 2000) colorimetric method, respectively. Antioxidant activity of the propolis extracts were examined by CUPRAC spectrophotometric methods (Apak et al. 2004). Statistical analysis of the data obtained in the study was performed with SPSS version 25.0 for Windows, and the data were expressed as arithmetic mean and standard error. One-way analysis of variance (ANOVA) was used for differences between groups, Duncan's Multiple Range Test was performed when F values were significant. Differences were considered significant when P values were less than 0.05.

5. **Results**

In this study, the total phenolic and total 104lavonoid amounts were determined between 5893.33-27138.19 mg/GAE/L and 14.58-118.39 mg/QE/L respectively. The antioxidant activity was found between 4.82-66.91 µg/ml when the CUPRAC method was used for the ethyl alcohol extracts of propolis were evaluated.

Table 2. Comparative study of some commercial ethanolic propolis extracts (PEE) of Turkey

Propolis Extract	Total Phenol (mg GAE/L)	Total Flavonoid (mg QEs/L)	Antioxidant Activity (µg/ml)
PEE1	18648±31.70^{c}	118.39±0.28^{a}	4.82±0.15^{f}
PEE2	27138.19±46.13^{a}	48.94±0.37^{b}	35.48±1.10^{c}
PEE3	22697.63±38.10^{b}	31.01±0.85^{c}	47.47±0.24^{b}
PEE4	5893.33±11.77^{d}	14.58±0.68^{d}	66.91±0.60^{a}
BHT			6.94±0.59^{e}
BHA			10.43±0.89^{d}
P	< 0.001	< 0.001	< 0.001

$^{a,b,c,d,e,f:}$ Mean values within the same column with different superscripts are significantly different (P < 0.001).

While the highest amount of TPC was found in P2 propolis extract, it was found in P4 at least. (P<0.001). Total flavanoid amount and antioxidant activity were found to be highest in P1 propolis and lowest in P4 propolis (P<0.001).

4. Discussion

The most important feature of propolis extracts is the polyphenols in their structure. These substances are the most important determinants for propolis. Because its high polyphenol value indicates high biological activity (Kolaylı et al. 2021). Kolaylı et al. (2021) reported that the highest total phenol value was in ethanolic samples, and the water and olive oil samples had the lowest activity. These researchers found that the total phenol and total flavonoid amounts were between 1093-5931 mg GAE/100 mL, 752±21-2114±39 mg QE/100 mL, respectively in Turkish propolis extracted in ethyl alcohol. Keskin (2018) analyzed the TPC contents of raw propolis and commercial products collected from different regions of Turkey in order to determine the quality parameters of propolis. In this study, TPC content of commercial EEP was determined as 6.83-77.68 mg GAE/mL, and TFC content as 1.24 -23.33 mg KE/ml. In the presented study, while the total phenol values were consistent with the values of Kolaylı et al. (2021), it was observed that the total flavonoid values were high. Total flavonoid values in the presented study are consistent with Keskin's (2018) findings. When studies on propolis are scanned in the literature, studies with mostly raw propolis draw attention. Since there were not many studies on commercial propolis, there was no possibility of comparison or discussion. In the ethanolic extraction of propolis samples collected from beekeepers from 54 different regions of Turkey using ultrasonic bath, TPC content was reported as 314.36 ± 3.65 mg GAE/g propolis and TFC amount was 522.71 mg QE/g propolis (Ozdal et al. 2018) . Studies have shown that the biological activity of propolis causes extracts prepared with different solvents to contain different amounts of polyphenolic compounds. Özkök et al. (2021) reported that CUPRAC antioxidant capacity of the propolis samples samples and antioxidant range was found from 95.35 to710 mg TE/g.

5. Conclusion

When commercial propolis samples are examined, it is seen that they contain propolis in different proportions and alcohol-derived substances such as alcohol, water or propylene glycol are declared in their contents. When choosing propolis samples in the market, the propolis content, type of solvent, total phenol, total flavonoid amounts and antioxidant activity should be taken into consideration.

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