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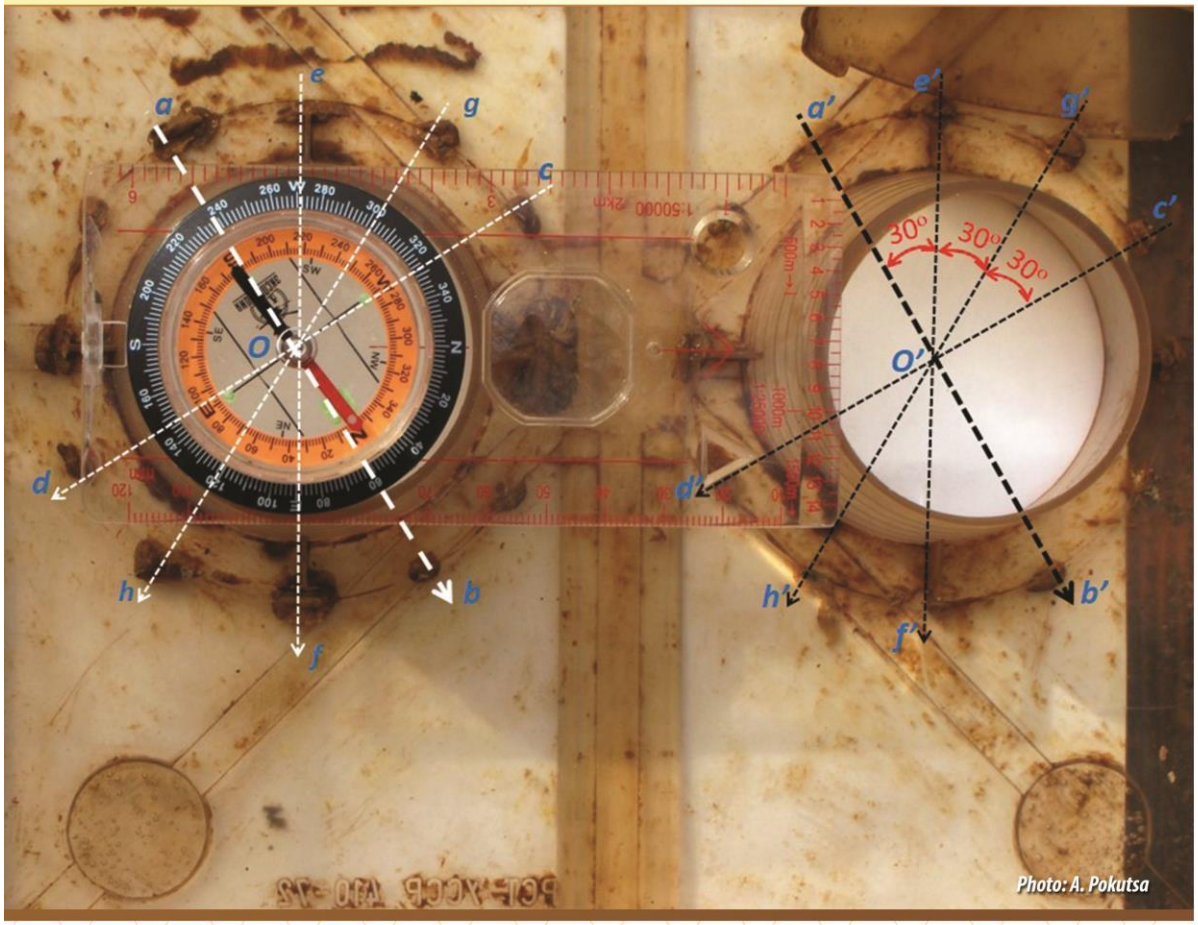

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ULUDAĞ Arıcılık DERGİSİ

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EDİTÖRE MEKTUP / LETER TO THE EDITOR

MAY THE HONEY BEES SENSITIVITY TO EARTH'S MAGNETIC FIELD ASSIST THEM FOR BUILDING?

Bal Arılarının Dünyanın Manyetik Alanına Duyarlılığı Yapımda Yardımcı Olabilirmi?

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ABSTRACT

A surprising D_{12} symmetry inside-hive constructions of honey bees were disclosed. The well-established sensitivity of bees to the magnetic field led us to supposition that it may play a key role in steering of such regular architecture.

Key Words: Magnetic field, *Apis mellifera carpatica,* Magnetic sensitivity, Inside-hive orientation, Uncommon architecture

ÖZ

Bal arılarının şaşırtıcı bir D12 simetrisi kovan içi yapıları açıklandı. Arıların manyetik alana karşı köklü duyarlılığı, bu tür düzenli mimarinin yönlendirilmesinde anahtar bir rol oynayabileceğini varsaymamıza neden oldu.

Anahtar Kelimeler: Manyetik alan, *Apis mellifera carpatica,* Manyetik hassasiyet, Kovan içi orayantasyon, Sıra dışı mimari

Gould and co-workers (1978) discovered the ability of the bees to feel subtle variations of the magnetic field. Gries's team demonstrated the honeybees sensitivity to the polarity of magnetic field (Lambinet et al. 2017) owing to the magneto-receptor located in their abdomens (Walker and Bitterman 1985, Liang et al. 2016). Such property of natural selection enables bees navigation in the opened environment and was intensively studied for decades mainly in that context (Gould, et al. 1980, Walker et al. 1989, Ferrari 2014). Nevertheless, as appeared, the established skills could also explain the revealed recently bees' behavior related to the unusual inner-hive engineering. In addition, the observations presented herein also supports the earlier expressed suggestions concerned the behavioral flexibility of honey bees which, on the other hand, may reflect their cognitive skills including individual recognition and observational learning (Tsvetkov et al. 2019, Feinerman and Korman 2017).

A few years ago, one of us (AP) fed his colonies of *Apis mellifera carpatica* (49°34' N, 22°47' E, Eastern Beskids, Ukraine) with sugar syrup poured in plastic feeders placed inside six hives The feeders possess twin truncated cones, each surmounted with a 90 mm top diameter cup (Fig. 1, S1, S2). A 70 mm

diameter hole at the base of each cone affords to the bee a safety round-trip from the hive to the syrup source (Fig. 1, S2). After two weeks, the examination of feeders led to a surprising observation. The edge of each cup of one of the feeder was glued to the feeder's bottom by propolis gobs, distinctly charting two perfect dodecagons symmetric to each other (Figures 1, 2, S1 and S2).

Despite the 115 mm distance between the centers of these regular dodecagons and shielding from each other by the walls of the plastic cups (Fig. S2), the figures (dihedral group D_{12} of order 24) created by propolis gobs were identical and furthermore mirror-image symmetrical (Figures 1, 2 and S1). The shape of these structures was unusual, differing from the regular hexagons (dihedral group D_6 of order 12) commonly constructed by the bees. Moreover, the direction of imaginary secant lines connecting the respective opposite propolis gobs coincide with the cardinal points (Fig. 2).

We assume that the architecture leading to formation of two symmetrical dodecagons arose from the bee sensitivity to the local geomagnetic field fluctuation (Liang et al. 2016). For example, the lines connecting the pairs of propolis gobs marked *ab* (left cone) and *a'b'* (right cone) are parallel and coincide with the N-S cardinal points. Remarkably, the other connecting lines (e.g. *cd, ef* and *gh* as well as *c'd', e'f'* and *g'h'*) create sectors with identical angles of 30° (Fig. 2). Such constructions, although not so evident, have been detected also from our other hives. For example, by inspection the manner by which the plastic cups were glued to the bottom of others feeders, one can discern the shapes of regular dodecagons too (Fig. S3).

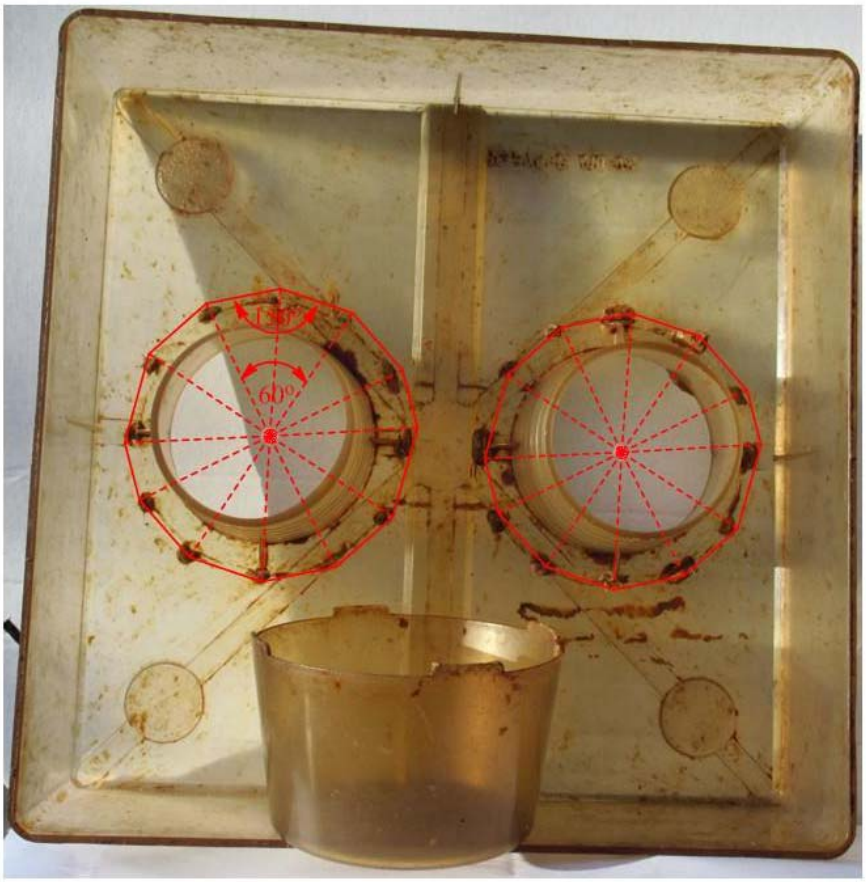

Fig. 1. The propolis gobs (brown patches) around the bottom border of the plastic truncated cones chart two regular dodecagons (red closed chains). For clarity, the cups which covered the cones (the formers were fixed by the gobs to the feeder's bottom) were disconnected and one of them was placed in the front of the feeder.

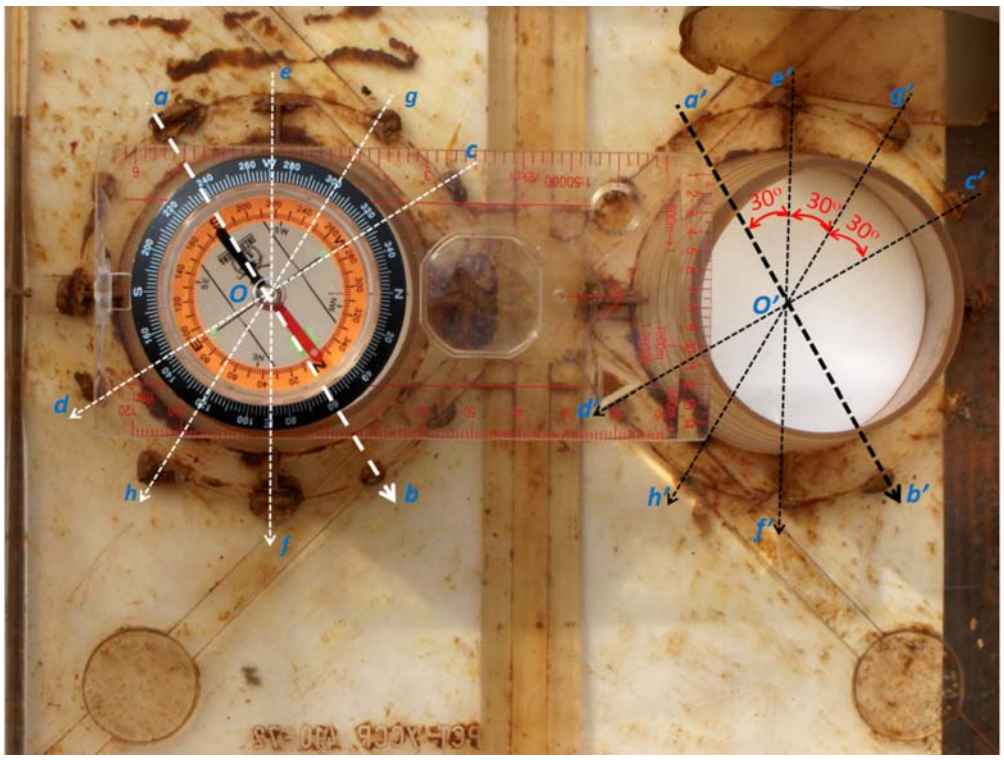

Fig. 2. Photo of the feeder with a compass over the left truncated cone. The secant *ab*, and *a'b'* dash-lines coincide with the Nord-South direction.

As we supposed, the circumstance furthered the developing of the revealed feature was, among others, the location of apiary in the rural region far from the facilities able to generate the electromagnetic field (i.e. radio, television or cell tower antennas, high-voltage lines, radar or satellite stations, etc. For instance, the shortest distance from the nearest cell tower to the colony was more than 2 km). The enlisted factors may be a crucial in view of impact on the bees behavior. According to Kirschvink et al. (19917) and Abou-Shaara (2018) investigation, honeybees are able to detect static intensity fluctuations as weak as 26 nT against the earth-strength magnetic field. Rendering of that the local magnetic anomalies may impact not only the bees navigation in the open environment, but predictively, inside the hive too. Elimination of such effect down to zero (i.e. placing the colony far away from any sources of artificial magnetic fields) enables the bees to follow of their natural engineering skills. And the last ones presumably assist them to construct the exciting figures were exhibited by this research. On the other hand, revealed constructional behavior of the honeybees presumably reflects not only the instinct activity but may be a result of their ingenuity and engineering prowess (Gallo and Chittka 2018, Nazzi 2016). For example, considering that the immobilization of regular large object (in our case the circle-shaped edge of cup) demands to use the figure with number of angles higher than six, they chosen the dodecagon-shaped one. The intra-hive orientation of the bees by their magnetoreceptors may please the execution of the last operation, what was actually demonstrated with this study.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

YEMLİK KOLZANIN FARKLI EKİM NORMLARININ BAZI VERİM ÖZELLİKLERİNE ETKİSİ VE ARI MERASI OLARAK DEĞERLENDİRİLMESİ

The Effect of Different Sowing Norms on Some Yield Traits of Forage Rape and Evaluation as a Bee Pasture

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ÖZ

Bu çalışma, yemlik kolzanın farklı ekim normlarının bazı verim özelliklerine etkisi, bal arıları arasındaki karşılıklı etkileşimlerinin belirlenmesi ve yemlik kolzanın arı merası oluşturulmasında alternatif bir bitki olarak değerlendirilmesi amacı ile yürütülmüştür. Çalışmada 400, 800, 1200, 1600, 2000 ve 2400 g/da ekim normu kullanılmıştır. Bitkinin çiçeklenmesi ile birlikte haftada iki defa gözlemler alınmış ve m^2 başına bal arısı sayısı, bal arılarının çiçekte kalma süresi, bitki boyu, m^2 başına bitki sayısı, bitki başına çiçek sayısı, m^2 başına çiçek sayısı, yan dal sayısı, bitki başına kapsül, kapsülde tohum, tohum verimi ve bin tane ağırlığı gibi özellikler ele alınmıştır. Araştırma sonucunda verim bakımından 1200-1600 g/da ekim normu öne çıkmıştır. 26 Nisan tarihi, arıların yemlik kolzayı en çok ziyaret ettiği, bitkinin en yüksek boyuna ulaştığı, bitki başına ve m^2 başına en fazla çiçeğe sahip olduğu tarih olarak görülmüştür. Yemlik kolzanın arıcılık faaliyeti açısından özellikle erken ilkbahar döneminde kolonilerde oluşan nektar ve polen yetersizliğinin çözümüne yönelik ideal bir arı merası bitkisi olduğu, Bingöl ve benzer ekolojik koşullara sahip bölgelerde 26 Nisan tarihi geçirilmeden bu bitkinin arıcılık açısından değerlendirilebileceği sonucuna varılmıştır.

Anahtar kelimeler: Yemlik kolza, *Brassica napus*, Arıcılık, Tohum verimi, Arı merası

ABSTRACT

In this study, the effects of different sowing norms of Forage Rape on some yield characteristics, the mutual interactions between honey bees and the evaluation of forage rape as an alternative plant in

the creation of bee pastures were carried out. Sowing norms of 400, 800, 1200, 1600, 2000 and 2400 g/da were used in the study. Data was taken twice a week. The flowering of the plant and the number of honeybees per m^2, the duration of the honeybee in bloom, the plant height, the number of plants per m^2, the number of flowers per plant, the number of flowers per m^2, the number of side branches, capsule per plant, seed in capsule, seed properties such as yield and thousand grain weight were discussed. This research observed that, 1200-1600 g/da sowing norm produces the highest yield. April 26th was observed as the date when bees visited the Forage Rape the most, reached the highest height of the plant, and had the most flowers per plant and per m^2. It has been concluded that Forage Rape is an ideal bee pasture plant for the solving nectar and pollen deficiency in beekeeping, especially in early spring periods in terms of beekeeping activity. İt is recommended that this plant should be further evaluated for beekeeping on dates prior to April 26th in Bingöl and regions with similar ecological conditions.

Keywords: Forage rape, *Brassica napus*, Beekeeping, Seed yield, Bee pasture

EXTENDED ABSTRACT

Aim: This study was carried out to examine the interrelationships between honey bees and forage rape cultivated in different seed norms and to evaluate Forage Rape as an alternative plant for creating bee pastures. In addition, it was aimed at determining the appropriate sowing norm within the scope of Forage Rape-bee relationship in Bingöl ecological conditions and to evaluate the effect of these sowing norms on the yield and yield elements of forage rape.

Materials and Methods: Forage rape (*Brassica napus* L. ssp. *oleifera* Metzg) used as plant material in the study was obtained from a private institution. A total of six different applications were made as 400 g, 800 g, 1200 g, 1600 g, 2000 g and 2400 g seeds per decare. Four rows of 20 m each with inter-row spacing of 40 cm were used on each plot. Sowing was made on the 2nd October, 2021. Data was taken between 18th April and 03rd May, 2021. The number of bees per m^2 and the duration of the bees in the flower were recorded by taking the average of five minute observations at 9:00, 12:00 and 15:00 hours of the day. After the flowering stage, the number of side branches, average number of capsules per plant and average number of seeds per capsule, seed yield and thousand-seed weight were determined by harvesting an area of 1 m^2 per plot.

Results: From the result of this research, highest number of honeybees per m^2 were observed on plots 1200, 1600 and 2400 g/da, highest plant height on plots 1200 and 1600 g/da, highest number of plants per m^2 on plot 2400 g/da, highest number of flowers per plant on plots 1600 and 2000 g/da, highest number of flowers per m^2 on plots 1200, 1600 and 2400 g/da and highest seed yield on plots 1200, 1600, 2000 and 2400 g/da respectively. It was observed that the sowing norm did not have any effect on the duration of the honeybee's stay in flower, number of side branches, number of capsule per plant, number of seeds per capsule and thousand-seed weight. The first flowers of the Forage Rape started sprouting as at 13th April with the ecological condition of the Bingöl province. Measurements started on April 18 and ended with the end of flowering on May 07. The average duration of flowering of Forage Rape in Bingöl conditions was observed to be 24 days. The highest number of bees visited the plants on April 26. It was observed that the duration of stay in flower of bees was longer between 22nd and 29th April. April 26 was also observed to be the date when the plants reached the highest plant-height and had the most flowers per plant and per m^2.

Conclusion: As a result, of the evaluated of Forage Rape for bee pasture from this research, it was concluded that sowing norms ranging from 1200 to 1600 g/da are ideal for the ecological condition of the Bingol province. Considering the duration of the flowering period of Forage Rape, it is understood that the bees can benefit from this plant significantly after the winter season, and therefore Forage Rape can be used as a bee pasture in Bingöl conditions before the 26th of April.

GİRİŞ

Meralar, sürekli bitki örtülerinden dolayı hayvanlar tarafından yaşam ve beslenme ortamı olarak, insanlar tarafından ise değişik tarımsal faaliyetler yapmak amacıyla kullanılmaktadır. Ayrıca doğal mera alanları, sürekli bitki örtüsü ile kaplı oldukları

için toprağın korunması, atmosferdeki dengenin sürdürülebilmesi ve ekosistemin sürekliliğinin sağlanmasında önemli rol oynamaktadır. Zengin bitki türünden meydana gelmiş olan meralar, çok yönlü faaliyete imkan sağlamakta olup, çiftlik hayvanlarını otlatmak suretiyle değerlendirilmelerinin yanı sıra arıcılık ve bal üretimi için de değerlendirilebilmektedir (Gökçe 2002, Gül v.d. 2005).

Bitkiler ve arılar arasında güçlü bağlar vardır ve arıların varlığı doğrudan bitkilere bağlıdır. Genellikle bu ortaklık karşılıklı fayda ilişkisine dayanmaktadır. Çiçeklerin tozlaşmak için arılara, arılarında beslenmesi için çiçeklere ihtiyacı vardır (Cengiz 2013). Mera alanlarında yapılan arıcılık faaliyeti, insan beslenmesi açısından değerli besin kaynağı olan bal ve arı ürünleri elde edilmesinde önemli rol oynamaktadır. Mera alanlarındaki arıcılık faaliyeti aynı zamanda mera bitki örtülerinde bulunan, yabancı tozlaşma özelliğine sahip ve üretim için mutlaka arılara ihtiyaç duyan bitki türlerinin verimi açısından önem taşımaktadır (Gençkan 1985, Özbek 2002). Ayrıca mera alanları, verimliliği artırmaya yönelik dışarıdan herhangi bir girdi kullanımı söz konusu olmayan, tamamen doğal alanlar oldukları için sağlıklı ve organik bal üretimi gerçekleştirme açısından da oldukça uygun alanlardır (Cengiz 2013).

Mera bitki örtüleri çok sayıda bitki türünden meydana gelmekte olup (Gökkuş ve Koç 2001), bu bitkiler polen üreterek nesillerini devam ettirmektedir. Polenler bitkilerin tozlaşma şansını artırırken aynı zamanda arıların bal üretimi için önemli bir besin maddesi görevini de üstlenmektedir (Delaplane ve Mayer 2000). Koloni popülasyon gelişimini sağlayan ana arının yumurta bırakması, koloniye gelen polen ve nektar miktarına bağlı olup, bunlardan birinin yetersizliği veya eksikliği durumunda ana arı yumurtlamayı durdurmaktadır. Polen aynı zamanda yavru arıların vücut gelişiminde protein kaynağı olarak görev yapmaktadır (Kutlu v.d. 2005). Bal arılarının polen kaynağı olarak tek kaynakları doğal floradır ve floranın polen değeri barındırdığı polenli bitki türlerinin çeşitliliği, yoğunluğu ve çiçeklenme periyodunun uzunluğuna eş değerdir (Cengiz 2013).

Ot tipi yem şalgamı, yemlik şalgam veya yemlik kanola gibi isimlerle tanımlanan yemlik kolza (*Brassica napus* L. ssp. *oleifera* Metzg) iklim değişikliğinden en az etkilenen ve polen zengini olan bitkiler arasında yer alması sebebiyle alternatif bir yem bitkisi olma potansiyeline sahiptir. Yemlik kolza, haçlıgiller veya turpgiller (*Cruciferae* veya *Brassicaceae*) familyasına ait tek yıllık bir bitkidir (Çaçan ve Nursoy 2021, Serin ve Tan 2001). Bu familyanın *Brassica* cinsi içerisinde yer alan yemlik kolzanın tohumu, yeşil otu, kuru otu, silajı, küspesi, yağı ve protein konsantreleri hayvan beslemede kullanılmaktadır (Nursoy v.d. 2018). Hayvan beslemenin yanı sıra yemlik kolza, floranın yeterli olduğu alanlarda bal arıları için iyi bir besin kaynağı, floranın yetersiz olduğu alanlarda ise iyi bir nektar ve polen kaynağıdır (Korkmaz 2003).

Farkas (2008) Macaristan'da üç farklı yağlık kolza (*Brassica napus* L. var. *napus*) çeşidinde nektar üretimi ve şeker bileşimini incelemiştir. Çalışmada genç ve polen döken çiçekler en iyi nektar üreticisi olarak belirlenmiştir. Polen saçan çiçekler, 14 Mayıs'ta güneşli ve rüzgârlı hava koşullarında genç çiçeklere göre iki kat, bulutlu koşullarda, 15 Mayıs 2005'te dört kat daha fazla nektar üretmiştir. Çalışmada diğer iki çeşitte de benzer eğilimler gözlenmiş olup, bu da hava koşullarının nektar üretimi/çiçek ve nektar şeker konsantrasyonu üzerinde belirgin bir etkisi olduğunu göstermiştir. Nedić v.d. (2013) tarafından yapılan çalışmada, yağlık kolza çiçeklerinin nektar özellikleri, nektar üretimi ve bal arısı ziyaretleri ile bal ve tohum verimine ilişkin gözlemler gibi morfofizyolojik özellikleri incelenmiştir. Çalışmada maksimum bal veriminin hesaplanması, çıkarılan balın gerçek miktarının potansiyel verimden çok daha düşük olduğu ortaya çıkmış ve bu arı merasının yeterince kullanılmadığını göstermiştir. Ayrıca bal arısı tozlaşmasının, kolza tohum verimini ve tohum üretimini, tozlayıcıların hariç tutulduğu işleme kıyasla %12 arttırdığı gözlemlenmiştir.

Bu çalışmada bal arıları ile farklı tohum normlarında ekimi yapılan yemlik kolza arasındaki karşılıklı ilişkileri incelemek ve yemlik kolzanın arı merası oluşturulmasında alternatif bir bitki olarak değerlendirilmesi hedeflenmiştir. Ayrıca Bingöl ekolojik koşullarında bal arılarının ihtiyaç duyduğu bitki popülasyonuna destek olarak uygun ekim normunda yemlik kolza ekiminin yapılması ve bu ekim normlarının verim ve verim öğeleri üzerinde etkisinin değerlendirilmesi amaçlanmıştır.

GEREÇ VE YÖNTEM

Gereç

Araştırmada bitkisel materyal olarak kullanılan yemlik kolza (*Brassica napus* L. ssp. *oleifera* Metzg)

özel bir kuruluştan temin edilmiştir. Araştırma Bingöl Üniversitesi Tarımsal Uygulama ve Araştırma Merkezinde yürütülmüştür. Bu alan Bingöl il merkezine 15 km uzakta olup, 38° 32' 41.85" K ile 40° 32' 25.58" D koordinatlarında yer almakta ve deniz seviyesinden yüksekliği ortalama 1080 m'dir.

Araştırma alanının iklim özellikleri

Bingöl ilinde yıllık ortalama sıcaklık değeri 12.1°C'dir. Ocak ve Şubat aylarında sıcaklık ortalaması sıfırın altında olmakta, Temmuz ve Ağustos ayları da en sıcak aylar olarak seyretmektedir. Bingöl ilinin yıllık toplam yağış miktarı da 948.4 mm'dir. En fazla yağış kış aylarında alınmaktadır. Temmuz ve Ağustos ayları en az yağış alan aylardır.

Araştırma alanının toprak özellikleri

Yapılan toprak analizine göre toprak yapısının killi-tınlı yapıda olduğu, hafif derecede asidik (pH: 6,26), tuzsuz (%0.014), organik madde içeriği az (%1,09), az kireçli (%0,41), potasyum içeriğinin az (18,27 kg/da) ve fosfor oranının orta (7,60 kg/da) olduğu tespit edilmiştir.

Yöntem

Araştırmada yemlik kolzanın farklı tohumluk miktarlarının verim ve verim öğeleri üzerinde etkisi ve aynı zamanda bitkinin arı merası olarak kullanılması değerlendirilmiştir. Bu amaçla araştırma alanının yaklaşık 50 m uzağına 10 adet bal arısı kovanı, yönleri güneye bakacak şekilde yerleştirilmiştir. Tohumluk miktarı olarak dekara 400 g, 800 g, 1200 g, 1600 g, 2000 g ve 2400 g olmak üzere toplam altı farklı uygulama yapılmıştır. Deneme 02 Ekim 2020 tarihinde, her uygulama parseli 4 sıra olarak, sıra arası mesafe 40 cm (Cacan ve Kokten 2017) ve her sıranın uzunluğu 20 m olacak şekilde kurulmuştur. Gözlemler üç tekerrür olacak şekilde parsellerde belirlenen 1 m^2'lik alan üzerinden yapılmıştır. İlk çiçeklenme 13 Nisan 2021 tarihinde görülmüştür. Parselin çiçeklenme oranının artmasıyla 18 Nisan tarihi itibariyle gözlemler alınmaya başlanmıştır. 18 Nisan, 22 Nisan, 26 Nisan, 29 Nisan ve 3 Mayıs olmak üzere beş farklı günde, her günün sabah saat 9:00, öğle saat 12:00 ve öğleden sonra saat 15:00'da olacak şekilde 5 dakika süre ile (Tansı ve Kumova 1999, Bakoglu ve Kutlu 2006, Kutlu v.d. 2018) üç farklı zamanda ve bu üç farklı zamanın ortalaması olarak m^2 başına bal arısı sayısı ve bal arılarının çiçekte kalma süresi hesaplanmıştır. Beş farklı günde bitki boyu cm olarak her tekerrürde 10 bitki olacak şekilde ölçülmüştür. Yine beş farklı günde m^2 başına bitki sayısı, bitki başına çiçek sayısı ve m^2 başına çiçek sayısı sayılarak ve ortalaması alınarak veriler elde edilmiştir. Çiçeklenme aşaması bittikten sonra bitkide yan dal sayısı, bitki başına kapsül sayısı, kapsülde tohum sayısı elde edilmiş ve her parselden 1 m^2'lik alan biçilerek bu alandan elde edilen tohum verimi, el ile harmanlanarak tohum verimi ve bin tane ağırlığı verileri elde edilmiştir.

Elde edilen verilere JMP istatistik paket programı yardımıyla varyans analizi uygulanmıştır. Ortalamaların farklılıkları 0.05 seviyesinde LSD testi ile karşılaştırılmıştır.

BULGULAR

Çalışmada yemlik kolzanın m^2 başına bal arısı sayısı, bal arılarının çiçekte kalma süresi, bitki boyu, m^2 başına bitki sayısı, bitki başına çiçek sayısı, m^2 başına çiçek sayısı, yan dal sayısı, bitki başına kapsül, kapsülde tohum, tohum verimi ve bin tane ağırlığı gibi özellikler incelenmiştir. Bu özelliklere ait varyans analizi Tablo 1'de verilmiştir. Tablo 1'de verildiği üzere yan dal sayısı, bitki başına kapsül sayısı, kapsülde tohum sayısı, bin tane ağırlığı ve m^2 başına bitki sayısının sayım zamanı dışında kalan diğer özelliklerin sayım zamanı ve ekim normu açısından istatistiksel olarak anlamlı farklılık gösterdiği görülmektedir.

1-m^2 başına tespit edilen bal arısı sayısı

Araştırmada farklı sayım zamanları ve farklı ekim normuna göre m^2 başına tespit edilen bal arısı sayısı Tablo 2'de verilmiştir. Tablo 2'de görüldüğü üzere farklı ekim normu ile farklı sayım zamanlarında tespit edilen m^2 başına arı sayıları arasındaki farkın istatistiksel olarak anlamlı olduğu görülmektedir. 26 Nisan tarihinde m^2 başına en yüksek arı sayısı tespit edilmiştir ve 29 Nisan-3 Mayıs tarihleri en düşük arı ziyaretinin olduğu tarihler olmuştur. Ekim normu açısından bakıldığında m^2 başına en yüksek arı sayısının 1200, 1600 ve 2400 g/da tohumluk kullanılan parsellerde tespit edildiği görülmektedir. Genel olarak tohumluk miktarlarının az kullanıldığı parselleri ziyaret eden arı sayılarının da az olduğu görülmektedir. Sayım zamanları ve ekim normu ortalaması olarak m^2 başına tespit edilen bal arısı sayısı 10,9 adet olmuştur.

Tablo 1. İncelenen özelliklerin varyans analizi sonucu elde edilen F değerleri ve önemlilik kontrolü
Table 1. The F values and the significance control obtained as a result of the analysis of variance of the examined features

	Serbestlik Derecesi	Arı sayısı	Arının çiçekte kalma süresi	Bitki boyu	Bitki sayısı	Bitki başına çiçek sayısı	m^2'de çiçek sayısı
Bloklar	2	0,60	0,04	2,33	2,66	14,2	9,90
Sayım Zamanı	4	39,3**	18,3**	11,3**	0,54	25,6**	17,5**
Ekim Normu	5	6,89**	1,66	15,6**	14,1**	4,85**	5,49**

	Serbestlik Derecesi	Yan dal sayısı	Bitki başına kapsül sayısı	Kapsülde tohum sayısı	Tohum verimi	Bin tane ağırlığı
Bloklar	2	3,45	0,19	0,58	0,75	0,93
Ekim Normu	5	1,66	1,18	1,21	3,45*	2,07

**: P≤0.01, *: P≤0.05 düzeylerinde önemli

Tablo 2. Farklı sayım zamanı ve ekim normuna göre m^2 başına tespit edilen bal arısı sayısı (adet)
Table 2. The number of honeybees detected per m^2 according to different count times and sowing norms (pieces)

Ekim Normu (g/da)	Sayım Zamanı					
	18 Nisan	22 Nisan	26 Nisan	29 Nisan	3 Mayıs	Ortalama
400	7,0	7,3	13,7	6,0	4,2	**7,6 bc****
800	4,8	6,2	17,8	2,0	0,0	**6,2 c**
1200	13,0	15,5	19,0	8,8	0,3	**11,3 abc**
1600	10,9	17,3	30,7	5,8	0,7	**13,1 ab**
2000	9,7	11,0	27,2	3,5	0,3	**10,3 bc**
2400	25,9	30,0	25,0	2,3	0,7	**16,8 a**
Ortalama	**11,9 b****	**14,6 b**	**22,2 a**	**4,7 c**	**1,0 c**	**10,9**

**: P≤0.01

2-Bal arılarınnın çiçekte kalma süreleri

Farklı sayım zamanları ve farklı ekim normuna göre bal arılarının çiçekte kalma süresi ortalamaları Tablo 3'te verilmiştir. Farklı sayım tarihlerinde bal arılarının çiçekte kalma süreleri arasındaki fark, istatistiki açıdan önemli bulunmuştur. 18 Nisan tarihinde arıların çiçekte kalma süresinin az olduğu, 22 Nisan, 26 Nisan ve 29 Nisan tarihlerinde arıların en fazla çiçekte kaldıkları zaman olduğu ve 03 Mayıs tarihi itibariyle de döllenmenin gerçekleşmesi nedeniyle arının çiçekte kalma süresinin oldukça azaldığı görülmektedir. Farklı ekim normunun uygulandığı parsellerde arıların çiçekte kalma süreleri istatistiki olarak bir farklılık göstermemiştir. Ekimde kullanılan tohumluk miktarının azlığı veya çokluğunun arının çiçekte kalma süresi üzerinde herhangi bir etkisinin olmadığı anlaşılmaktadır. Farklı ekim normu ve sayım zamanlarında bal arılarının çiçekte kalma süreleri ortalama 5,4 saniye olmuştur.

Tablo 3. Farklı sayım zamanı ve ekim normuna göre bal arılarının çiçekte kalma süreleri (saniye)
Table 3. Duration of honeybees in flower according to different count times and sowing norm (seconds)

Ekim Normu (g/da)	Sayım Zamanı					
	18 Nisan	22 Nisan	26 Nisan	29 Nisan	3 Mayıs	Ortalama
400	3,7	7,5	7,5	8,5	5,3	**6,5öd**
800	3,2	6,8	9,3	6,1	2,6	**5,6**
1200	4,7	7,8	7,0	6,4	0,0	**5,2**
1600	4,7	7,2	4,9	6,8	4,9	**5,7**
2000	3,8	5,3	6,5	8,7	0,0	**4,9**
2400	3,3	5,5	5,5	6,2	2,3	**4,6**
Ortalama	**3,9 b****	**6,7 a**	**6,8 a**	**7,1 a**	**2,5 b**	**5,4**

**: P≤0.01, öd: Önemli değil

3-Bitki boyu

Farklı sayım zamanı ve ekim normunda tespit edilen bitki boyu ortalamaları Tablo 4'te verilmiştir. Tabloda farklı ekim normu ile farklı sayım zamanlarında tespit edilen bitki boyları arasındaki farkın istatistiksel olarak anlamlı olduğu görülmektedir. En düşük bitki boyları 18 Nisan ve 22 Nisan tarihlerinde ölçülmüştür. Zaman ilerledikçe bitki boyunda artış olduğu, 26 Nisan, 29 Nisan ve 03 Mayıs tarihlerinde bitki boyunun istatistiksel olarak aynı grupta olduğu ve en yüksek değerleri verdiği görülmektedir. Ekim normu açısından bakıldığında en yüksek bitki boyu ortalamasının dekara 1200 g ve 1600 g tohumluk atılan parsellerden alındığı görülmektedir. Bu miktarlardan daha az ve daha çok atılan tohumluk miktarlarının bitki boyu üzerindeki etkisinin daha az olduğu görülmektedir. Ekim sıklığı ve sayım zamanlarının ortalaması olarak bitki boyu 69,8 cm olarak elde edilmiştir.

Tablo 4. Farklı sayım zamanı ve ekim normuna göre bitki boyu ortalamaları (cm)
Table 4. Plant height averages (cm) according to different count times and sowing norms (cm)

Ekim Normu (g/da)	Sayım Zamanı					
	18 Nisan	22 Nisan	26 Nisan	29 Nisan	3 Mayıs	Ortalama
400	50,2	52,2	56,7	42,3	65,1	**53,3 d****
800	65,0	70,0	71,0	81,3	70,7	**71,6 bc**
1200	62,7	69,0	85,0	88,2	85,5	**78,1 ab**
1600	63,8	67,8	81,3	96,0	95,5	**80,9 a**
2000	55,8	60,2	68,6	65,7	74,9	**65,0 c**
2400	61,9	65,7	71,8	71,1	79,6	**70,0 c**
Ortalama	**59,9 b****	**64,2 b**	**72,4 a**	**74,1 a**	**78,6 a**	**69,8**

**: P≤0.01

4-m^2 başına bitki sayısı

Farklı sayım zamanı ve ekim normundaki m^2 başına bitki sayıları Tablo 5'te verilmiştir. Tabloda farklı ekim normları ile tespit edilen m^2 başına bitki sayısı arasındaki farkın istatistiksel olarak anlamlı olduğu görülmektedir. Sayım zamanları arasında m^2 başına bitki sayıları istatistiksel olarak herhangi bir farklılık göstermemiştir. Ekim normunda kullanılan tohumluk miktarının artması ile parsellerde m^2 başına tespit edilen bitki sayısının arttığı ve en yüksek sayının 2400 g/da uygulama yapılan parselden elde edildiği belirlenmiştir. Sayım zamanları arasında m^2 başına bitki sayıları 138,8-151,1 adet arasında değişim göstermiş ve ortalaması 142,2 adet olarak tespit edilmiştir.

Tablo 5. Farklı sayım zamanı ve ekim normunda m^2 başına tespit edilen bitki sayısı (adet)
Table 5. The number of plants determined per m^2 at different count times and sowing norms (pieces)

Ekim Normu (g/da)	Sayım Zamanı					
	18 Nisan	22 Nisan	26 Nisan	29 Nisan	3 Mayıs	Ortalama
400	82,7	108,8	106,3	111,3	87,5	**99,3 c****
800	97,7	102,5	97,5	175,0	80,0	**110,5 c**
1200	95,7	187,5	140,0	118,8	92,5	**126,9 bc**
1600	170,7	145,2	143,8	91,3	170,0	**144,2 b**
2000	201,0	136,3	128,8	142,5	147,5	**151,2 b**
2400	209,3	226,2	170,0	193,8	305,0	**220,9 a**
Ortalama	**142,8**	**151,1**	**131,0**	**138,8**	**147,1**	**142,2**

**: P≤0.01

5- Bitki başına çiçek sayısı

Araştırmada farklı sayım zamanı ve ekim normundaki bitki başına çiçek sayısının ortalamaları Tablo 6'da verilmiştir

Tablo 6. Farklı sayım zamanı ve ekim normundaki bitki başına çiçek sayıları (adet)
Table 6. Number of flowers per plant at different count times and sowing norms (pieces)

Ekim Normu (g/da)	Sayım Zamanı					
	18 Nisan	22 Nisan	26 Nisan	29 Nisan	3 Mayıs	Ortalama
400	5,1	7,8	8,7	6,6	1,5	**5,9 c****
800	4,9	6,9	10,5	6,6	4,2	**6,6 c**
1200	7,3	11,0	10,8	5,6	1,6	**7,3 bc**
1600	6,5	9,2	14,1	10,5	2,8	**8,6 ab**
2000	7,6	11,3	16,7	8,4	7,1	**10,2 a**
2400	5,4	8,7	10,0	9,9	1,7	**7,1 bc**
Ortalama	**6,1 c****	**9,2 b**	**11,8 a**	**7,9 bc**	**3,2 d**	**7,6**

**: P≤0.01

Farklı ekim normu ile farklı sayım zamanlarında tespit edilen bitki başına çiçek sayıları arasındaki farkın istatistiksel olarak anlamlı olduğu görülmektedir. Bitki başına çiçek sayısının 18 Nisan tarihinden sonra arttığı, en yüksek değerine 26 Nisan tarihinde ulaştığı ve bu tarihten sonra da azaldığı görülmektedir. En yüksek bitki başına çiçek sayısı da 1600 g/da ve 2000 g/da ekim normu uygulanan parsellerden alındığı görülmektedir. Ortalama bitki başına çiçek sayısı 7,6 adet olarak tespit edilmiştir (Tablo 6).

6- m^2 başına çiçek sayısı

Farklı sayım zamanı ve farklı ekim normundaki m^2 başına çiçek sayısının ortalamaları Tablo 7'de verilmiştir. Tabloda farklı ekim normu ile farklı sayım zamanlarında tespit edilen m^2 başına çiçek sayıları arasındaki farkın istatistiksel olarak anlamlı olduğu görülmektedir. Tabloya göre en fazla m^2 başına çiçek sayılarının 22 Nisan ve 26 Nisan tarihlerinde yapılan sayımlarından elde edildiği görülmüştür. Ekim normu açısından bakıldığında m^2 başına çiçek sayısının en çok 1200, 1600 ve 2400 g/da tohumluk uygulanan parsellerden elde edildiği görülmektedir. Ortalama m^2 başına çiçek sayısı 1123 adet olarak tespit edilmiştir.

Tablo 7. Farklı sayım zamanı ve ekim normundaki m^2 başına çiçek sayıları (adet)
Table 7. Number of flowers per m^2 at different count times and sowing norms (pieces)

Ekim Normu (g/da)	Sayım Zamanı					
	18 Nisan	22 Nisan	26 Nisan	29 Nisan	3 Mayıs	Ortalama
400	501	825	1306	876	142	**730 c****
800	446	904	1166	1135	317	**794 c**
1200	1302	2517	1399	799	234	**1250 ab**
1600	1167	1800	2034	1113	476	**1318 ab**
2000	623	1215	1918	963	620	**1068 bc**
2400	1114	2682	1725	1862	513	**1579 a**
Ortalama	**859 b****	**1657 a**	**1591 a**	**1125 b**	**384 c**	**1123**

**: P≤0.01

7-Yan dal sayısı, bitki başına kapsül, kapsül başına tohum, tohum verimi ve bin tane ağırlığı

Farklı ekim normunda yemlik kolza bitkisinde tespit edilen yan dal, bitki başına kapsül, kapsül başına tohum, tohum verimi ve bin tane ağırlıkları Tablo 8'de verilmiştir. Bu özellikler arasında sadece tohum veriminin ekim normu açısından istatistiksel olarak farklılık gösterdiği görülmüştür. Yan dal sayısı 1,33-1,93 adet, bitki başına kapsül sayısı 6,8-12,5 adet ve kapsülde tohum sayısı 10,5-14,1 adet arasında değişim göstermiştir. Ekim normunun bu özellikler arasında istatistiksel olarak herhangi bir farklılığa yol açmadığı görülmektedir. En yüksek tohum verimi 1200 g/da tohum atılan parselden elde edildiği tespit edilmiştir. Bin tane ağırlıkları açısından da istatistiksel olarak bir farklılık olmadığı, bin tane ağırlıklarının 2,718-3,043 g arasında değişim gösterdiği ve ortalamasının 2,834 g olduğu belirlenmiştir.

Tablo 8. Farklı ekim normunda bitkinin yan dal sayısı, bitki başına kapsül sayısı, kapsülde tohum sayısı, tohum verimi ve bin tane ağırlığı ortalamaları
Table 8. The number of side branches of the plant, the number of capsules per plant, the number of seeds in the capsule, the seed yield and the average weight of one thousand seeds in different sowing norms

Ekim Normu (g/da)	Bitkide Yan dal sayısı (adet)	Bitki başına kapsül (adet)	Kapsülde tohum (adet)	Tohum verimi (kg/da)	Bin tane ağırlığı (g)
400 g/da	1,33	6,8	10,5	19,7 c*	2,768
400	1,87	8,7	12,1	24,6 bc	2,879
800	1,87	11,0	12,3	40,7 abc	2,725
1200	1,93	12,5	14,1	58,2 a	2,718
1600	1,53	8,9	11,4	36,7 abc	3,043
2000	1,40	9,9	12,8	47,8 ab	2,872
Ortalama	**1,66**	**9,6**	**12,2**	**37,9**	**2,834**

*: P≤0.05

TARTIŞMA

Farklı ekim normlarında yetiştirilen yemlik kolzada, m^2 başına ortalama 10,9 adet bal arısı sayısı tespit edilmiş ve arıların çiçekte kalma süreleri ortalama 5,4 saniye olarak belirlenmiştir. Rosa v.d. (2010) Güney Brezilya'da, bal arılarının *Brassica napus*'un başarılı bir şekilde tozlaşması için yeterli davranış gösterip göstermediğini ortaya koymak ve bal arılarının beslenme davranışını değerlendirmek amacı ile yürüttükleri çalışmada, bal arılarının bir bitkide ortalama iki çiçeği ziyaret ettiğini ve çiçek üzerindeki kalış süresinin 1-43 saniye arasında değiştiğini beyan etmişlerdir.

Çalışmada yemlik kolzanın boyu ortalama 69,8 cm olarak belirlenmiştir. Başalma v.d. (2003), tarafından Ankara koşullarında kolza ile yapılan 2 yıllık çalışma sonucunda, bitki boyu 123,4-129,1 cm olarak tespit edilmiştir. Gizlenci v.d. (2005) tarafından Samsun koşullarında üç kışlık kolza çeşidi ile yedi farklı ekim zamanında yürütülen çalışmada, çeşitlerin bitki boyu ortalamalarının 124,6-169,1 cm, Gizlenci v.d. (2011) tarafından Samsun koşullarında, 52 kolza hat/çeşidi kullanarak yürütülen iki yıllık çalışmada, bitki boyunun 132,1-178,2 cm arasında olduğu rapor edilmiştir. Sargın (2012), Ordu koşullarında yaptığı araştırmada, kışlık kolza çeşitlerinde bitki boyunun 172,4-202,1 cm arasında tespit edildiğini bildirmiştir. Cacan ve Kokten (2017) tarafından Bingöl ilinde yürütülen bir çalışmada yemlik kolzanın bitki boyu ortalama 135 cm, Köymen (2018) tarafından 3 kışlık kolza çeşidinde, azotun verim ve verim öğeleri üzerine etkisini belirlemek amacıyla yapılan çalışmada bitki boyunun 174,16-190,55 cm arasında değiştiği gözlemlenmiştir. Özyazici v.d. (2020), Siirt koşullarında kışlık kolza üzerinde yürüttükleri çalışmada, bitki boyunun 144,3-152,5 cm arasında değiştiğini bildirmişlerdir. Bingöl ilinde yürütülen bir çalışmada ise yemlik kolzanın bitki boyu ortalama 139 cm (Çaçan ve Nursoy 2021) olarak tespit edilmiştir. Bitki boyunun daha önce yapılan çalışmalar ile kıyaslandığında oldukça düşük olduğu görülmektedir. Bunun nedeni 2020-2021 yılı yetiştirme periyodunda karşılaşılan kuraklıktır. Yağış miktarının azlığı bitkinin boylanmasını engellemiş, bitkinin vejetatif gelişmesinin doruğuna ulaşmadan,

çiçek açarak generatif döneme geçmesine yol açmıştır. Dolayısıyla bitki boyu düşük kalmıştır.

Çalışmada yemlik kolzanın ortalama 24 gün çiçekte kaldığı, m^2 başına 142,2 adet bitki, bitki başına 7,6 adet e m^2 başına 1123 adet çiçeğin olduğu belirlenmiştir. Koltowski (2002), Polonya'da, altı kolza çeşidi ile yürüttüğü çalışmada; kolza çeşitlerinin, Mayıs ayında çiçeklenmeye başladığını, ortalama 15-20 gün çiçekte kaldıklarını ve çiçek sayısının 9.150-12.180 adet/m^2 arasında olduğunu beyan etmiştir. Korkmaz (2003) Çukurova koşullarında, arı otu ve yemlik kolza ile bal arılarının bazı ilişkilerini belirlemek amacıyla iki yıl süreyle yürüttüğü çalışmasında, yemlik kolza bitkisinin Şubat ortalarında çiçeklendiğini, bitkinin 47 gün süre ile çiçekte kaldığını ve 2955,85 adet/m^2 çiçeğe sahip olduğunu belirlemiştir. Kumova ve Korkmaz (2003) Çukurova bölgesinde, kolza bitkileri ile bal arılarının bazı ilişkilerini belirlemek amacıyla yürüttükleri çalışmada, kolza bitkisinin Şubat-Nisan ortasına kadar çiçeklendiğini ve 47-65 gün çiçekte kaldığını belirlemişlerdir. Ayrıca kolza bitkisinde çiçek sayısını ortalama 271,20-2955,85 adet/m^2 olduğunu beyan etmişlerdir. Kumova ve Korkmaz (2007) Çukurova koşullarında kolzanın çiçeklenme fenolojisi, çiçek sayısı, nektar ve polen miktarı ile nektar ve polen potansiyellerini karşılaştırmak amacıyla yaptıkları iki yıllık çalışmada, çiçeklenme başlangıç ve bitiş tarihlerinin her iki yılda farklı aylarda gerçekleştiğini, ilk yıl kolzanın 47 gün çiçekte kaldığını ve ortalama 2955,85±111,11 adet/m^2 çiçeğe sahip olduğunu gözlemlemişlerdir. Ayrıca araştırmanın ikinci yılında, kolza bitkisinin 45 gün çiçekli kaldığını, ortalama 271,20±43,70 adet/m^2 çiçeğe sahip olduğunu rapor etmişlerdir.

Çalışmada yemlik kolzada ortalama yan dal sayısı 1,66 adet, bitki başına kapsül 9,6 adet, kapsülde tohum 12,2 adet, tohum verimi 37,9 kg/da ve bin tane ağırlığı 2,834 g olarak tespit edilmiştir. Başalma v.d. (2003) tarafından Ankara koşullarında kolza ile yapılan 2 yıllık çalışma sonucunda, yan dal sayısını 4,59-5,59 adet, Gizlenci v.d. (2011) Samsun koşullarında 52 kolza hat/çeşidi kullanarak yürüttükleri iki yıllık çalışmada yan dal sayısını 5,0-8,5 adet, Sargın (2012) Ordu koşullarında yaptığı araştırmada, kışlık kolza çeşitlerinde ana sapa bağlı yan dal sayısını 4,7-8,9 adet ve Özyazici v.d. (2020) Siirt koşullarında kışlık kolza üzerinde yaptıkları araştırmada yan dal sayısını 5,9-9,1 adet olarak tespit etmişlerdir.

Öztürk (2000) tarafından Konya koşullarında dört farklı ekim zamanı, dört yemlik kolza çeşidi ve üç farklı sıra aralığında yaptığı araştırmada, sayısının 243,6-308,1 adet, kapsüldeki tohum sayısının 26,7-28,3 adet, tohum veriminin 391,9-435,4 kg/da ve bin tane ağırlığının 4,69-5,06 g arasında olduğunu rapor etmiştir. Başalma v.d. (2003), Ankara koşullarında kolza üzerinde yapmış oldukları iki yıllık çalışma sonucunda, ana sapta kapsül sayısını 50,24-54,66 adet, kapsülde tohum sayısını 24,96-26,13 adet, en yüksek tohum verimini dekara 243,59-249,17 kg ve bin tane tohum ağırlığını 3,27-4,34 g olarak beyan etmişlerdir.

Blažytė-Čereškienė v.d. (2010) Litvanya'da, SW Savann ve Ural kolza çeşitlerine ait çiçekler üzerinde, bal arılarının besin arama davranışlarını incelediklerinde, yüksek sıcaklıklarda kolza çiçekleri üzerindeki böcek yoğunluğunun azaldığını ve tohum veriminde negatif yönde bir etkileşimi gözlemlemişlerdir. Gizlenci v.d. (2011) Samsun koşullarında, kolza bitkisi üzerinde yapmış oldukları iki yıllık çalışmada kapsülde tohum sayısının 16,5-29,6 adet, Sargın (2012) Ordu koşullarında yaptığı araştırmada, kışlık kolza çeşitlerinde bitkide kapsül sayısının 175,2-535.3 adet, kapsülde tohum sayısının 15,1-19,8 adet, tohum veriminin 128,2-372,3 kg/da ve bin tane tohum ağırlığının 3,36-4,39 g arasında olduğunu bildirmişlerdir.

Farklı iklim ve toprak koşullarında bitkiler, verim açısından farklı sonuçlar vermektedir. Bu durumun yanı sıra özellikle 2020 yılında yaşanan kuraklığın ve yağış azlığının, mevcut çalışmada elde edilen bitkide yan dal sayısı, bitki başına kapsül, kapsülde tohum, tohum verimi ve bin tane ağırlığının literatür bulgularından daha düşük olarak elde edilmesine yol açtığı düşünülmektedir.

Sonuç: Yemlik kolzada farklı ekim normlarının bazı verim özellikleri üzerine olan etkisinin belirlenmesi ve yemlik kolzanın arı merası olarak Bingöl koşullarında değerlendirilmesi amacıyla bu çalışma yürütülmüştür. Çalışmada m^2 başına en fazla bal arısı sayısı 1200, 1600 ve 2400 g/da, en yüksek bitki boyu 1200 ve 1600 g/da, en fazla m^2 başına bitki sayısı 2400 g/da, en fazla bitki başına çiçek sayısı 1600 ve 2000 g/da, en fazla m^2 başına çiçek sayısı 1200, 1600 ve 2400 g/da ve en fazla tohum verimi 1200, 1600, 2000 ve 2400 g/da ekim normu uygulanan parsellerden alındığı belirlenmiştir. Bal arılarının çiçekte kalma süresi, yan dal sayısı, bitki başına kapsül sayısı, kapsülde tohum sayısı ve bin tane ağırlığı üzerinde ekim normunun herhangi bir etkisinin olmadığı görülmüştür.

Yemlik kolzada Bingöl koşullarında 13 Nisan tarihi itibariyle ilk çiçekler görülmeye başlanmıştır. Sayımlar, 18 Nisan tarihinde başlamış ve 07 Mayıs tarihi itibariyle çiçeklenmenin son bulmasıyla bitmiştir. Yemlik kolzanın Bingöl koşullarında çiçekli kalma süresi ortalama 24 gün olarak belirlenmiştir. Bitkiyi en fazla arı 26 Nisan tarihinde ziyaret etmiştir. 22-29 Nisan tarihlerinde arıların çiçekte kalma sürelerinin daha fazla olduğu görülmüştür. 26 Nisan tarihi, aynı zamanda bitkinin en yüksek bitki boyuna ulaştığı, bitki başına ve m^2 başına en fazla çiçeğe sahip olduğu zaman olarak belirlenmiştir.

Sonuç olarak yemlik kolza arı merası olarak değerlendirildiğinde 1200-1600 g/da ekim normunda ekim yapılmasının Bingöl koşulları için ideal ekim normu olduğu sonucuna varılmıştır. Yemlik kolzanın çiçeklenme periyodundaki süre dikkate alındığında arıların kış mevsiminden sonra bu bitkiden önemli oranda yararlanabileceği ve bu nedenle yemlik kolzanın Bingöl koşullarında 26 Nisan tarihi geçirilmeden arı merası olarak kullanılabileceği anlaşılmaktadır.

Katkı ve çatışma durumu: Yazarlar eşit durumda katkı sağlamışlardır ve yazarlar arasında herhangi bir ihtilaflı durum bulunmamaktadır.

Mali kaynak: Bu çalışma, Bingöl Üniversitesi Pilot Üniversite Koordinasyon Merkez Birimi Tarafından (Proje No: PİKOM-Bitki.2019.001) desteklenmiştir.

Etik belgesi: Gerekli değildir.

Teşekkür: Bu çalışma, Bingöl Üniversitesi Pilot Üniversite Koordinasyon Merkez Birimi Tarafından (Proje No: PİKOM-Bitki.2019.001) desteklenmiştir. Verilen destekten dolayı Pilot Üniversite Koordinasyon Merkez Birimine teşekkür ederiz.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

USING GEOGRAPHICAL INFORMATION SYSTEMS AND REMOTE SENSING TO DETECT RESIN-RICH AREAS FOR PROPOLIS PRODUCTION FROM APIARIES

Arı Kovanlarından Propolis Üretimi için Reçine Bakımından Zengin Alanları Tespit Etmek için Coğrafi Bilgi Sistemlerini ve Uzaktan Algılamayı Kullanma

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ABSTRACT

Propolis (bee glue) is considered among bee products with medicinal properties. The majority of plants in Egypt are field crops which cultivated by farmers. Such crops may supply bee colonies with nectar/pollen, but are not good sources for resin due to their short life cycles. This study aimed to explore potential sites for producing high amounts of propolis from bee colonies based on a remote sensing analysis. The study was performed on a Governorate with intensive agricultural activity in Egypt (Assiut Governorate). The methodology depended on isolating trees from the satellite image using unsupervised classification followed by supervised classification based on numerous geographical coordinates of trees. Trees were transferred into points, and then point density was calculated to classify the study location into classes according to density. Sites with the very high numbers of trees were especially recommended for producing propolis on a commercial scale by beekeepers. Giving more attention to propolis production can enhance the income of beekeepers and maximize the economic benefit from bee colonies.

Keywords: Resin, GIS, Trees, Density, Beekeeping

ÖZ

Propolis (arı tutkalı), tıbbi özellikleri olan arı ürünleri arasında sayılmaktadır. Mısır'daki bitkilerin çoğu, çiftçiler tarafından yetiştirilen tarla bitkileridir. Bu tür mahsuller, nektar/polen ile arı kolonilerine besin sağlayabilir, ancak kısa yaşam döngüleri nedeniyle reçine için iyi kaynaklar değildir. Bu çalışma, uzaktan algılama analizine dayalı olarak arı kolonilerinden yüksek miktarda propolis üretmek için potansiyel alanları keşfetmeyi amaçladı. Çalışma Mısır'da yoğun tarımsal faaliyet gösteren bir Valilik (Assiut Valiliği) üzerinde gerçekleştirilmiştir. Metodoloji, ağaçların çok sayıda coğrafi koordinatlarına dayalı olarak denetimli sınıflandırmanın ardından denetimsiz sınıflandırma kullanılarak uydu görüntüsünden ağaçların yalıtılmasına dayanıyordu. Ağaçlar noktalara aktarılmış ve daha sonra çalışma yerinin yoğunluğa göre sınıflara ayırmak için nokta yoğunluğu hesaplanmıştır. Çok yüksek sayıda ağaç bulunan alanlar, özellikle arıcılar tarafından ticari ölçekte propolis üretimi için tavsiye edildi. Propolis üretimine daha fazla önem verilmesi, arıcıların gelirini artırabilir ve arı kolonilerinden ekonomik faydayı en üst düzeye çıkarabilir.

Anahtar kelimeler: Reçine, CBS, Ağaçlar, Yoğunluk, Arıcılık

GENİŞLETİLMİŞ ÖZET

Çalışmanın amacı: Propolis (arı tutkalı), antimikrobiyal özellikleri nedeniyle özel bir öneme sahiptir. Arı kolonilerinden propolis üretimi, çoğunlukla ağaçlardan elde edilen reçine kaynaklarının mevcudiyetine bağlıdır. Mısır'daki bitkilerin çoğu, çiftçiler tarafından yetiştirilen tarla bitkileridir. Bu tür mahsuller, nektar/polen ile arı kolonilerine besin sağlayabilir, ancak kısa yaşam döngüleri nedeniyle reçine için iyi kaynaklar değildir. Bu nedenle, bu çalışma, çok sayıda ağaç içeren alanları belirleyerek uzaktan algılama görüntülerini analiz etmeye dayalı olarak arı kolonilerinden yüksek miktarda propolis üretmek için potansiyel alanları keşfetmeyi amaçladı.

Gereç ve yöntem: Çalışma Mısır'da yoğun tarımsal faaliyet gösteren bir Valilik (Assiut Valiliği) üzerinde gerçekleştirilmiştir. Bu Valiliği kapsayan Landsat 8 Operasyonel Arazi Görüntüleyici (OLI)/ Termal Kızılötesi Sensör (TIRS) Koleksiyon 1 (C1) Seviye 1 görüntülerinden alınan uydu görüntüsü 30 metre çözünürlüklü analizde kullanılmıştır. Uydu görüntüsünün bantları (1'den 5'e ve 7) ArcGIS'te birleştirildi. 1'den 5'e kadar olan bantlar sırasıyla ultra mavi, mavi, yeşil, kırmızı ve yakın kızılötesidir; 7 numaralı bant ise kısa dalga kızılötesidir. Metodoloji, ağaçların çok sayıda coğrafi koordinatlarına dayalı olarak denetimli sınıflandırmanın ardından denetimsiz sınıflandırma kullanılarak uydu görüntüsünden ağaçların yalıtılmasına dayanıyordu. Ağaçlar noktalara aktarılmış ve daha sonra çalışma yerini yoğunluğa göre sınıflara ayırmak için nokta yoğunluğu hesaplanmıştır.

Bulgular: Çalışma alanı için haritalar analizlerden oluşturulmuştur. Bu haritalar, çalışma alanını ağaçların mevcudiyetine göre kategorilere ayırdı. Ayrıca, çalışma alanı için çok yüksek sayıda ağaç, çok sayıda ağaç ve orta sayıda ağaç bulunan yerleri gösteren çokgenler oluşturulmuştur. Arı kolonilerinden ticari yolla propolis üretimi için dört alan çok yüksek olarak kabul edildi. Çok yüksek ve yüksek derecede uygun sitelerin çokgenleri, orta derecede uygun sitelerden farklı olarak ayrıldı. Propolis üretimine çok uygun olarak sınıflandırılan dört alanın toplam alanı 76.83 km2'dir. Bununla birlikte, orta derecede uygun olarak sınıflandırılan siteler, oldukça uygun veya çok uygun olarak kabul edilenlere göre en yüksek toplam alana sahipti.

Tartışma ve sonuç: Propolis üretimi için potansiyel alanları belirlemek için çalışma yerine (Assiut Valiliği) uzaktan algılama tekniği uygulandı. Dört bölge reçine kaynakları olarak çok uygun olarak kabul edildi. Bu sahaların ticari miktarlarda propolis üretmeyi amaçlayan arıcılar tarafından kullanılması tavsiye edilmektedir. Seçilen dört site için saha ziyaretleri ve gerçek görüntüler, buralarda çok sayıda ağacın varlığını desteklemektedir. Gelecekteki bir adım olarak, sorumlu kurumlar, özellikle çalışmadan belirtilen alanları ziyaret ederek, arıcıların gelirlerini artırmak için kolonilerinden propolis üretimine yönelik farkındalıklarını artırmalıdır. Gelecekteki çalışmalarda mükemmel yerlerde kovan başına ortalama propolis üretimi hesaplanabilir

Çok yüksek sayıda ağaç bulunan alanlar, özellikle arıcılar tarafından ticari ölçekte propolis üretimi için önerildi. Propolis üretimine daha fazla önem verilmesi, arıcıların gelirini artırabilir ve arı kolonilerinden ekonomik faydayı en üst düzeye çıkarabilir. Ayrıca, arı sağlığını geliştirmek için propolisten arı hastalıkları için bazı doğal tedaviler hazırlanabilir.

INTRODUCTION

Beekeeping is among the agricultural activities which assist in alleviating poverty and boost national income in many countries (Qaiser et al. 2013, Al-Ghamdi et al. 2016, Amulen et al. 2019). Beekeeping is also essential for crop production due to the role of honeybees, *Apis mellifera* L., in pollinating plants (Morse and Calderone 2000, Reyes-Carrillo et al. 2007, Blazyte-Cereskiene et al. 2010, Calderone 2012, Klatt et al. 2014). The major products from honeybee colonies are honey and pollens. So, beekeepers transfer their colonies from location to another in search for good sources of nectar/pollen (Sharma and Bhatia 2001, Güler and Demir 2005, Pilati and Prestamburgo 2016). There are other valuable products with medicinal properties which can be produced from bee colonies including bee venom, royal jelly and propolis (bee glue) (Nagai et al. 2006, Jingli and Zhsgg 2008, Fratellone et al. 2016, Habryka et al. 2016). These products can increase the profitability form bee colonies especially beekeepers can produce them after or between honey seasons. The production of royal jelly and bee venom depends on the strength of bee colonies while the production of propolis depends on the availability of resin-rich plants (resin is the raw material of propolis) (Bankova et al. 2000, Bankova et al. 2019). Therefore, propolis can be produced

from specific sites with resin-rich plants (Abou-Shaara and Eid 2019). The production of propolis is common in countries with forests while searching for resin sources is essential in countries without forests such as Egypt.

There are various sources of resin especially trees (Bankova et al. 2000, Salatino et al. 2005), as resin from wounds in tree trunks is collected by bees (Drescher et al. 2014). The short life cycles of some plants negatively affect their suitability as major sources of resin to bee colonies including field crops which cultivated seasonally by farmers (Abou-Shaara and Eid, 2019). Honeybees tend to collect materials resemble resin from wired sources such as asphalt and artificial paints (König 1985) or recycle previously used propolis when very few resin resources are available around colonies (Abou-Shaara 2014a). In fact, bees use propolis to reduce hive entrance, fill in hive cracks, cover hive intruders to prevent their spoilage inside beehives, and to strength wax combs (Abou-Shaara 2014a, Conrad 2016, Bankova et al. 2019), and can enhance bee immunity and colony health (Borba et al. 2015). Propolis has been used by researchers to control some bee diseases including Varroa mites (Garedew et al. 2002, Damiani et al. 2010, Abou-Shaara 2017). Moreover, propolis has anti-microbial, anti-viral and anti-fungal properties (Grange and Davey 1990, Cheng and Wong 1996, Jingli and Zhsgg 2008). Thus, it has many medicinal usages. Indeed, the commercial production of propolis from bee colonies requires more attention from beekeepers especially in developing countries to increase the profitability from their bee colonies especially after or between honey seasons.

Recently, computer sciences have been used to develop beekeeping. In fact, Geographical Information System (GIS) in combination with remote sensing have many applications in beekeeping (Abou-Shaara 2019, Abou-Shaara and Kelany 2021) including the analysis of land cover around apiaries (Jo et al. 2001, Abou-Shaara et al. 2013,* Fernandez et al. 2016, Ambarwulan et al. 2017, Zoccali et al. 2017, Ausseil et al. 2018). It is possible to identify specific plant types from the satellite images (Abou-Shaara 2013, Abou-Shaara and Kelany, 2020). Potential resin sources (mainly trees) can be identified from satellite images using unsupervised and supervised classification tools (Abou-Shaara and Eid 2019). Therefore, this study aimed to classify the study area (Assiut Governorate) according to the availability of major resin sources (trees) using geographical information system and remote sensing technique. Beekeepers can increase the profitability from their colonies by producing propolis on a commercial scale especially from sites with high resin sources as classified from this study.

MATERIALS AND METHODS

Location

This study was performed on Assiut Governorate (Lat: 27° 15' 7.2" °N, Lon: 31° 5' 24" °E) located towards the South of Egypt (Figure 1). This Governorate has a good vegetation cover especially with field crops cultivated seasonally alongside the Nile River by farmers.

Isolating trees

The analysis was done following the method by Abou-Shaara and Eid (2019) using the ArcGIS 10.5. A satellite image from Landsat 8 Operational Land Imager (OLI)/ Thermal Infrared Sensor (TIRS) Collection 1 (C1) Level 1 images covering this Governorate was used in the analysis with 30-meter resolution. The bands (1 to 5 and 7) of the satellite image were combined (Figure 2A). These bands are band 1 (ultra-blue), band 2 (blue), band 3 (green), band 4 (red), band 5 (near infrared), and band 7 (shortwave infrared). Then, the unsupervised classification considering high number of classes (15 classes) was used to discriminate between different land objectives (Figure 2B). After that, coordinates of trees were tested on the classified image to separate only classes that represent them (supervised classification based on field visits and Google earth). The reclassify tool was used to reclassify all classes into No Data except those representing trees (i.e. to show only trees). Then, the trees were converted into points (Figure 2 C).

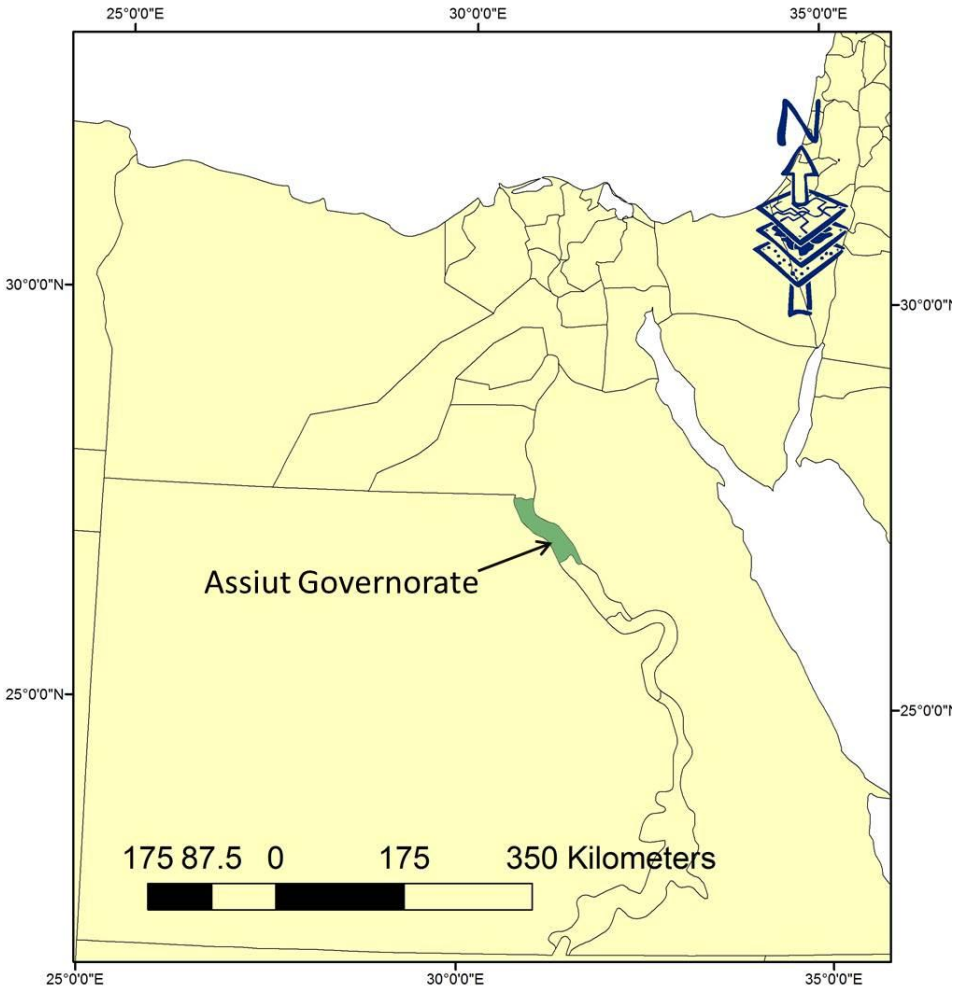

Figure 1: Map showing location of Assiut Governorate (green color).

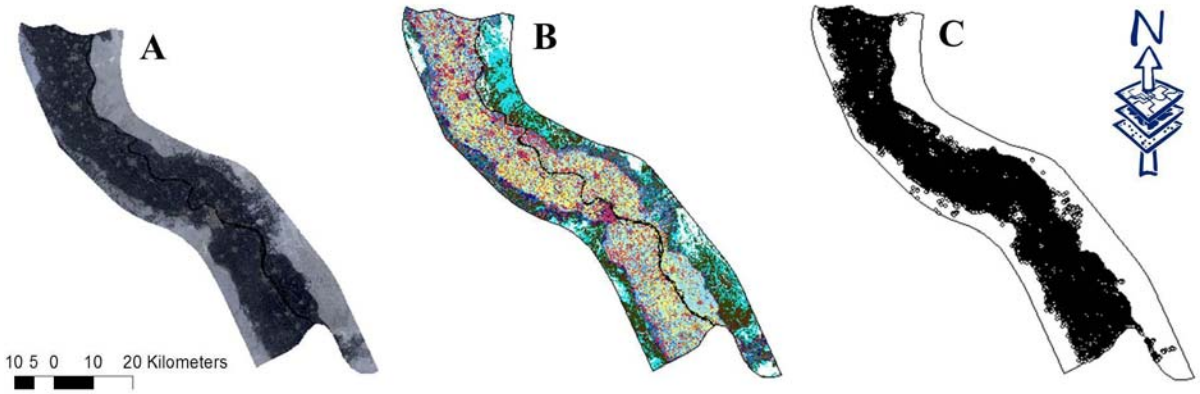

Figure 2: The satellite image of the study location (A), the unsupervised classification of the image (B), and trees as points (C).

Classifying sites according to their suitability for propolis production

Points represent trees were analyzed using point density tool. This tool calculates density as number of points per square map unit. Accordingly, sites were classified into four equal degrees: very high suitable (332.81-443.75), high suitable (221.87-332.81), moderately suitable (110.93-221.87), and low suitable (0 -110.93) according to the method by Abou-Shaara and Eid (2019). The very high suitable sites are especially anticipated to be the perfect places for beekeepers wishing to produce propolis from their colonies. Hence, the results focused on these sites. The four classes were converted into polygons. Then, areas of these polygons were calculated in square kilometers.

Verification of the analysis

Real images from Google earth for the sites classified as very high suitable for producing propolis were firstly inspected to be sure from the presence of high number of trees at them. This was done according to Abou-Shaara and Eid (2019). Additionally, several field visits were conducted to observe the availability of trees in these sites.

RESULTS

Suitability maps

The study location was divided into four classes according to numbers of trees (Figure 3A and B). It is clear that four sites were exclusively considered as very high suitable for propolis production due to the presence of high tree numbers at them. Indeed, these sites are surrounded by other sites with relatively high numbers of trees enveloped with sites classified as with moderate numbers of trees. The rest of the study location was considered as with low numbers of trees which is not suitable for propolis production on the commercial scale.

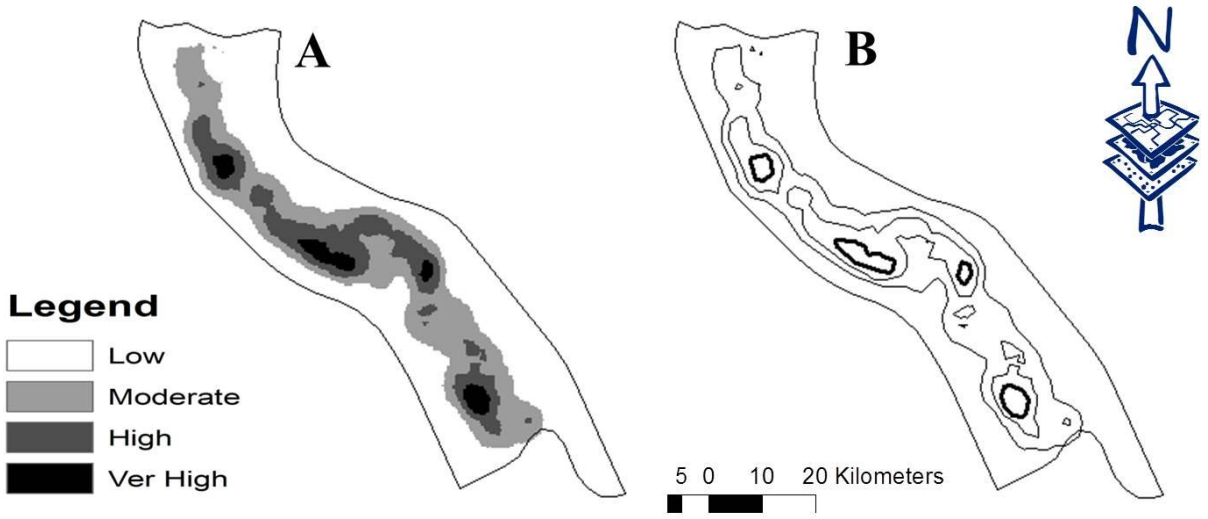

Figure.3: The study location classified into four classes according to numbers of trees: raster image (A), and image as polygons (B).

In fact, four sites were considered as very high suitable for commercial production of propolis from bee colonies (Figure 4A). The polygons of the very highly and highly suitable sites are separated clearly than each other (Figure 4A and 4B) unlike the moderate suitable sites (Figure 4C). The areas of the four sites classified as very high suitable for propolis production were 7.56, 15.03, 22.57, and 31.67 km^2 in ascending order with total of 76.83 km^2. However, the sites classified as moderately suitable had the highest total area than those considered as highly suitable or very highly suitable (Table 1).

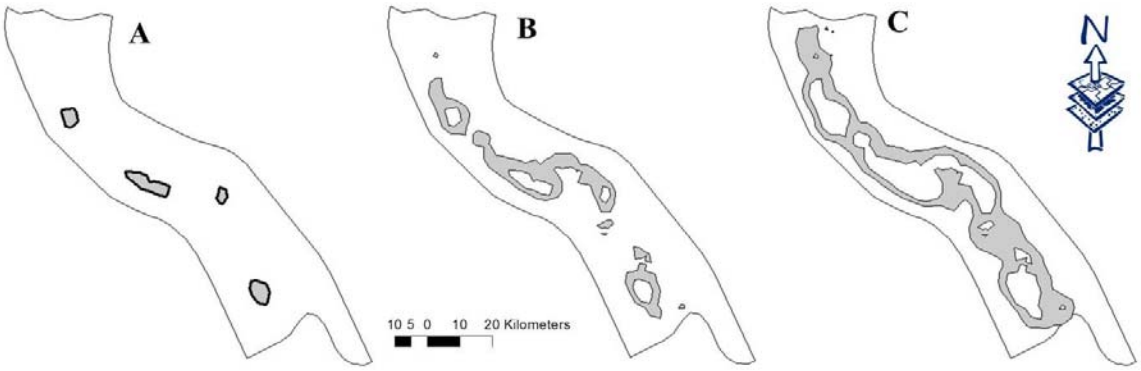

Figure 4: Polygons showing sites with very high numbers of trees (A), high numbers of trees (B), and moderate numbers of trees (C).

Table 1. Information about sites with different numbers of trees.

Numbers of trees	Number of polygons	Mean of areas ± S.D.	Sum of area (km^2)
Very high	4	19.21±10.32	76.83
High	9	36.94±65.56	332.49
Moderate	4	168.83±337.36	675.32

Verification of locations

The four sites classified as very high and suitable for propolis production were inspected to ensure the validity of the study. The four sites are typically located in agricultural lands with high numbers of trees (Figure 5). The first three sites (Figure 5 A to C) contain villages which may cause some limitation in utilizing the whole site area for beekeeping. The 4th site (Figure 5D) is approximately empty of villages except some individual houses and can be utilized completely by beekeepers.

DISCUSSION

It is apparent that agricultural crops are available beside trees. This indicates that various food resources are available for bee colonies at these sites beside permanent resin sources. Generally, there are various plant species suitable as food sources for honey bees in Egypt but there are no forests (Abou-Shaara 2015). Thus, fruit and roadside trees represent the major resin sources (Abou-Shaara and Eid, 2019). Accordingly, date palm, banana, casuarina and eucalyptus are the main resin sources in Assiut.

The first three sites contain villages. It is better for beekeepers to place their colonies away from inhabited locations as much as possible. Fortunately, the foraging distance of honey bees is high (Abou-Shaara 2014b), and beehives can be placed anywhere within the very high suitable sites. Although the presence of wild bees beside honey bees in the study location (Assiut area), honey bees represent the major visitor to many crops (Hussein and Abdel-Aal, 1982). This reflects the importance of beekeeping in Assiut for crop production. Therefore, producing propolis from bee colonies is anticipated to be an additional benefit for beekeepers. Additionally, Egyptian propolis has shown antimicrobial activity against some pathogens (Hegazi et al. 2014). This encourages production and studies on propolis from different Egyptian locations.

Remote sensing technique was applied on the study location (Assiut Governorate) to identify potential sites for propolis production. Four sites were considered as very high suitable as sources for resin. These sites are recommended to be utilized by beekeepers aiming to produce commercial amounts of propolis. Field visits and real images for the four selected sites support the presence of high numbers of trees at them. As a future step, responsible agencies should increase the awareness of beekeepers towards the production of propolis from their colonies to increase their income, especially by visiting the sites specified from the study. The average production of propolis per beehive at the perfect sites can be calculated in future studies.

Figure 5: Google images for the four sites classified as very high suitable for propolis production. Site 1 (A and A1), site 2 (B and B1), site 3 (C and C1), and site 4 (D and D1).

Conclusions

It can be concluded that the study area contains some locations which can be further utilized by beekeepers to produce significant amount of propolis. In the future studies, bee colonies will be placed in the slected locations to evluate propolis production per colony. Also, the perfect season for the production will be specified. Without any doubt, the use of geogrphical information system and remote sesning can help in locating the areas that conatin potential sources for resin. The methodology described herein are benificial to other researchers as they can use it in their local areas to boost propolis production from bee colonies.

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Conflict of Interest: Authors declare that they have no any conflict of interests to be reported.

Author contribution: The authors contributed equally in the study. They designed, performed, analyzed the data, wrote and revised the manuscript.

Ethical issue: Not applicable because this study on honey bees and not animals or humans.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

DETERMINATION OF ANTI-UREASE ACTIVITY OF PROPOLIS FROM MARMARA REGION OF TURKEY

Türkiye'nin Marmara Bölgesinden Elde Edilen Bazı Propolislerin Anti-üreaz Aktivitesinin Belirlenmesi

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ABSTRACT

Propolis is a natural product collected by honeybees from plants especially flowers and buds by mixing with wax and resin and used for many purposes in hive. It is known that propolis has been used in the treatment of various diseases in traditional medicine for many years and has biological activities such as antioxidant, antimicrobial, antiulcer, antitumor, anti-inflammatory. There is increasing interest in alternative approaches to inhibit *Helicobacter pylori* (*H. pylori*) and thereby treat many gastric diseases. This study investigated various properties of raw propolis samples from the Marmara region of Turkey. Anti-urease activity was investigated in propolis samples. The anti-urease activity of IC50 values ranged from 1.110 to 5.870 mg/mL. As a result, it can be said that propolis extract is a good inhibitor that can be used in the treatment of *H. pylori* to improve human health.

Keywords: Propolis, Urease, Enyzme inhibition

ÖZ

Propolis, bal arıları tarafından bitkilerden özellikle çiçek ve tomurcuklardan balmumu ve reçine ile karıştırılarak toplanan ve kovanda birçok amaç için kullanılan doğal bir üründür. Propolisin uzun yıllardır geleneksel tıpta çeşitli hastalıkların tedavisinde kullanıldığı ve antioksidan, antimikrobiyal, antiülser, antitümör, antiinflamatuar gibi biyolojik aktiviteleri olduğu bilinmektedir. Helicobakter pilori'yi (*H. pylori*) inhibe etmeye ve böylece birçok mide hastalığını tedavi etmeye yönelik alternatif yaklaşımlara artan bir ilgi vardır. Bu çalışma, Türkiye'nin Marmara bölgesinden alınan ham propolis örneklerinin çeşitli özelliklerini araştırmıştır. Propolis örneklerinde anti-üreaz aktivitesi araştırıldı. IC_{50} değerlerinin anti-üreaz aktivitesi 1.110 ila 5.870 mg/mL aralığındaydı. Sonuç olarak propolis ekstraktının *H. pylori* tedavisinde insan sağlığını iyileştirmek için kullanılabilecek iyi bir inhibitör olduğu söylenebilir.

Anahtar Kelimeler: Propolis, Üreaz, Enzim inhibisyonu

GENİŞLETİLMİŞ ÖZET

Giriş: Mide ülserine genellikle asidik ortamlarda yaşayan ve büyüyen *Helicobakter pylori'nin* neden olduğu bilinmektedir. *H. pylori* üreaz enzimi salgılar, üreyi amonyağa dönüştürür ve ürettiği amonyak ile mide asidinin etkilerinden kendini korur. *H. pylori* ve üreazı, kronik gastrit, peptik ülser, mide kanseri ile güçlü bir şekilde ilişkilidir. Üreaz enzimi (E.C. 3.5.1.5.), ürenin karbon dioksit ve amonyağa hidrolizini katalize eden çok spesifik bir enzimdir.

Amaç: Propolis, bal arılarının (*Apis mellifera* L.) ağaç, bitkilerin tomurcuklarından topladıkları öz sularını, kendisinde bulunan salgı bezlerinden salgılanan enzimlerle işleyerek oluşturdukları açık sarı renginden koyu rengine kadar değişim gösteren yapışkan doğal bir üründür. Yapılan araştırmalarda propolisin antioksidan, antibakteriyal, antienflamatuar, antifungal, antitumor ve antiülser gibi biyolojik aktiviteye sahip olduğu bildirilmiştir. Çoğu hastalığın tedavisi onunla ilişkili olan enzimlerin inhibisyonu ile mümkündür. Bu çalışmada, Marmara bölgesinin farklı illerindeki propolislerin potansiyel üreaz inhibitörü olup olamayacağı incelendi.

Gereç-Yöntem: 2018-2019 yılları arasında Türkiye'nin Marmara bölgesinin farklı yerlerinden (Bursa Uludağ Üniversitesi Kampüsü, Bursa (Merkez), Kapıdağ peninsula (Balıkesir), Bandırma, İznik, Marmara adası, İstanbul, Çanakkale, Tekirdağ, Yalova, Sakarya) Arı Yetiştiricileri Birliklerinden propolis örnekleri temin edildi. Herbir propolis örneği %70'lik etanol çözücüsünde ekstraktlar hazırlandı. Hazırlanan propolis örneklerinde üreaz enzim inhibisyonuna karşı inhibitor etkisi araştırıldı. Standart olarak tiyoüre kullanıldı.

Bulgular: Etanolik propolis ektraktlarının üreaz enzim varlığında IC_{50} 1.110 to 5.870 mg/mL. değerleri arasında tespit edildi. Propolis ekstraktlarının IC_{50} değeri rakamsal olarak ne kadar düşük ise enzimi inhibe etme oranı da o derece yüksektir.

Tartışma ve Sonuç: Bu çalışmada polifenoller açısından zengin propolis örnekleri için inhibisyon etkileri oldukça güçlü olduğu görülmektedir. Önceki çalışmada, çeşitli Türk propolis numunelerinin anti-üreaz aktivitesi 0.080 ile 1.560 mg/mL arasında değişmekteydi ve yüksek fenolik içerikli numuneler daha yüksek anti-üreaz aktiviteleri sergiledi (Baltaş ve ark., 2016a). Başka bir çalışmada Baltaş ve ark. (2016b), tüm EPE'lerin *H. pylori* üreazını 0.260 ila 1.525 mg/mL gibi geniş bir inhibitör aralıkla inhibe ettiğini bildirmiştir. Literatürdeki veriler mevcut çalışmadaki verilerden daha farklı görülmektedir. Bunun nedeni propolis örneklerinin alındığı bölgeden ve toplanma şeklinden kaynaklandığını söyleyebiliriz.

H. pylori'ye karşı ortaya çıkan antibiyotik direnci, araştırmacıları ülser iyileşmesini hızlandırmak ve enfeksiyonu ortadan kaldırmak için bir ajan olarak propolisi keşfetmeye teşvik ediyor. Yapılan çalışmalar 15 farklı propolisin üreaz enziminin aktivitesini önemli ölçüde inhibe ettiğini göstermiştir (Baltas ve ark., 2016a). Üreaz inhibitörlerinin bazı toksik etkileri göz önüne alındığında, doğal bir ürün olan propolis, *H. pylori* ile ilişkili mide-duodenal enfeksiyonu baskılamak ve kontrol etmek için çok güvenli bir kaynak olma potansiyeline sahiptir.

Bu çalışmada, Türkiye'nin Marmara Bölgesinin faklı illerinden temin edilen propolis örneklerinin farklı oranlarda üreaz enziminin aktivitesini engellediği tespit edildi. Hergeçen gün önemi daha çok artan propolisin, *Helikobakteri pilori* bakterisinin sebep olduğu enflamasyon hastalıklarına karşın doğal bir inhbitör olarak kullanılarak ilaçların oluşturmuş olduğu yan etkilerini ortadan kaldırabileceğini

INTRODUCTION

Bee products such as honey, polen, propolis have been used for traditional and complementary treatment since ancient times (Sahin, 2016). In particular, propolis, one of the bee products, has antioxidant, antimicrobial, anti-inflammatory and anticancer properties due to the presence of phenolic acids, flavonoids (Catchpole et al. 2015). The chemical composition of propolis varies depending on the season, geographical and botanical origins, and the mechanisms used to collect the material (Bankova, 2005, Ulloa et al. 2017). Until this time, the chemical contents of propolis samples from different parts of the world have been investigated and most of them have been found to have a unique and region-specific phytochemical profile (Stavropoulou et al. 2021). The characteristic constituents of propolis are favonoids such as chrysin, galangin, pinocembrin, and pinobanksin. Caffeic acid phenethyl ester is a major constituent of propolis with broad biological activities (Alanazi et al. 2021). There are about 250

and even more chemicals in propolis and and most of them are not known sufficiently.

It is now known that gastric ulcer is generally caused by *Helicobakter pylori,* which survives and grows in acidic environments (Dunn et al. 1997, Mégraud et al. 1999, Amin et al. 2013). *H. pylori* secretes urease enzyme, converts urea into ammonia and protects itself from the effects of stomach acid with the ammonia it produces. *H. pylori* and its urease are strongly associated with chronic gastritis, peptic ulcers, gastric cancer. The urease enzyme (E.C. 3.5.1.5.) is a very specific enzyme that catalyzes the hydrolysis of urea to carbon dioxide and ammonia (Mareska et al. 2013, Modakh et al. 2015).

H. pylori infection remains a worldwide public health problem, particularly due to current treatment inadequacies. This bacterium is an important cause of ulcer and gastric cancer. Antibiotic treatment is widely used in the treatment of this disease. However, some drugs used in the treatment of this disease have a toxic effect. Increasing antibiotic resistance is one of the reasons why *H. pylori* eradication has failed in most countries (Baltas et al. 2016a). Therefore, it becomes necessary to search for new *H. pylori* urease inhibitors with improved stability and low toxicity in natural products such as plant extracts, honey, pollen, and propolis.

This study intended to survey the potential inhibitor activity of the Marmara Region propolis against urease enzyme inhibition, expanding the therapeutic use of propolis and thus consolidating the medicinal properties of propolis type as a possible new therapy for *H. pylori* eradication.

MATERIAL AND METHODS

Chemicals

Jack bean urease, urea, acetohydroxamic acid, sodium nitroprusside and organic solvent were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck.

Propolis Samples

Propolis samples from 11 different areas in the Marmara region of Turkey were obtained from experienced beekeepers between 2018 and 2019 in Figure 1. The samples, sample codes, and their geographic origin are given in Table 1. Extraction, approximately 3 g each propolis sample was taken and onto 30 mL 70% ethanol was added and stirred on a shaker (Heidolph Promax 2020, Schwabach). After 24 hours of extraction at room temperature, filtration was performed.

Urease enzyme inhibition assay

Urease enzyme inhibition of propolis samples was determined in UV-Vis spectrophotometer. (Weatherburn 1967). In summary, 500 µL of buffer solution (pH 8.2), 200 µL urease enzyme solution and 100 µL sample were incubated for 15 min in test tube. Then a phenol reagent 500 µL, and alkali reagent 600 µL were added to each tube absorbance reading was performed at 625 nm than incubated 50 min. Thiourea was used as standard inhibitor. The IC_{50} results are given in mg/mL.

RESULTS

Anti-Urease Activity

The urease inhibition potential of propolis extracts was evaluated *in vitro* by Jack bean urease assay. The result of urease inhibitor activity of the test samples was shown as IC_{50} (mg/mL). The propolis samples totaling 11 belonged to 10 different locations. (Figure 1). Exhibited inhibition effects with IC_{50} values ranged from 1.110 to 5.870 mg/mL in all samples, and with a mean value of 2.493±1.678 mg/mL (Table 1).

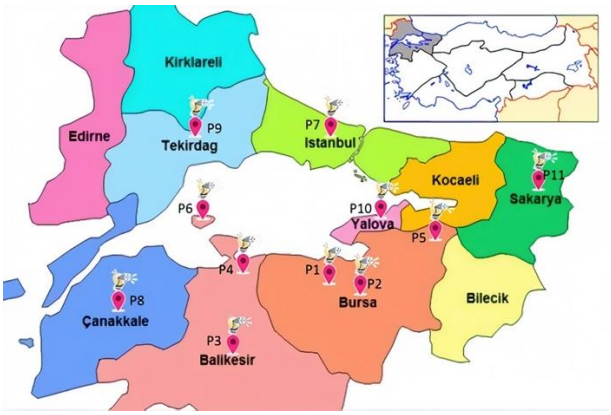

Figure 1. Distribution of propolis samples locations.

(https://www.alfayapimuhendislik.com/bolge/marmara-bolgesi.html)

Table 1. Code, collected region of propolis samples and enzyme inhibition IC50 values.

Code	Regions	Anti-Urease IC_{50} mg/mL
Pr1	Bursa Uludag University Campus	1.420±0.090
Pr2	Bursa (Central)	2.230±0.110
Pr3	Kapidag peninsula (Balikesir)	1.542±0.100
Pr4	Bandırma	1.534±0.080
Pr5	İznik	1.460±0.130
Pr6	Marmara island	1.900±0.070
Pr7	Istanbul	4.600±0.050
Pr8	Canakkale	5.870±0.700
Pr9	Tekirdag	1.200±0.010
Pr10	Yalova	1.110±0.020
Pr11	Sakarya	4.560±0.130
Mean±SD		**2.494±1.678**
Range		**1.110-5.870**
Thioure (µg/mL)		12.110±0.090

DISCUSSION

The enzyme urease is a nickel-containing metalloenzyme belonging to the hydrolase class. Ammonia released as a result of the breakdown of urea by urease assists the colonization of *H. pylori* by shifting stomach acid to neutral pH (Baltas et al. 2016a). Urease provides *H. pylori* with the opportunity to live in the stomach at low pH, causing gastritis peptic ulceritus, and especially cancer (Morishita et al. 2008). A disease caused by *Helicobacter pylori* can be prevented through urease inhibition (Baltas et al. 2016a). Numerous urease enzyme inhibitors are used as medical drugs, but many are costly and have side-effects (Romeno et al. 2019, Song et al. 2020). In recent years, many investigations have indicated that polyphenol-rich extracts such as bee products (especially propolis) have substantial inhibitory effects (Manyi- Loh et al. 2012).

The inhibition effects are quite strong for propolis samples which are rich in polyphenols in this study. In previous study, the anti-urease activity of various Turkish propolis samples ranged from 0.080 to 1.560 mg/mL, and samples with high phenolic contents exhibited higher anti-urease activities (Baltas et al. 2016a). In another study, Baltas et al. (2016b) reported that all of the EPEs inhibited *H. pylori* urease with wide inhibitory ranges that is from 0.260 to 1.525 mg/mL. This data is a little different from ours. The reason for this, we can say that it is caused by the region where the propolis samples were taken and the way it was collected.

Şahin, (2016) reported that was investigate oak, chestnut and polyfloral honeys the inhibition effect of oak honey was found to be higher than other honeys. Another study, it was reported that chestnut honey inhibition urease with 12.36– 34.20 mg/mL inhibition values (Kolayli et al. 2016). Another study reported that the urease enzyme inhibition IC_{50} result of buckweat polen ana chesnut polen 7.41-5.23mg/mL respectively (Can, 2018). It is clearly seen that, propolis a bee product is more effective in urease inhibition compared to other bee products.

The emerging antibiotic resistance to *H. pylori* prompts researchers to explore propolis as an agent to speed up ulcer healing, and eradicate the infection. Studies have shown that 15 different propolis significantly inhibits the activity of urease enzyme (Baltas et al. 2016a). Considering some of the toxic effects of urease inhibitors, propolis, a natural product has the potential to be a very safe source for suppressing and controlling gastro-duodenal infection associated with *H. pylori.* The

future studies should focus on urease inhibitors of propolis from different regions.

Future studies should be focusing on propolis from different regions to treat different health problems particularly microbial infection problems.

Author contribution: Zehra Can: Laboratory part of the study, evaluation of the results, manuscript writing, submitting to the journal, Yakup Kara: Laboratory part of the study, evaluation of the results İbrahim Çakmak: Design and planning of the study, site selection and sample collection, manuscript editing, and correction, Sevgi Kolaylı: Design and planning of the study

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Ethical issue: Not applicable.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

ECOLOGICAL NICHE MODELING: AN EMPIRICAL STUDY ON *Apis mellifera* POPULATION DISTRIBUTION

Ekolojik Niş Modelleme: *Apis mellifera* Popülasyon Dağılımı Üzerine Deneysel Çalışma

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ABSTRACT

***Apis mellifera* is one of the species that aid in the maintenance of ecosystem diversity and spread all around the world, yet their numbers are subjected to a decline in the near future. In this study, *A. mellifera* species' occurrence data obtained from the United Kingdom, Netherlands, France, Germany, Zambia was studied by using the present and future projection models generated by using principal component analysis (PCA) on 19 bioclimatic variables. Regarding the future projections, the honey bee species will be significantly decreased in number in Europe, whereas in North America, the change from current to future was somewhat ambiguous. Therefore, the growth rates of the bioclimatic variables from present to the future for both Europe and North America were compared with each other to conclude the most effective bioclimatic variables on the species' occurrences. In conclusion, the most effective bioclimatic variables that caused the major decline in European clades of this species were assessed as the severity of winters, precipitation seasonality, temperature seasonality, and the mean temperature of the wettest quarter.**

Keywords: *Apis mellifera*, Climate change, PCA ecological niche modeling, Projections, Bioclimatic variables, Occurrences

ÖZ

Ekosistem çeşitliliğinde en önemli türlerden olan bal arısı, *Apis mellifera* türü dünya genelinde birçok bölgeye yayılım göstermiştir fakat iklim değişikliği sebebiyle yakın gelecekte sayılarının azalma tehdidi ile karşı karşıyadır. Bu nedenle iklimsel değişkenlerin bal arıları üzerindeki etkileri daha detaylı incelenmeli ve potansiyel negatif etki faktörleri belirlenmelidir. Bu çalışmada Birleşik Krallık, Hollanda, Fransa, Almanya ve Zambiya'da bulunduğu kaydedilen *A. mellifera* verileri iki farklı izdüşümü modeli yardımıyla şimdiki zaman ve gelecek tahminlerine yansıtıldı. Gelecek tahmininde Avrupa genelinde bal arılarında gözle görülür bir nüfus azalışı kaydedilirken Kuzey Amerika'da değişim, günümüze göre, çok azdı. Daha sonra PCA analiz yöntemi kullanarak 19 iklimsel değişken bir arada değerlendirildi ve günümüzden geleceğe büyüme oranları hesaplandı. Bu çalışma doğrultusunda genel olarak Avrupa'da gelecekte en çok değişiklik gösteren iklimsel değişkenlerin Kuzey Amerika'da gelecekte kritik bir değişikliğe uğramayacağı sonucuna ulaşıldı. Sonuç olarak, bu tür için yüksek orandaki nüfus azalışında etkili olan ve etkilerini yakın gelecekte Avrupa'da gösterecek olan değişkenler kışların şiddeti, en soğuk bölgenin ortalama sıcaklığı, yağış ve sıcaklık mevsimselliği olarak belirlendi.

Anahtar Kelimeler: *Apis mellifera*, İklim değişikliği, PCA, İklimsel değişkenler, Ekolojik niş modelleme

GENIŞLETILMİŞ ÖZET

Amaç: Bu çalışmada Birleşik Krallık, Hollanda, Fransa, Almanya ve Zambiya'da bulunduğu kaydedilen *Apis mellifera* verileri Gbif veri tabanından alındı ve iki farklı izdüşümü modeli kullanarak şimdiki zaman ve gelecek tahminlerine yansıtıldı.

Giriş: Polen taşıyıcı türler biyolojik çeşitliliğin sağlanması ve devamlılığını sağlayan en önemli etkenlerdendir. Ekosistem çeşitliliğinde en önemli türlerden olan Apis mellifera, arıcılık faaliyetleri başlıca olmak üzere insan etkisi ile dünya genelinde birçok bölgeye yayılım göstermiştir fakat yine de insan etkisi ile yakın gelecekte nüfuslarında bir azalma beklenmektedir. Bal arısı türleri için en önemli olumsuz etken faktörlerinden biri de bünyesindeki birçok değişkenin etkisi ile birlikte, bütün halinde ekosisteme zarar veren iklim değişikliğidir. Bu nedenle iklimsel değişkenlerin bal arıları üzerindeki etkileri daha detaylı incelenmeli ve potansiyel negatif etki faktörleri belirlenmelidir.

Yöntem ve Gereç: Gelecek tahminleri için, daha gerçekçi bir gelecek üzerine, araştırılan tür için tahminde bulunulması amacı ile en olası senaryo koşullarını içeren yakın gelecekteki iklimsel değişkenleri ifade eden WorldClim veri tabanı modeli tercih edildi. A. mellifera türünün potansiyel nüfus dağılımını analiz edebilmek amacıyla kullanılan iki model random forest (RF) ve generalized linear model (GLM) olarak belirlendi ve şimdiki zaman projeksiyonları için kullanıldı. Ayrıca genetik ve coğrafi olarak 3 e ayrılan, A. mellifera türüne ait, Avrupa, Afrika ve Büyük Britanya nesilleri ayrı ayrı GLM modeli kullanarak günümüze ve geleceğe yansıtıldı.

Bulgular: Gelecek tahmininde *A. mellifera* türündeki nüfus azalışı, Avrupa genelinde gözle görülür düzeyde, yakın gelecekteki tür kaybının ciddiyet düzeyini belirtebilecek ölçüde iken Kuzey Amerika'da kaydedilen değişim günümüze göre çok azdı. Daha sonra tahmin analizinde belirleyici olarak kullanılan iklim değişkenlerinin veri boyutu ve doğrusallığı değişkenler arasında düşürüp aynı zamanda bilgi kaybından en az miktarda etkilenmek amacıyla PCA analiz yöntemi kullanarak 19 iklimsel değişken bir arada değerlendirildi ve günümüzden geleceğe büyüme oranları hesaplandı. Tüm iklimsel değişkenler için hesaplanan büyüme oranları, gelecekte en çok değişime uğrayacak Avrupa bölgesi, en az değişikliğe uğrayacak Kuzey Amerika Bölgesi ve dünya geneli arasında karşılaştırma yöntemi ile araştırıldı. Bu çalışma doğrultusunda, yakın gelecekte Avrupa'da en çok değişikliğe uğrayacak olan iklimsel değişkenlerin Kuzey Amerika'da gelecekte kritik bir değişikliğe uğramayacağı sonucuna ulaşıldı.

Sonuç: Bu tür için yüksek orandaki nüfus azalışında etkili olan ve etkilerini yakın gelecekte Avrupa'da gösterecek olan değişkenler kışların şiddeti, en soğuk bölgenin ortalama sıcaklığı, yağış ve sıcaklık mevsimselliği olarak belirlendi. Büyüme oranları doğrultusunda en çok farkı yaratacağı tespit edilen değişkenlerin Avrupa'da, Kuzey Amerika'dan ve dünyanın geri kalanından fark edilir derecede artış veya azalış gösterdiği analizler sonucunda doğrulanmış ve literatür bilgileri ile karşılaştırılarak bu değişkenlerin araştırılan tür üzerinde nüfus ve ekolojik niş korumada hâlihazırda yüksek tesirli olduğu tespit edilmiştir.

INTRODUCTION

Apis mellifera is the western honey bee species that are spread across the world mostly as a result of beekeeping activities due to its environmental, agricultural, and economical importance (Han et al., 2012). Honey bee species are a prerequisite for the maintenance of the ecosystem and diversity of many field crops of flowering plants, fruits, nuts, and vegetables as being one of the most important pollinators on Earth. However, honey bees are facing various threats as a result of the exclusively human effect of habitat destruction, pesticides, loss of genetic diversity, parasites, and climate change (Paudel et al., 2015). As a result of the decrease in the numbers of this species, the world's economy is affected negatively due to the decrease in ecological services. As a result of the beekeeping activities, *A. mellifera* species are spread across the world and diverged over time in Africa, the Middle East, and Europe, yet their numbers are decreasing with the changing climatic conditions and human activities (Arias and Sheppard, 2005). In addition to the direct effect of climate change, the honey bee populations are in decline worldwide also due to the indirect effects such as the infestations of pests and pathogens that cause honey bee loss (Sharif et al., 2021). Therefore, comprehensive research should be focusing on the future projections of quantitative

analysis on the *A. mellifera* species and the possible sources of decline response; in order to protect the colonies from the current degradation more importantly in the rather sensitive regions to the climatic changes such as Mediterranean zones (Flores et al., 2019).

Due to the diversity of the species' population that followed their globalization, the genetic difference between different lineage groups should also be considered for the population studies. In accordance with the comparisons of recent mtDNA and nuclear diversity data of honey bee species from distinct regions, several lineage groups were identified for this species that consist of mainly African and European clades.

With regard to the previous studies, the African origin of *A. mellifera* and the early colonization of west Europe has followed a regional differentiation during the intermediate Pleistocene glaciation. As a result, the extant haplotype frequency and distribution were influenced at a regional scale following the adaptations to local climatic conditions, along with the local beekeeping practices during the last decades (Jaffé et al, 2019). The significant evolutionary events that affected the genetic structure of *A. mellifera* species are; dispersal and differentiation after their divergence from the other cavity-nesting honeybee species, and differentiation of different clades from their native ranges of Europe, Africa, and the Middle East followed by the further dispersal and differentiation by human activities. The population size is further increased in Africa while European colonies were restricted in lower densities (Cánovas et al, 2008).

As shown by the previous studies, the 19 bioclimatic variables are highly correlated in terms of informativeness. Therefore, the linear combination of the 19 bioclimatic variables can be used more efficiently as the indicators for species' distribution, instead of selecting all separately (Makori et al., 2017).

The distribution modeling of species is carried out by utilizing a special package for programming in R. The biomod2 package illustrates larger areas than the initial occurrence data distribution provided by random sampling of the distribution and is used for ensemble forecasting of species distributions (Thuiller et al., 2009). According to the inquired data and the required output, various predictors are used in this package, such as Random forest (RF) and generalized linear model (GLM), and can be used together to assess the fitness of the environmental conditions of the species occurrences to the projection model and for their comparisons. RF is an ensemble learning algorithm that can be applied only to decision trees. Since decision trees are likely to overfit, RF aims to increase the prediction through random subsampling of rows and columns by introducing a bias to the training phase. This bias results generally in higher test accuracy (Breiman, 2001). GLM, on the other hand, assumes the linear relationship between the expected response and the explanatory variables (PennState, 2022).

In this study, the present and the future projection models were applied to *A. mellifera* occurrence data by using one of the dimensional reduction methods, principal component analysis (PCA), on 19 bioclimatic variables. Here the aim was to explain more than 90% of the 19 bioclimatic variables by excluding multicollinearity. Additionally, based on the genetic information of difference and the distribution based on the location, the occurrence data of the *A. mellifera* species was classified as representing three lineage groups; African clade, Great Britain clade, and European clade.

METHODS

Acquiring the occurrence data of *Apis mellifera*

The occurrence data of *A. mellifera* is downloaded from the Gbif database (Global Biodiversity Information Facility, 2022). The date was restricted from 1981 to 2021, and the countries were restricted to the United Kingdom, Netherlands, France, Germany, and Zambia.

Distribution of the lineages on the occurrence data based on genetic information

The African species occurrence data was considered as a distinct clade from the European occurrences. Accordingly, the lineages were decided based on the geographical occurrence data and the genetic background of the species. In this study, the data were taken and classified based on the countries of origin as follows;

Clade 1: Great Britain

Clade 2: Germany, Belgium, France, and the Netherlands

Clade 3: South Africa

The distribution map of the *A. mellifera* species is plotted by using R studio based on the occurrence data obtained from Gbif and the 3 different lineages are colored differently as shown below by the world

map (Figure 1). The African and European clades are focused in figures (Figure 2 and 3) respectively with the European clade having 2 distinct clades also that were colored differently.

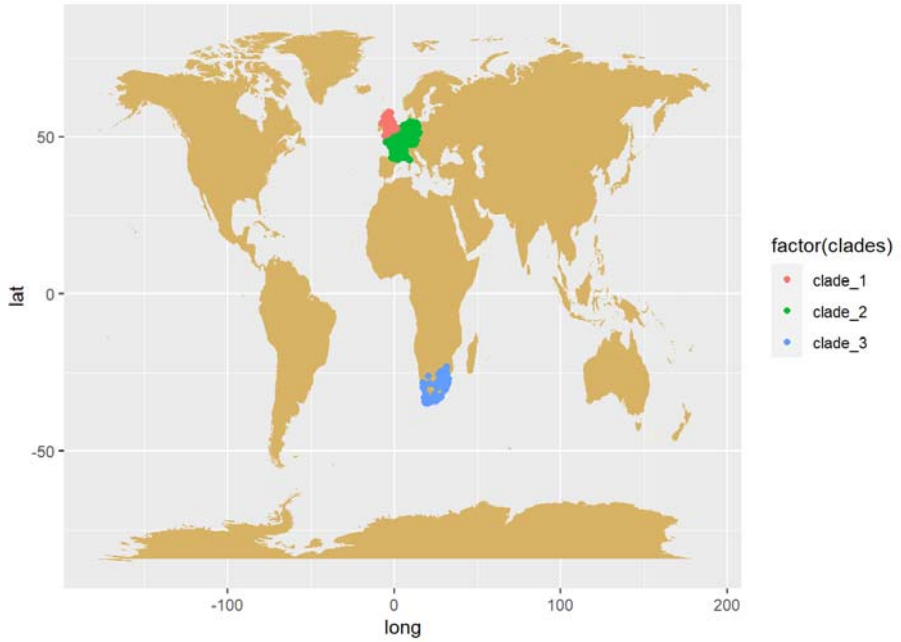

Figure 1. The distribution map of the *A. mellifera* shows the occurrences in Great Britain –as clade_1 in pink color, Germany, Belgium, France, and Netherlands –as clade_2 in green, and Africa –as clade_3 in blue.

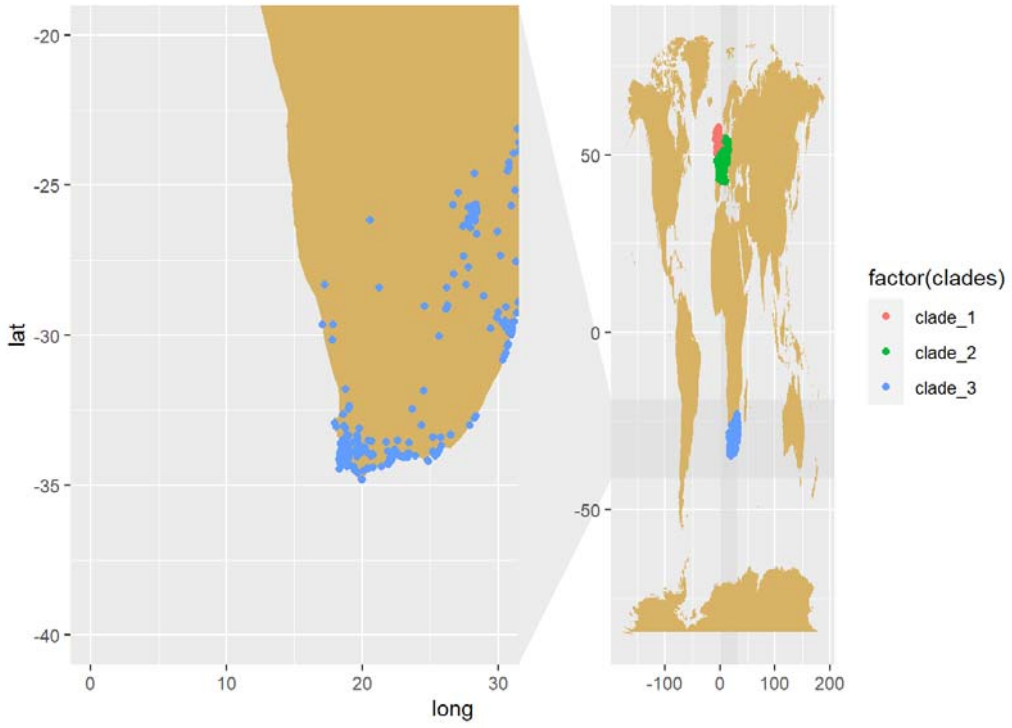

Figure 2. The focused picture of African clade_3.

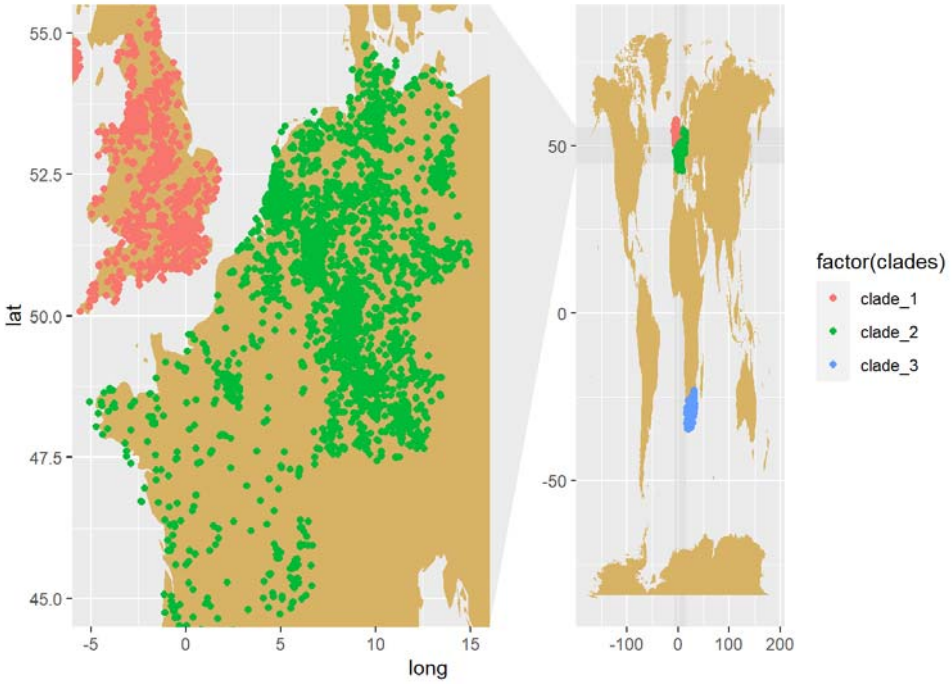

Figure 3. Focus on the European clades, clade_1, and clade_2, depicting the occurrences in Great Britain, Germany, Belgium, France, and the Netherlands.

Acquiring the historical and future bioclimatic variables

The data for each of the 19 bioclimatic variables, BIO 1 to 19, for 5-minute spatial resolutions were obtained manually from the WorldClim database for the historical bioclimatic variables (WorldClim, 2020). The future bioclimatic variables were also acquired from the WorldClim database for longitude/latitude degree of 5-minute spatial resolutions about 9 km at the equator future (2021-2040), and for CNRM-CM6-1 estimations of ssp370 (World Climate Research Programme, 2016). SSP370 scenario was selected because it is the closest to the current CO_2 emissions, due to the assumption that in the near future the climatic conditions will not be able to change drastically in 20 years of the time period. Also, because of the small lifespan of honey bees, a closer future scenario was selected to accomplish more realistic results from the projections. **Performing principal component analysis (PCA) on the bioclimatic variables**

In this study, for the utilization of the honey bee ecological niche model, PCA was performed on all of the 19 bioclimatic variables and the resulting linear combination was used as the indicator variables. As a result, 4 dimensions were used instead of 19, since 4 eigenvectors were obtained having the eigenvalue greater than 1, meaning that they can explain more variability than what they are supposed to explain. The original space of 19 dimensions was reduced to 4 because the previous dimensions were highly correlated with each other since they are all coming from the same family namely the bioclimatic variable; thus, they all are linear combinations of each other. The reduced dimension space of 4 provided adequate information by explaining 90% variability. In terms of interpretability, here, computational cost and multicollinearity problems have been excluded along with the problem of biased results in the algorithm that were used for the current and future projections. Then, the current and the future projection models were constructed by using the 4 dimensions of the explanatory variables. Figure 4 shows the eigenvalues where the red line is 1 which is the limit for eigenvalues and the cumulative variance that was explained by the PCA respectively in the left and right scree diagrams.

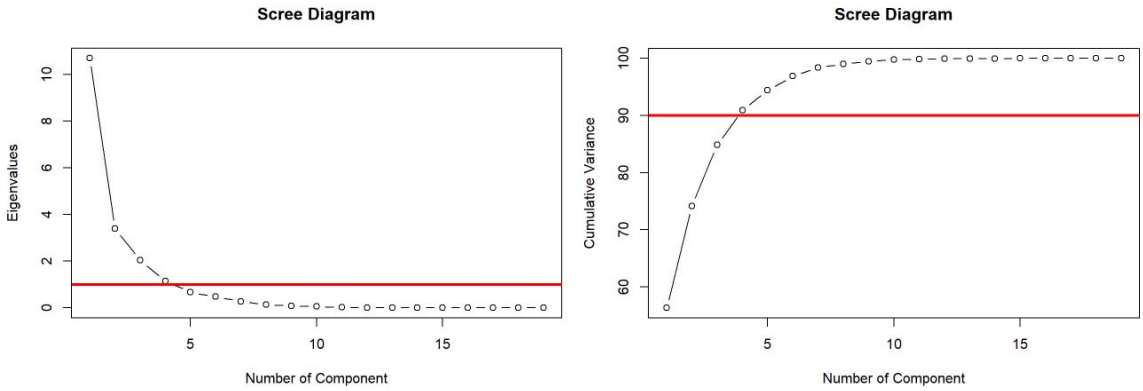

Figure 4. The eigenvalues are shown with the red line showing the limit for eigenvalues, 1 in the left plot. The cumulative variance is shown in the right plot with the red line showing 90% of the variability.

Additionally, PCA was performed on the future environmental variables, as well as the historical bioclimatic variables both by transforming data of the raster-layer object into a raster-stack object in order for BIOMOD species distribution models to work and to perform the current and future projections.

Species distribution modeling and projections

For forecasting of *A. mellifera* species distribution, initially, two model classes were used as part of the biomod2 package processing of modelling in order to test the fitness of the environmental conditions of the species occurrences to the projection model. Also, the pseudo-absence data was generated by using biomod2 projection models since the occurrence data acquired for *A. mellifera* was presence-only, and the undefined locations in the map were excluded by this way, otherwise, they cause manipulations in the projection and decrease the accuracy of the results.

Even if discussing all the details of such algorithms is beyond the scope of this paper, we can mention the bias-variance trade-off in machine learning algorithms. In general, when one is higher the latter is lower and vice versa. RF tries to find a good balance between them by introducing bias through random subsampling. The strength of the individual trees and the correlation between them cause generalization errors in tree classifiers (Thuiller et al., 2009). Thus, GLM was also used as a model in the projection.

Analysis of some of the differences between the present and the future projections

Based on the changes observed for the present and the future projections for the species, analysis was carried out by using the bioclimatic variables. Firstly, the mean values of the bioclimatic variables for each location of the presence data were calculated. Then the comparisons for the calculated growth rate of each bioclimatic variable from present to future projections were done between North America, namely Mexico, and Europe in order to further study the underlying climatic reasons for the major declines that were observed for the European lineages by comparisons done with the regions that showed no observable change in the near future.

RESULTS

Present and future projections for *Apis mellifera*

The occurrence status map in Figure 5 illustrates both current and future distributions of *A. mellifera* species, generated by using both RF and GLM models.

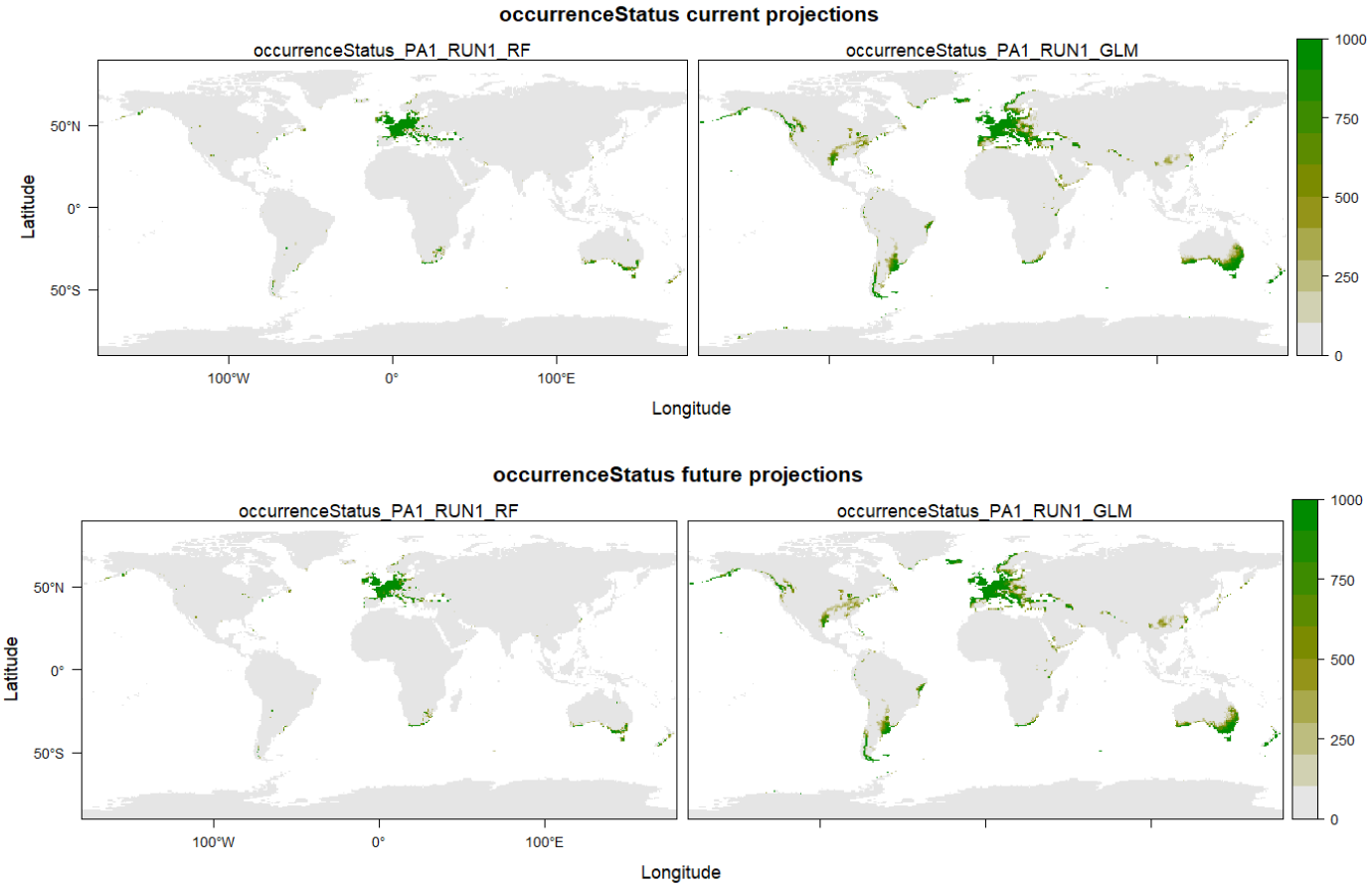

Figure 5. The distribution maps of the present projection of the species are shown above, and that of the future projection of the species are shown in the below pictures. Each projection was generated by using RF and GLM models as labeled RUN1_RF and RUN1_GLM.

Firstly, the GLM model used for both current and future projections projected the occurrences over a larger area than the RF model. Secondly, for both of the model projections, a significant decrease in occurrences was observed worldwide for the future projections. Especially, the major decrease was observed in the locations where the species were found less abundant than the locations where more abundance is projected.

Present and future projections for the different lineages of *Apis mellifera*

The GLM model was used for the present and future projections of the 3 lineages separately. Initially, the present and current projections of the first lineage of Great Britain are shown below (Figure 6).

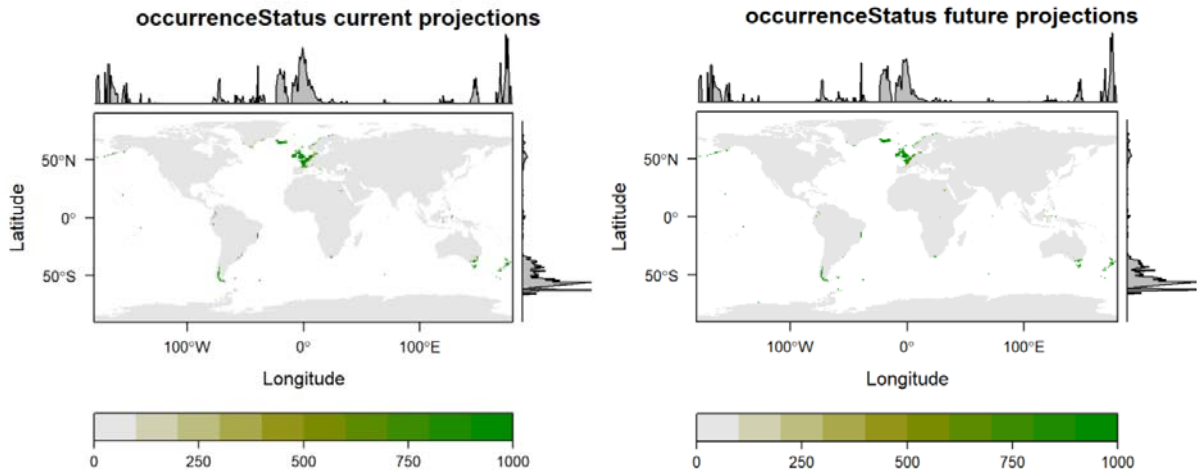

Figure 6. The distribution maps of the present projection of the species were generated by using the GLM model for the first lineage, clade_1.

For the clade_1, some slight changes in distribution from present to future were obtained. Again for the locations where the species are more abundant, no change in occurrence was observable, yet in the locations where the species are found less abundant, the future projections showed less occurrence especially around coastal regions of southeast America, Italy, and Turkey Black Sea region.

Secondly, the present and the future projections for the second lineage of the occurrences in Germany, Belgium, France, and the Netherlands are depicted on the map (Figure 7).

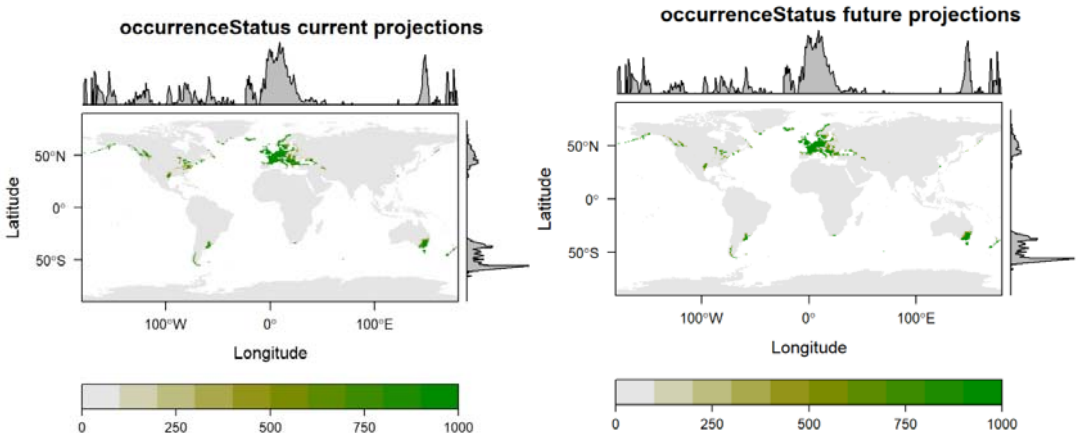

Figure 7. The distribution maps of the present projection of the species were generated by using the GLM model for the second lineage, clade_2.

For the second clade (Figure 7), a significant decrease was observed in the future projection compared to the present around the eastern coastal region of North America whereas the changes in the rest of the world were somewhat ambiguous.

Lastly, the third lineage of South Africa is shown below in Figure 8 as projected by the GLM model to the present and the future.

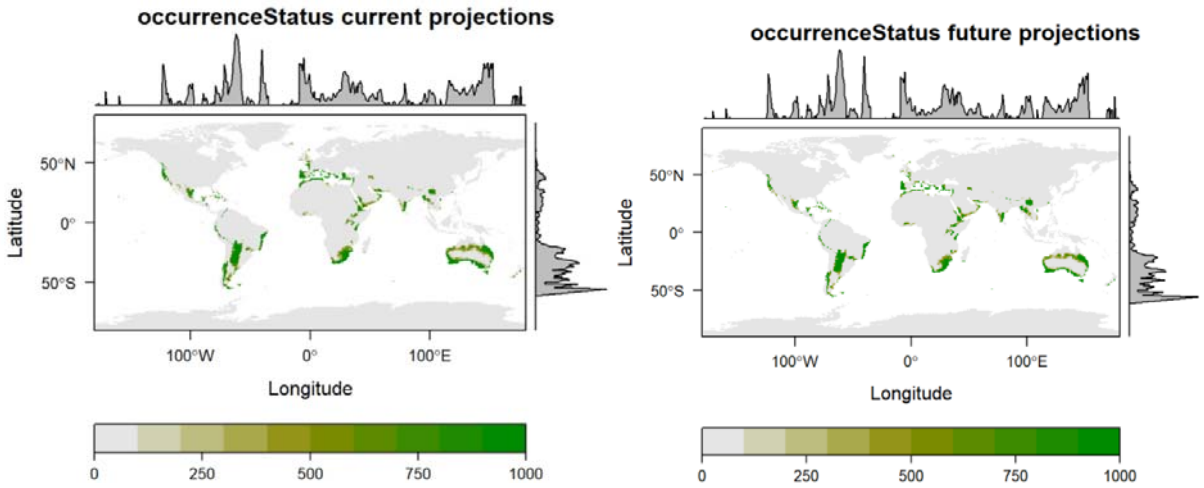

Figure 8. The distribution maps of the present projection of the species were generated by using the GLM model for the first lineage, clade_3.

For the 3rd lineage group, a slight increase in the occurrences in Argentina, Ghana, Nigeria, and Mozambique was observed in the future projection compared to the present, whereas the decrease was observed in the midland regions of Australia, South Africa, Mexico, and Europe.

Analysis on the bioclimatic variables that lie on the locations of the lineages

The mean values of the bioclimatic variables of present and future that are calculated for each location are shown below in Table 1.

Table 1. The mean values for current and future bioclimatic variables for each location in the presence data.

	means_current	means_future
BIO1	10.3136025664475	11.4260536559162
BIO2	8.12932007065567	8.23656814539118
BIO3	36.0917182126033	35.5872033440604
BIO4	553.814401011695	571.786200171954
BIO5	23.0217727425183	24.554102847215
BIO6	0.206130673364563	1.10327672907893
BIO7	22.8156420571953	23.4508261379741
BIO8	11.9309419711625	13.2118662566453
BIO9	7.76488228768998	8.77732713429388
BIO10	17.2515062962582	18.6451892693464
BIO11	3.73434130250618	4.73332450768689
BIO12	760.742233880989	775.627674945988
BIO13	84.7117216622946	87.1066431822113
BIO14	44.5579179897832	44.8745800569606
BIO15	21.0383202996386	21.8114940604337
BIO16	238.510561921856	245.029821884364
BIO17	147.859036310921	149.315166361207
BIO18	198.466519398039	201.801785625609
BIO19	190.357586635372	195.477184878682

Table 1 showed the mean values of the bioclimatic variables for both present and future. According to Table 1, the Annual Mean Temperature will be increased by 1.1 degrees (BIO1), Mean Diurnal

Range (BIO2) will not change remarkably, (BIO3) isothermality, will decrease by 0.5 points, (BIO4) temperature seasonality will increase by almost 28 points, Max Temperature of Warmest Month (BIO5) will increase by 1.5 points, Min Temperature of Coldest Month (BIO6) will increase by 0.9 points, Temperature Annual Range (BIO7) will increase by 0.6 points, Mean Temperature of Wettest Quarter (BIO8) will increase by 1 point, Mean Temperature of Driest Quarter (BIO9) will increase by 1 point, Mean Temperature of Warmest Quarter (BIO10) will increase by 1.4 points, Mean Temperature of Coldest Quarter (BIO11) will increase by 1 points, Annual Precipitation (BIO12) will increase by 15 points, Precipitation of Wettest Month (BIO13) will increase over 2 points, Precipitation of Driest Month (BIO14) and Precipitation Seasonality (BIO15) will be almost identical, Precipitation of Wettest Quarter (BIO16) will increase by 6.5 points, Precipitation of Driest Quarter (BIO17) will increase by 1.5 points, Precipitation of Warmest Quarter (BIO 18) will increase by almost 3 points, and Precipitation of Coldest Quarter (BIO19) will increase over 5 points.

Analysis on the differences between present and future projections

The reasons behind the certain differences in the certain locations were searched by comparing the growth rates of the bioclimatic variables in such regions due to the expectation that they differ significantly. In Europe, there is a visible difference in the distribution of honey bees therefore, the analysis was focused in there to conclude whether they make any difference from present to future. On the other hand, in North America the change between the present and future projections was somewhat ambiguous, the comparisons were done between the Europe and the North America growth rates –percentage change. Table 2 shows the growth rates of Europe and North America, namely Mexico.

Table 2. The means calculated for the current, and the future bioclimatic variables along with the growth rates for Europe and North America, Mexico.

	means_europe_current	means_future	growth_rate
BIO1	10.8418817653946	12.2636349510256	11.5932445095494
BIO2	9.00521080189194	9.06274363948366	0.634828037516847
BIO3	31.7373581274422	31.1618018660173	-1.84699287897233
BIO4	745.662088198472	768.72410220004	3.00003784655199
BIO5	26.3403537017054	28.2765418924246	6.84733019364696
BIO6	-2.51988145970525	-1.22012367564396	-106.526724299099
BIO7	28.8602351615028	29.4966655818685	2.15763513539957
BIO8	11.881519628254	12.9270631148142	8.08802028174513
BIO9	11.1298639062733	13.0459083642299	14.6869378847561
BIO10	20.0183582609496	21.7974579238216	8.16195938576725
BIO11	1.82852994269513	3.0926703327782	40.8753683405845
BIO12	678.735606651858	681.715615077802	0.437133661021393
BIO13	90.5038722459026	91.9474845392017	1.5700400076563
BIO14	29.178843729363	29.2231644337408	0.151662919593282
BIO15	41.7415474941302	42.2321388960643	1.16165416850316
BIO16	248.402353364952	250.771527479453	0.944754031015557
BIO17	99.6213003542054	100.138640412219	0.516623808635956
BIO18	151.975745932641	149.756073538114	-1.4821919018613
BIO19	184.982169658402	187.802915693467	1.50197137496495

	means_mexica_current	means_future	growth_rate
BIO1	21.2794842010401	22.6086139103462	5.87886420006423
BIO2	9.85852981587287	9.86313929034231	0.0467343543850897
BIO3	44.6733844362456	44.3597974318558	-0.706917124388466
BIO4	503.715544421963	509.941685536379	1.22095158937002
BIO5	32.4455969450478	33.8370765687766	4.11229268255634
BIO6	9.13348229702168	10.4436592651183	12.54519067347
BIO7	23.3121146515363	23.3934173168591	0.34754505603658
BIO8	26.4151459549386	27.9140879921818	5.36984062550416
BIO9	17.9666572097155	19.3600442166121	7.19723049858009
BIO10	27.2069105444283	28.6150868376024	4.92109739581015
BIO11	14.8921839345767	16.1823395490646	7.97261490266094
BIO12	1090.4224137931	1096.7277679928	0.574924277811894
BIO13	160.376588021779	163.690865130693	2.02471720475545
BIO14	46.4460072595281	45.4166666561646	-2.26643802627857
BIO15	46.7948444062699	47.8607001828195	2.22699578668575
BIO16	412.872050816697	420.377646742196	1.78544125351697
BIO17	164.409255898367	164.65237459297	0.147655747573708
BIO18	369.714609800363	369.734800473748	0.00546085284890373
BIO19	190.533121597096	190.288377313562	-0.128617568234835

From the above table, it was concluded that more drastic changes were observed from the present to future bioclimatic variables in Europe, than North America. For example, the Mean Temperature of Coldest Quarter (BIO11) increases over 40% in Europe whereas that increases almost 8% in North America, and the Min Temperature of Coldest Month (BIO6) decreases by 106% in Europe whereas that increases 12% in North America in the future.

Accordingly, the comparisons of the growth rates were done between North America, Europe, and the world as shown below in Table 3.

Table 3. The comparison of the growth rates of Europe, the world, and North America respectively.

	EU_Growth	World_Growth	NA_Growth
BIO1	11.5932445095494	12.7544732551088	5.87886420006423
BIO2	0.634828037516847	-1.18264984163205	0.0467343543850897
BIO3	-1.84699287897233	-2.41309437332736	-0.706917124388466
BIO4	3.00003784655199	-0.672934445273483	1.22095158937002
BIO5	6.84733019364696	5.88263420049868	4.11229268255634
BIO6	-106.526724299099	-532.499634244125	12.54519067347
BIO7	2.15763513539957	-0.474435526128063	0.34754505603658
BIO8	8.08802028174513	8.51412271860269	5.36984062550416
BIO9	14.6869378847561	17.7520395422023	7.19723049858009
BIO10	8.16195938576725	7.56957335204494	4.92109739581015
BIO11	40.8753683405845	32.5500125543644	7.97261490266094
BIO12	0.437133661021393	1.55001518849285	0.574924277811894
BIO13	1.5700400076563	3.1035018628058	2.02471720475545
BIO14	0.151662919593282	-0.169734199429162	-2.26643802627857
BIO15	1.16165416850316	1.36669412550563	2.22699578668575
BIO16	0.944754031015557	2.32598070054787	1.78544125351697
BIO17	0.516623808635956	0.755622928510687	0.147655747573708
BIO18	-1.4821919018613	0.0820956787201721	0.00546085284890373
BIO19	1.50197137496495	2.05168091391371	-0.128617568234835

The above table showed how drastic the changes will take place in the future for Europe, from the comparison that the changes are close to the world growth rates or exceed them even if the regional changes are in question.

DISCUSSION

From the present and future projections generated of *Apis mellifera* species and the 3 lineages of the species, by both RF and GLM models, overall a significant decrease in the occurrences was observed. However, by using the GLM model for the projections, 3 different lineages were also projected separately from each other and the whole species occurrences. From the separate projection models of each 3 lineages, the changes in the occurrences were observed more clearly since a limited region was in question and the projections were more realistic by working with a smaller group of occurrences. Additionally, since the lineages were decided based on the genetic information, for the ecological niche modeling of the species the genetic background and the different clade groups should also be considered alongside the occurrence only information. The comparative analysis of the changes in the bioclimatic variables for the least affected and the most affected regions provided detailed information on the possible sources of the major declines within the distinct lineages and revealed the most vulnerable regions to the climatic changes in the near future. Also, while performing PCA on the bioclimatic variables enabled collective assessment of the effects in the future, the separate comparisons of each variable enabled to identify which variable results in more severe effects in the future.

Regarding the bioclimatic variables used for the projections, PCA was performed for each of the 19 bioclimatic variables, so that 90% of the variability was explained by 4 dimensions, instead of using 19 bioclimatic variables separately. As a result, the projections were done by using the information from all of the bioclimatic variables as well as using them all for the research of the factors that caused the changes between the projections, without facing the multicollinearity problem or dealing with the computational problems in terms of interpretability.

The GLM model was preferred over the RF model for the present and future projections of the 3 lineages since the model projected the occurrence data over a larger area than the RF model. Even if the size of the projected area does not necessarily reflect the accuracy of the method used, since the distribution of the *A. mellifera* species was recorded as they spread all around the world (Techer et al., 2017), and regardless of the model used, in this study, the differences in the occurrence projections over a large area was preferred for comparability of the results.

By the bioclimatic variables' growth rate comparisons done for Europe, North America, and the whole world, the reason behind the significant decrease in this species' occurrences in Europe was investigated. Overall, more drastic changes were calculated in the bioclimatic variables from present to future projections for Europe; thus, it was concluded that climate change will be affecting Europe more than North America which in turn appeared to affect the species distribution negatively. Although Europe lineage (clade_2) accounts for the occurrences in North America, as a result of the severity of the difference between the climatic conditions, the same genetic lineage group responds differently in different locations.

For Europe, some of the bioclimatic variables were changed severely and predicted as the main causes of the decreases in the occurrences of the species. Although the *A. mellifera* species spread all around the world and shows great adaptive potential in all those diverse climates, the evidence demonstrated that climate change is directly affecting the honey bee development, behavior, physiology, and distribution (Le Conte and Navajas, 2008). In the past, high mortality rates were explained by longer cold, rainy, and hot weather periods (Kauffeld et al., 1976), also severe winter weather is reported to be the most important factor in the winter mortality rates of honey bees by the direct effect of the weather on colony productivity (VanEngelsdorp et al., 2008). This effect is also assessed in this study that the Min Temperature of Coldest Month (BIO6) decreases by 106% in Europe whereas 12% increase is seen for North America, also the Mean Temperature of Coldest Quarter (BIO11) increases over 40% in Europe whereas that increases almost 8% in North America. Therefore, Europe will have more severe winters in the future and this will be observable in the decrease of the numbers of *A. mellifera* species.

The other effect is the high temperatures and precipitation effects that are positively related to nectar production, accordingly colony productivity and increase in numbers in honey bees (Shuel, 1992). Therefore, insufficient precipitation at inappropriate times is expected to affect the numbers negatively in the future (Meixner, 2010). However, this precipitation effect is barely seen in the results, since a minor change is observed in the precipitation-related bioclimatic variables or it can be also concluded that, honey bees are vulnerable to minor changes in precipitation amounts and seasonality.

According to previous studies, the Temperature Seasonality (BIO4), and the Mean Temperature of Wettest Quarter (BIO8) affect the numbers of honey bees negatively, whereas the effect of the Precipitation of Wettest Month (BIO13) is positive (Delgado et al., 2012). In conjunction with the results of this study, it can be said that the major decrease in honey bee occurrences in Europe is also correlated with the effects of the changes on BIO4 and BIO8. Temperature seasonality is changed by 3 points for Europe, and the mean temperature of the wettest quarter was increased by 8 points both are remarkably high in Europe compared to North America and the world whereas precipitation of the wettest month increased by 1.5 points less than North America and the world. Another study showed that the most effective bioclimatic variables are mean temperature in the wettest quarter and mean annual temperature in honey bee numbers (Peil and Aranda, 2021); for which the severity of the effects was explained as the reasons for the future decrease in honey bee occurrences.

Other than the effects of the climatic condition variations solely on the *A. mellifera* species itself, expectedly, these changes will also influence the other interacting species with honey bees and influence the honey bee occurrences indirectly. Such an effect can be observed with the changes in the annual mean temperature, mean diurnal range, minimum temperature of the coldest month, and the mean temperature of the warmest quarter. Even if the population distribution was assessed based only on the bioclimatic variables in this study, further decline effects will be much more likely in the future. For instance, in the population distribution of invasive species such as parasitic flies the major contributions belong to the BIO1, BIO2, BIO6 and BIO10 variables in the Mediterranean region (Abou-Shaara and Darwish, 2021). As a result, since these variables already showed a higher growth rate in the European clades and contributed to the major decline in the distribution within the scope of this study, due to the interconnected effects, these bioclimatic variables will appear to be more disruptive in the future.

Regarding the regional future occurrence changes, whereas the major declines are likely to be observed in the coastal regions in the future, the South Africa region was assessed as if the population size there will be increased in the near future. *A. mellifera* species prefer warmer, fewer variable climates and mainland rather than island sites for visitation (Hung,

2018) since the species response to temperature increase are increasing flight departures (Burrill and Dietz, 1981). This may explain the higher decrease in numbers around coastal regions and a slight increase in the numbers of the mainland for the African clade.

Lastly, step declines are the problem of North America for a long time as well as Europe and the risk factors are still affecting the populations' occurrences (Watanabe, 1994). Thus, in this study, the severity of the effects was compared between Europe and North America, yet the changes in North America cannot be assessed that might cause by the model performances the fitness to the data, as well as lack of repetition of the projections while using the models, and the insufficiency of the data.

Conclusions

Honey bees are one of the most important species in maintaining biodiversity as pollinators. The *Apis mellifera* species has great adaptive potential since they are spread all around the world mostly by human activities. However, the numbers of these species are decreasing mostly because of climate change, and again mostly because of human activities. In conclusion, conservation measures should be taken in order to decrease the loss of this species that in turn cause the disruption of ecotypes that are highly dependent on world biodiversity which is maintained by honey bees all around the world.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

PALYNOLOGICAL ANALYSIS, PHENOLIC COMPONENTS AND ANTI-INFLAMMATORY ACTIVITY OF SOME BEE POLLENS COLLECTED FROM THE NORTHEAST REGION OF ALGERIA

Cezayir'in Kuzeydoğu Bölgesinden Toplanan Bazı Arı Polenlerinin Palinolojik Analizi, Fenolik Bileşenleri ve Anti-İnflamatuar Aktivitesi

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ABSTRACT

Bee pollen is multiplex blend of floral pollen and nectar agglutinated by bee salivary substances. It is famously known for being high in proteins, carbs, lipids, vitamins, and phenolic compounds, among other physiologically dynamic components. Its composition fluctuates incredibly agreeing to both botanical origins and edaphoclimatic conditions. In this work, the botanical origin, the phenolic components and the anti-inflammatory activity *in vivo* of eight bee pollens intended for human consumption were taken from distinctive apiaries in Algeria's northeast, were determined and compared. All samples were detected heterofloral based on the identification of forty pollen types belonging to 22 botanical families. Total phenolic contents varied between 752.94 ± 17.78 and 12247.06 ± 40.04 mg GAE/ 100g, while the total flavonoid contents ranged from 2680.55 ± 12.02 to 8506.94 ± 15.56 mg QE/ 100g, and the total flavonol contents were in the interval between 4978.87 ± 33.39 and 7903.75 ± 24.39 mg QE/ 100g. The obtained results showed that the bulk of the ethanolic extracts had a good anti-inflammatory activity. As a conclusion, all the aforementioned heterofloral bee pollen samples could significantly be a wealthy source of polyphenols with a potential anti-inflammatory activity.

Key words: Bee pollen, Palynological analysis, Phenolic contents, Anti-inflammatory activity

ÖZ

Arı poleni, çiçek poleni ve nektar ile arı tükürük maddelerinin karmaşık bir karışımıdır. Karbonhidratlar, proteinler, lipidler, vitaminler ve fenolik bileşikler gibi birçok biyolojik olarak aktif maddenin zengin bir kaynağı olarak ünlüdür. Bileşimi, botanik kökenlere ve edafoklimatik koşullara göre büyük ölçüde değişir. Bu çalışmada, Cezayir'in kuzeydoğusunda bulunan farklı arılıklardan toplanan insan tüketimine yönelik sekiz arı poleninin botanik orijini, toplam fenolik içerikleri, toplam flavonoid içerikleri, toplam flavonol içerikleri ve *in vivo* antiinflamatuar aktiviteleri belirlendi ve karşılaştırıldı. Tüm örnekler, 22 botanik familyaya ait kırk polen türünün tanımlanmasıyla heterofloral olarak saptandı. Toplam fenolik içerik 752,94 ± 17,78 ile 12247,06 ± 40,04 mg GAE/ 100 arasında değişirken, toplam flavonoid içeriği 2680,55 ± 12,02 ila 8506,94 ± 15,56 mg QE/ 100g arasında değişmekte ve toplam flavonol içeriği 4978,87 ± 33,39 ile arasında değişmektedir. 7903,75 ± 24,39 mg QE/ 100g. Sonuçlar etanolik ekstraktların büyük kısmının iyi bir anti-inflamatuar aktiviteye sahip olduğunu göstermektedir. Sonuç olarak, yukarıda bahsedilen tüm heterofloral arı poleni örnekleri, potansiyel bir anti-inflamatuar aktiviteye sahip zengin bir polifenol kaynağı olabilir.

Anahtar kelimeler: Arı poleni, Palinolojik analiz, Fenolik içerik, Antiinflamatuar aktivite

GENİŞLETİLMİŞ TÜRKÇE ÖZET

Çalışmanın amacı: Arı poleni nektarın arı tükürük maddeleriyle aglutine edilmiş multipleks karışımıdır. Proteinler, karbonhidratlar, lipidler, vitaminler ve fenolik bileşikler gibi etkileyici fizyolojik olarak dinamik bileşenlere sahiptir. Arı poleni, bağışıklık uyarıcı, antimikrobiyal, antienflamatuar, antioksidan ve antinosiseptif özelliklere sahip olduğundan, geleneksel Çin tıbbında genellikle apiterapötik bir çare olarak tavsiye edilir. Bununla birlikte, bileşimi, botanik kökenlere ve edafoklimatik koşullara göre inanılmaz derecede dalgalıdır. Buna göre bu çalışmada sekiz Cezayir arı poleninin in vivo botanik orijini, toplam fenolik içerikleri, toplam flavonoid içerikleri, toplam flavonol içerikleri ve antiinflamatuar aktiviteleri belirlenmiş ve karşılaştırılmıştır.

Gereç ve Yöntem: Cezayir'in kuzeydoğu bölgesindeki farklı arı kovanlarından insan tüketimine yönelik sekiz arı poleni örneği toplandı. Daha sonra, Louveaux ve diğerleri tarafından belirtildiği gibi slaytlarda (asetoliz olmadan hazırlanmış) 500'den fazla polen tanesinin değerlendirilmesiyle palinolojik tanımlama doğrulandı (1978) ve polen frekansları sınıflandırıldı (de França Alves ve de Assis Ribeiro DosSantos 2014). Toplam fenolik, flavonoid ve flavonol içerikleri üç yöntem kullanılarak belirlendi: sırasıyla Folin–Ciocalteu kolorimetrik yöntem (Singleton ve Rossi 1965) ve trikloroalüminyum kolorimetrik yöntemler (Topçu ve diğerleri 2007) ve (Kumaran ve Joel Karunakaran 2007). Olası antienflamatuar aktivite, sıçanlarda formalin testi kullanılarak in vivo olarak değerlendirildi (Arzi ve ark. 2015).

Bulgular: Mikroskobik analiz, tüm örneklerin heterofloral olduğunu ortaya koydu. 22 botanik familyaya ait toplam kırk polen tipi tespit edilmiş olup, örneklerin tamamında Cistus tipi mevcuttur. Toplam fenolik içerikler 752.94 ± 17.78 ve 12247.06 ± 40.04 mg GAE/ 100g arasında değişirken, toplam flavonoid içerikleri 2680.55 ± 12.02 ila 8506.94 ± 15.56 mg QE/ 100g arasında değişmektedir. Ayrıca, toplam flavonol içerikleri 4978.87 ± 33.39 ve 7903.75 ± 24.39 mg QE/ 100g aralığındaydı. Son olarak, numunelerimizin etanolik ekstraktlarının büyük kısmının iyi bir anti-inflamatuar aktiviteye sahip olduğu bulundu. Ek olarak, en iyi anti-enflamatuar aktivite, tüm değerlendirilen sürelerde, Diklofenak ile gözlemlenenden önemli ölçüde daha düşük şişme yüzdeleriyle ekstrak E'de gözlendi.

Sonuç: Bu çalışmada incelenen heterofloral arı poleni örnekleri, önemli bir anti-inflamatuar aktivite sergileyen zengin bir polifenol kaynağı olabilir. Bu nedenle, ilaç ve gıda endüstrilerinde kullanımı kesinlikle umut vericidir.

INTRODUCTION

In Algeria, beekeeping is a genealogical hone although its origin is lost in the mists of time. It has gained prominence over the last two decades and has become an indispensably portion for the sustainable economical advancement of agricultural and provincial exercises. The Algerian’s apiarian livestock has jumped from 360000 colonies in 2000 to over 1300000 colonies in 2014, thanks to the various agricultural and rural development plans

connected within the nation (Tamali and Özkırım 2019).

Bee pollen is one of the hive-derived products (together with honey, propolis, beeswax, royal jelly, beebread and bee venom) that is expecting more noteworthy popularity among beekeepers as a result of the growing request on it from agri-food, pharmaceutical and cosmetics industries around the world. Bee pollen is advertised as a nutrient-dense food with a wide range of medicinal and nutritional benefits, which is driving up demand (Nogueira et al., 2012). Commonly designated as “the life-giving dust”, it is floral pollen gathered by foraging bees, mixed with nectar then agglutinated by bee salivary substances. This bee product is harvested in a form of pellets using a trap installed at the entrance of the beehives (Thakur and Nanda 2020).

Bee pollen is usually recommended as an apitherapeutic remedy in traditional Chinese medicine since it has immune-stimulating, antimicrobial, anti-inflammatory, antifungal, antioxidant, antinociceptive and antiviral properties (Komosinska-Vassev et al. 2015, Tutun et al. 2021).

It has the potential to provide vital nutrients like proteins, carbs, lipids and minerals. Furthermore, it contains some antioxidant vitamins, mainly vitamin C, E, all B-complex vitamins and provitamin A. Besides vitamins, it is considered as a rich source of polyphenols, mainly flavonoids (Khalifa et al. 2021). The chemical composition of this bee product fluctuates incredibly agreeing to plant origin and its nutrient status, geographical region, edaphoclimatic conditions, storage and degree of processing (Aylanc et al. 2021, Sattler et al. 2015)

In this context, the current work is devoted to determine the botanical origins, assess the phenolic components and evaluate the anti-inflammatory activities *in vivo* of eight bee pollens intended for human consumption with various botanical and geographical origins. As far as we are mindful, this could be the first ponder conducted on Algerian bee pollen aimed to compare and to look up for a possible relationship between the anti-inflammatory potential, the botanical origin, and the total phenolic, flavonoid and flavonol contents. In this regard, this study has the characteristic of being a preliminary study.

MATERIALS AND METHODS

Bee pollen samples

Eight bee pollen samples (A-H) intended for human consumption were collected by beekeepers, during 2019 flowering season, from different apiaries in Algeria’s northeastern region: sample A (Constantine), sample B (Guelma), sample C (Boumerdès), sample D (Sétif), sample E (Jijel), sample F (Skikda), sample G (Khenchla) and sample H (Tizi Ouzou) (Figure 1). After the beekeepers had dried the bee pollen, the samples were delivered and kept in the dark at ambient temperature until their used.

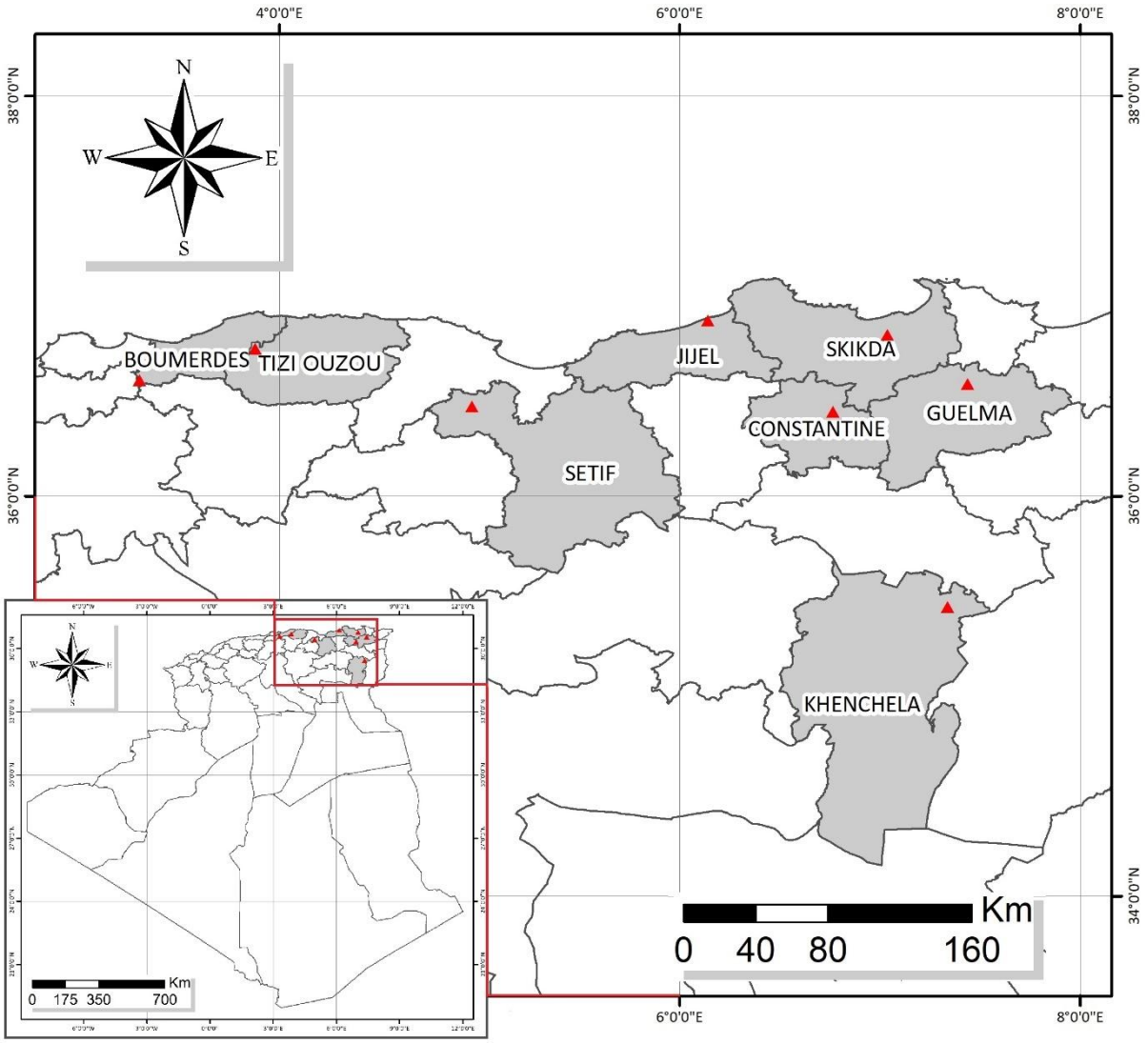
Palynological analysis

A ten-gram sample of each specimen was mixed with 100 milliliters of warm water. Two hours later, the pollen was totally macerated with a glass stick. The supernatant was drawn off after centrifugation (10 min, 2500 r/min). Water: glycerin solution (50 mL), at ratio 1:1 (v/v), was added to the pollen residue. After 2 hours and 24 hours, another centrifugation was done following the same conditions. Finally, the slides were prepared using the glycerol jelly method (Riding 2021) with the final pollen residue. During the slides mounting process, basic fuchsine was used as dye to stain pollen grains. Palynological identification of bee pollen samples was ascertained by examining over 500 pollen grains in slides prepared without acetolysis as outlined by Louveaux et al. (1978) with some modifications.

The pollen grains observation was carried out using an optical microscope, BIOSTAR B4 SP Microscope (Exacta Optech, München, Germany), at 400X and a picture analysis system EOS 450 D (CANON Inc., Japan). The authors' reference collection was used to identify pollen types, as well as specialized atlases and literature when necessary.

When the pollen grains were identified as a belonging to a particular genus and a specific species, their scientific names; consisting of the genus and the species, were applied. However, when the pollen grains had a similar morphology for some species and genus or even botanical families, the nomenclature pollen type was used. Therefore, a pollen type can be defined as a group of plants with similar pollen grains under a light microscope.

Figure 1: Apiaries geopositioning in the study area (Source: Arc Gis 10.7).



The frequency of occurrence (FO) was assigned to the types in each sample based on the percentage of each pollen type's occurrence in the sample set: De França Alves and de Assis Ribeiro DosSantos (2014) defined Rare (less than 10 percent), Less Frequent (10–20 percent), Frequent (21–50 percent), and Very Frequent (more than 50 percent).

Extracts preparation

The extracts were prepared by macerating 300 g of the powdered bee pollen samples with Ethanol (96%) at a ratio of 1:2 (w/v) for 5 days at room temperature, with solvent renovation in the third day and every 24 hours thereafter. The ethanolic extracts of bee pollen were obtained by mixing, filtering, and then concentrating the products of all extractions for each sample in a rotavap at 40 °C.

Phenolic components

Total phenolic content (TPC)

The total phenolics contents of the eight (8) extracts were assessed spectrophotometrically using the Folin–Ciocalteu method (Singleton and Rossi 1965). In every well of the microtiter plate, 100 µL of 1/10 (v/v) diluted Folin–Ciocalteu reagent and 7.5µL of sodium carbonate (7.5%) were added to 20 µL extract. After incubating the microtiter plate for 2

hours in the dark, the absorbances of each mixture were measured at 765 nm using a 96-well microtiter plate reader (Perkin Elmer, Enspire). The results were expressed as milligram gallic acid equivalents per 100 gram of bee pollen extract (mg GAE/100 g).

Total flavonoid content (TFC)

The total flavonoids contents were determined by the trichloroaluminum colorimetric method (Topçu et al. 2007). In every well, 130 µL of methanol, 10 µL of aluminum nitrate (10%) and 10 µL of aqueous potassium acetate (1 M) were added to 20 µL extract. After a 40-minutes incubation period, the absorbances were read at 415 nm using the microtiter platereader. Results were expressed as milligram quercetin equivalent per 100 gram of bee pollen extract (mg QE/100 g).

Total flavonols content (TFIC)

The total flavonols contents were carried out using the trichloroaluminum colorimetric method with slight modifications (Kumaran and Joel Karunakaran 2007). In every well, 50 µL of each extract were placed then 50 µL of aluminum nitrate (2%) and 150 µL of aqueous sodium acetate (5%) were added. After a 2-hours incubation period, the absorbances were recorded at 440 nm using the microtiter platereader. Results were expressed as milligram equivalent per 100 gram of bee pollen extract (mg QE/100 g).

Animals

Fifty male Wistar rats weighing 250–300 g, aged 3–4 months, were used for *in vivo* anti- inflammatory activity. The animals were produced and raised at the laboratory animal facility of the "Department of Animal Biology, Faculty of Natural and Life Sciences, Frères Mentouri Constantine 1 University, Algeria". The rats were kept in groups of five per cage, for acclimatization for seven days before the start of the experiment, under standard laboratory condition (temperature 22 ± 2 °C, photoperiodic cycle (light/darkness) of 12h and relative humidity 50 ± 5%) and unrestricted access to food and water *ad libitum*. This protocol was used in accordance with the Laboratory Animals Care and Use guidelines and approved under the PRFU project (D01N01UN250120210003) by the Ethical Committee of the DGRSDT at the Algerian Ministry of Higher Education and Scientific Research.

***In vivo* Anti-Inflammatory Activity**

Formalin-induced paw edema test

The formalin-induced inflammation test was carried out based on the method of Arzi et al. (2015) with slight modifications. The Wistar rats were randomly allocated into ten homogeneous groups (n=5). After a 12-hours fast period, the rats of each group received the tested samples intraperitoneally. Group 1 received normal saline solution (5 mL/kg) and treated as negative control. Group 2 was administered by an anti-inflammatory drug (Diclofenac) in a dose of 20 mg/kg of Body Weight (BW) and considered as standard. Group 3 to 10 were given different bee pollen extracts in a dose of 200 mg/kg of BW. Thirty minutes later, acute paw edema was induced on the right hind paw by a subplantar injection of 1% formalin (100 µL). Paw volume was measured before and after formalin injection at 0, 30, 60, 120, 180 and 240 minutes, using a water displacement plethysmometer (Fereidoni et al. 2000). The results (% swelling) were expressed as the proportional rise in paw volume before formalin injection. The following formula was used to calculate it:

$$\%\, swelling = \left[\frac{V_t - V_i}{V_i}\right] \times 100$$

Where V_i is the paw volume before formalin injection and V_t is the paw volume after formalin injection at different time points.

Statistical analysis

The *in vivo* experiment was carried out in quintuplicate and the data were recorded as means ± standard deviation. The one-Factor ANOVA test was used to determine whether there were significant differences between pollen samples, with the Tukey test serving as post-hoc test. Moreover, the principal components analysis (PCA) was used to check the relationships between pollen types, phenolic components and anti-inflammatory activity. All statistical analyses were performed using IBM SPSS Statistics 23.0 software (IBM® SPSS Inc.). For all analyses, the level of significance was set at 5% ($p < 0.05$).

RESULTS

Pollen analysis revealed that all samples were heterofloral. A total of 40 pollen types belonging to 22 botanical families were identified in eight pollen

samples (Table 1). The most diverse families were *Asteraceae* (8 types), *Brassicaceae* (4 types), *Cistaceae*, *Fabaceae*, and *Fagaceae* (3 types) respectively. The other families were represented by one or two types only.

Cistus type was the most represented, found in all samples. *Bellis*, *Boraginaceae*, *Bryonia*, *Buxus*, *Centaurea*, *Cerinthe major,* *Convolvulus*, *Cucurbitaceae*, *Euphorbiaceae*, *Fraxinus*, *Galactites*, *Helianthemum*, *Juglans*, *Myrtus communis*, *Oxalis*, *Poaceae*, *Quercus suber*, *Raphanus*, *Rhamnus*, *Sonchus*, *Verbenaceae*, *Vicia* and *Vitex* pollen types were in turn observed in only a single sample.

For sample A, eight different pollen types were identified, of which *Brassicaceae* and *Cistus* types were the two most frequent pollen types. Twelve pollen types were recorded in sample B without any frequent pollen types. The *Pistacia lentiscus* was the most frequent pollen type among 10 different pollen types recorded in sample C. In sample D, the two most frequent pollen types were *Cistus* and *Quercus*, out of a total of 9 pollen types. Thirteen different pollen types were found in sample E where *Quercus* type was the most frequent type. For sample F, *Brassica* was the most repeated type out of a total of 14 pollen types. The recurrent pollen types in sample G were *Brassica* and *Pistacia lentiscus* types from 7 pollen types. Finally, ten different pollen types were found in sample H, among which three pollen types were frequent: *Bryonia*, *Cistus* and *Quercus ilex*.

Table 1: Frequency of occurrence data recorded in the eight bee pollen samples

Family	Pollen Type	Samples (%)							
		A	B	C	D	E	F	G	H
Anacardiaceae	***Pistacia lentiscus*** type	-	R. 9.12%	F. 45.70%	-	-	L.F. 14.97%	F. 43.04%	L.F. 11.46%
Asteraceae	***Anthemis*** type	-	-	R. 0.17%	-	-	R. 0.66%	R. 0.72%	-
	Bellis type	-	-	-	-	-	-	-	R. 0.83%
	Carduus type	-	-	R. 0.33%	-	R. 0.18%	R. 0.49%	-	-
	Centaurea type	-	-	-	-	L.F. 19.85%	-	-	-
	Cichorium type	-	-	-	-	-	R. 0.33%	R. 0.36%	R. 0.66%
	Galactites type	L.F. 10.07%	-	-	-	-	-	-	-
	Picris type	-	R. 5.34%	-	R. 0.64%	R. 0.72%	-	-	-
	Sonchus type	R. 0.72%	-	-	-	-	-	-	-
Boraginaceae	***Boraginaceae*** type	-	-	-	-	-	-	-	R. 2.49%
	Cerinthe major type	-	-	L.F. 19.25%	-	-	-	-	-
Brassicaceae	***Brassica*** type	-	-	R. 4.51%	R. 5.14%	-	F. 30.60%	F. 41.96%	L.F. 13.95%
	Brassicaceae type	F. 39.76%	L.F. 10.53%	-	L.F. 12.05%	-	R. 3.95%	-	-
	Other Brassicaceae type	-	-	-	-	R. 3.43%	-	L.F. 12.11%	R. 3.99%
	Raphanus type	-	-	-	-	-	R. 3.29%	-	-
Buxaceae	***Buxus*** type	-	R. 7.07%	-	-	-	-	-	-
Cistaceae	***Cistus*** type	F. 21.40%	L.F. 16.20%	R. 1.64%	F. 24.30%	R. 3.97%	R. 9.55%	R. 0.36%	F. 20.10%
	Halimium type	-	R. 9.90%	-	-	R. 3.43%	R. 2.96%	-	-

	Helianthemum type	-	R. 5.97%	-	-	-	-	-	-
Convolvulaceae	***Convolvulus*** type	-	-	-	-	-	-	-	R. 5.81%
Cucurbitaceae	***Bryonia*** type	-	-	-	-	-	-	-	F. 20.61%
	Cucurbitaceae type	-	-	-	-	R. 3.79%	-	-	-
Ericaceae	***Erica arborea*** type	-	-	R. 6.03%	-	-	R. 4.28%	-	-
Euphorbiaceae	***Euphorbiaceae*** type	-	-	-	-	L.F. 19.68	-	-	-
Fabaceae	***Fabaceae*** type	-	L.F. 18.88%	R. 8.49%	L.F. 13.18%	-	-	R. 1.45%	-
	Lotus type	-	R. 5.19%	-	-	-	-	-	-
	Vicia type	-	-	-	-	R. 7.04%	-	-	-
Fagaceae	***Quercus ilex*** type	R. 1.08%	-	-	R. 8.84%	-	R. 5.26%	-	F. 20.10%
	Quercus suber type	-	R. 2.99%	-	-	-	-	-	-
	Quercus type	L.F. 10.07%	R. 4.25%	-	F. 20.74%	F. 22.92%	L.F. 16.43%	-	-
Juglandaceae	***Juglans*** type	-	-	-	R. 7.88%	-	-	-	-
Lamiaceae	***Vitex*** type	R. 5.57%	-	-	-	-	-	-	-
Myrtaceae	***Myrtus communis*** type	-	-	R. 2.78%	-	-	-	-	-
Oleaceae	***Fraxinus*** type	-	-	-	-	R. 2.17%	-	-	-
Oxalidaceae	***Oxalis*** type	-	-	-	R. 7.23%	-	-	-	-
Papaveraceae	***Papaver*** type	L.F. 11.33%	-	-	-	R. 2.53%	-	-	-
Poaceae	***Poaceae*** type	-	-	-	-	-	R. 3.78%	-	-
Rhamnaceae	***Rhamnus*** type	-	R. 4.56%	-	-	-	-	-	-
Rosaceae	***Rosaceae*** type	-	-	L.F. 11.10%	-	-	R. 3.45%	-	-
Verbenaceae	***Verbenaceae*** type	-	-	-	-	L.F. 10.29%	-	-	-
Number of pollen types		8	12	10	9	13	14	7	10

* F.: Frequent, L.F.: Less Frequent, R.: Rare

The results of phenolic components of the eight ethanolic extracts indicated significant differences in their total contents. Whereby, high total phenolics contents were recorded in the extract D followed by the extract G then the extracts C and E (12247.06 ± 40.04, 9050.98 ± 17.93, 7854.90 ± 33.05, and 7541.27 ± 48.71 mg GAE/100 g; respectively) with insignificant difference in the two last ones (Table 2), while the lowest content was obtained in the extract A (752.94 ± 17.78 mg GAE/100 g). For flavonoids, the extract D provides the highest content (8506.94 ± 15.56 mg QE/100 g), followed by the extract G (6076.39 ± 20.01 mg QE/100 g), whereas low values were registered in extracts A and B (2694.44 ± 22.85 and 2680.55 ± 12.02 mg QE/100 g; consecutively). Flavonols contents values ranged from 4978.87 ± 33.39 mg QE/100 g (extract B) to 7903.75 ± 24.39 mg QE/100 g (extract D).

In the anti-inflammatory activity, after formalin injection into the hind paw, the paw edema in the normal saline group; increased along with the time course and the peak edema, was registered after 240 min in which the mean of swelling percentage was 63.99 ± 2.98 % (Table 3). Our results showed that the extract E had the best anti-inflammatory

activity with the least significant swelling percentage mean in all assessed time. Followed by extract F which had also strongly and significantly activity, compared to the control group, inhibited the formalin induced paw edema, with comparable effect to the reference drug (diclofenac at 20 mg/kg BW). Furthermore, extracts A, D, G, and H exhibited mild anti-inflammatory effect, with significant statistical difference when compared to the normal saline group. However, extracts B and C did not affect the paw edema with non-significant difference compared to the control group.

Table 2: Phenolic components contents in the pollen types ethanolic extracts

Extracts	TPC (mg GAE/100 g)	TFC (mg QE /100 g)	TFIC (mg QE /100 g)
A	752.94 ± 17.78 ^{e}	2694.44 ± 22.85 ^{e}	5058.68 ± 12.97 ^{d}
B	5913.72 ± 19.13 ^{d}	2680.55 ± 12.02 ^{e}	4978.87 ± 33.39 ^{d}
C	7854.90 ± 33.05 ^{c}	5201.39 ± 37.87 ^{c}	6950.70 ± 31.72 ^{a,b}
D	12247.06 ± 40.04 ^{a}	8506.94 ± 15.56 ^{a}	7903.75 ± 24.39 ^{a}
E	7541.27 ± 48.71 ^{c}	4847.22 ± 36.10 ^{c,d}	7406.10 ± 17.13 ^{a}
F	6482.35 ± 15.5 ^{d}	4965.28 ± 27.43 ^{c}	5744.13 ± 50.46 ^{c,d}
G	9050.98 ± 17.93 ^{b}	6076.39 ± 20.01 ^{b}	6208.92 ± 43.97 ^{b,c}
H	6178.43 ± 34.45 ^{d}	4347.22 ± 27.43 ^{d}	7485.91 ± 54.82 ^{a}

*The outcomes were presented as Means ± SD of three measurements. Analysis of variance (One-Factor ANOVA and Tukey tests) revealed statistical difference ($P < 0.05$). Different superscripts (a, b, c…) for the values in the same columns are statistically different.

The obtained data on the main pollen types, the total bioactive components and the swelling percentages were ordinated with PCA analysis. In total, 48 standardized variables were introduced to create covariance matrix. Whereby, Varimex method was used as a factor analysis rotation technique and the number of extracted factors was fixed at two principal components (PCs). The PCA indicated that the two PCs accounted for 71.16% of the total variance. The first principal component (PC1) represented 40.42% and had the highest positive correlation coefficients with the swilling percentages (t+30: 0.975; t+120: 0.972; t+60: 0.964; t+180: 0.960 and t+240: 0.947). The PC2 (30.74% of the variance) had the main correlation coefficients with the total bioactive components (TPC: 0.985; TFC: 0.908 and TFIC: 0.689). The variables with high correlation coefficients appeared together in the biplot (Figure 2). Focusing on the PC1, the PCA demonstrated four bee pollen types; *Centaurea*, *Cucurbitaceae*, *Euphorbiaceae* and *Fraxinus*, with negative correlation coefficient (-0.796) with the swilling percentages which means that these pollen types were characterized by potent anti-inflammatory activity. Regarding PC2, it can be concluded that *Oxalis* and *Juglans* types are positively correlated (0.575) with the total bioactive components (TPC, TFC, and TFIC) and that their presence matches up with high total bioactive components. Whereas, the three pollen types; *Galactites*, *Sonchus* and *Vitex* had a negative relationship (-0.777) with the total bioactive components.

Table 3: Effects of bee pollen and diclofenac on formalin-induced paw edema in rats.

Extracts and standards	T+30 (%)	T+60 (%)	T+120 (%)	T+180 (%)	T+240 (%)
A	19.80 ± 1.29 ^{d,e}	26.42 ± 2.89 ^{b,c}	32.49 ± 2.57 ^{c}	32.86 ± 2.60 ^{c,d}	42.38 ± 2.06 ^{c}
B	30.84 ± 2.31 ^{a}	40.01 ± 1.74 ^{a}	44.44 ± 2.55 ^{b}	55.59 ± 2.96 ^{a}	61.08 ± 1.90 ^{a}
C	29.75 ± 2.25 ^{a,b}	42.40 ± 2.51 ^{a}	53.49 ± 2.76 ^{a}	55.68 ± 3.75 ^{a}	63.71 ± 3.28 ^{a}
D	15.12 ± 1.78 ^{e,f}	23.87 ± 1.93 ^{b,c}	35.14 ± 1.51 ^{c}	38.10 ± 1.67 ^{b}	49.53 ± 1.03 ^{b}
E	3.89 ± 0.66 ^{h}	3.67 ± 0.66 ^{e}	4.22 ± 1.27 ^{f}	7.71 ± 1.46 ^{h}	10.17 ± 2.31 ^{h}
F	12.68 ± 2.74 ^{f,g}	21.97 ± 1.77 ^{c}	25.54 ± 2.47 ^{d}	28.04 ± 1.89 ^{d}	31.95 ± 2.14 ^{d}
G	18.73 ± 2.09 ^{e,}	26.75 ± 1.52 ^{b}	30.18 ± 2.07 ^{c,d}	37.70 ± 2.55 ^{b,c}	43.71 ± 1.90 ^{c}
H	23.04 ± 2.90 ^{c,d}	24.29 ± 2.48 ^{b,c}	34.97 ± 2.92 ^{c}	39.02 ± 2.21 ^{b}	49.73 ± 2.60 ^{b}
Control	26.61 ± 2.90 ^{b,c}	37.99 ± 2.37 ^{a}	57.81 ± 2.57 ^{a}	59.74 ± 1.17 ^{a}	63.99 ± 2.98 ^{a}
Diclofenac	8.74 ± 1.17 ^{g}	12.54 ± 2.98 ^{d}	11.53 ± 2.63 ^{e}	16.50 ± 2.73 ^{e}	22.49 ± 1.94 ^{e}

* The swelling percentages values mean ± SD (n = 5) were checked by One-Factor ANOVA test followed by multiple comparison test Turkey ($p < 0.05$). Outcomes with distinct superscript letters are statistically different.

DISCUSSION

The botanical identification of our samples confirmed the richness of the northeastern region of Algeria in important species resulting from the particular climate (Mediterranean climate for both tell and steppe regions), orography and human impact. Most of the found pollen types were from spontaneous species known by their high melliferous potential (Ghorabet al. 2021b, Saadia TamaliandÖzkırım 2019, Zerrouk et al. 2014). Therefrom, this floral diversity represents a great feature that favours the sustainable development of beekeeping activities in Algeria.

Even if *Asteraceae* family was the most diverse (8 types), it was not the most frequent plant family (represents just 5.25% of identified pollen types). The most abundant families in our samples were *Brassicaceae* (its 4 pollen types constituted 23.15% of identified pollen types), followed by *Anacardiaceae* (represented by its unique type *Pistacia lentiscus* with 15.53% of identified pollen types) then *Cistaceae* (14.99%) and *Fagaceae* (14.10%). Excluding *Brassicaceae* family, all the cited families are well known as good polliniferous species. However, most species of *Brassicaceae* and *Asteraceae* families were considered as nectariferous. *Fabaceae* family members (6.68% of identified pollen types) were known as good nectar and pollen producers (Ghorabet al. 2021a).

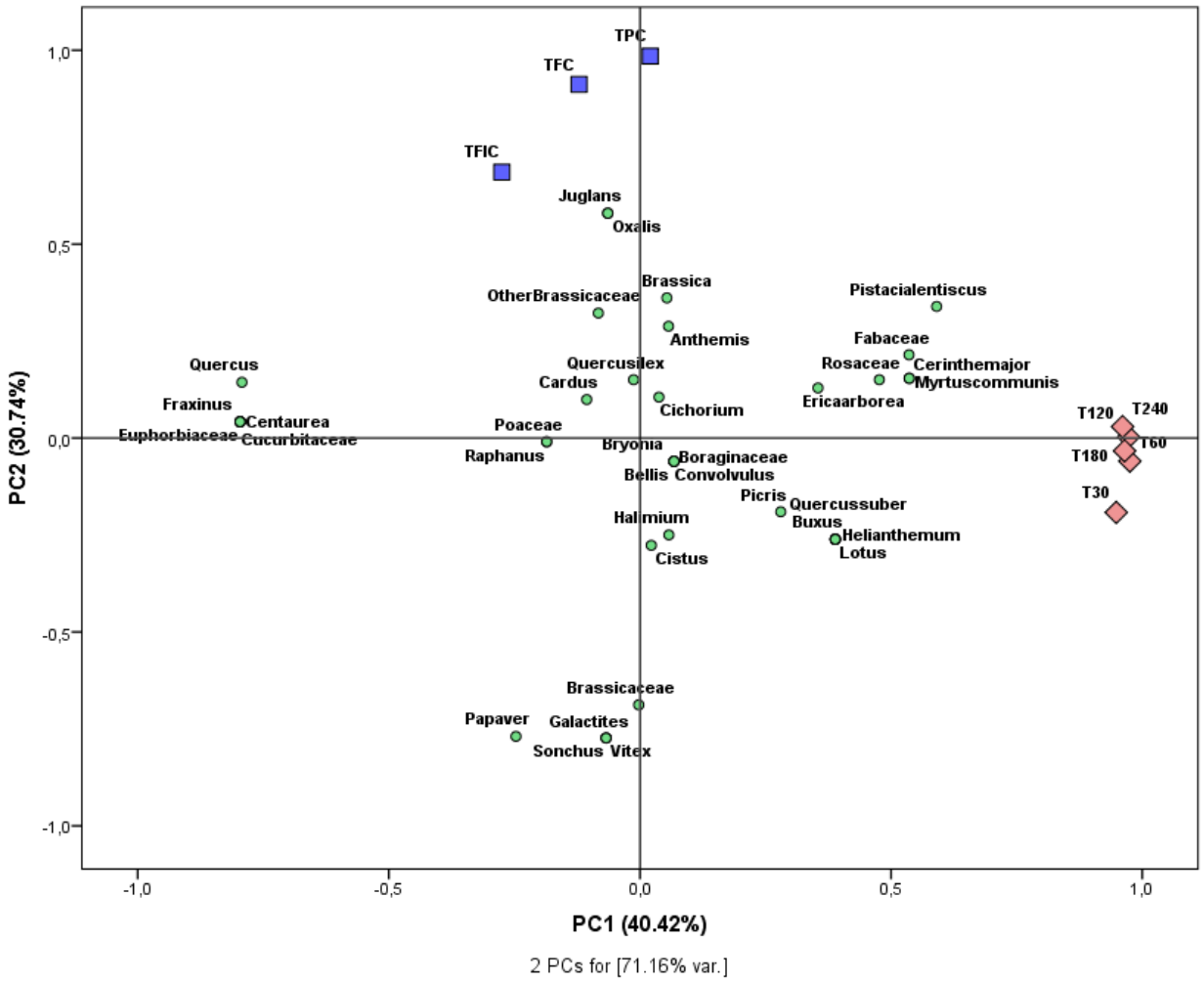


Figure 2: Loading biplot of variables (Pollen types, phenolic components and swilling percentages) included in the Principal Component Analysis (PCA)

Numerous studies in many countries have been conducted on bee pollen total bioactive components. Concerning our bee pollens, the TPCs were ranging from 752.94 to 12247.06 mg GAE/100 g, TFC ranging from 2680.55 to 8506.94 mg QE/100 g and TFIC ranging from 4978.87 to 7903.75 mg QE/100 g. Although different extraction and assay methods were used, these results were tad higher than those reported by (Asmae et al. 2021, Eraslan et al. 2009, Fatrcová-Šramková et al. 2013, LeBlanc et al. 2009, Yildiz et al. 2013). Our results can also be comparable with those obtained by (ŞahinandKarkar 2019, Žilić et al. 2014). Nevertheless, the present findings were moderately lower than those found on Turkish bee pollen (Gercek et al. 2021).

This variation is common and may be ascribed to variations in geographical origin and edaphoclimatic condition (Araújo et al. 2017, Nogueira et al. 2012). However, the most influencing factor remains botanical origin (Bogdanov 2004, Daoud et al. 2019, Estevinho et al. 2012).

The anti-inflammatory effect of our ethanolic bee pollen extracts was investigated by a method of formalin-induced paw edema in rats. This method is commonly used as a model for anti-nociceptive and anti-inflammatory activities assessment which mainly results from a neurogenic inflammation mediated by neuropeptides such as substance P (Damas et al. 1999).

The diclofenac was chosen as an anti-inflammatory drug reference. It is a proven nonsteroidal anti-inflammatory drug (NSAID) with antipyretic, anti-inflammatory and analgesic properties. Diclofenac exerts its action via cyclooxygenases (COX-1 and 2) inhibition with relative equipotency. However, extensive research shows that the mechanisms of action of diclofenac goes beyond COX inhibition and confirms that it can inhibit substance P, inhibit lipoxygenase enzymes, influence the release and uptake of arachidonic acid, activate the nitric oxide–cGMP antinociceptive pathway and alter the interleukin-6 production (Gan 2010).

The bulk of the tested bee pollen ethanolic extracts suppressed paw edema. Homogeneous effect was found in an ethanolic bee pollen extract from *Cistus sp.* of Spanish (Maruyama et al. 2010), hydroethanolic extracts of bee pollen from *M.fasculata* (Lopes et al. 2019) and from *S.aff.postica* (Lopes et al. 2020) at comparable doses showed high anti-inflammatory activity in rat carrageenan-induced paw edema models. These results confirm the hypothesis that bee pollen extract may act by COX-2 inhibition and also probably acts by NO release inhibition and as H1 histamine receptor antagonist (Lopes et al. 2019).

In mouse formalin-induced paw edema model, Choi (2007) noted that the ethanolic extract of pine (*Pinus densiflora*) bee pollen has demonstrated at the same dose (200mg/kg BW) a strong anti-inflammatory activity significantly better than that of the used anti-inflammatory drug reference (indomethacin at 10 mg/kg BW). This finding corroborates with the result observed with extract E.

The results of the PCA showed that there is no correlation between the total phenolic, flavonoid and flavonol contents of the samples with the anti-inflammatory activity (0.057, -0.042 and -0.167 respectively). Therein, the registered variation of the anti-inflammatory activity in our results between the eight bee pollen extracts could be explained by the different botanical origins of our bee pollen which certainly implies a variation in their secondary metabolites composition. The high anti-inflammatory activity registered with extract E compared to remaining extracts could be attributed to its possible richness in flavonoids from the subgroup of flavonols with potent anti-inflammatory effect like quercetin and kaempherol, likewise their glycosides especially rutin (Panche et al. 2016; Rzepecka-Stojko et al. 2015). Therefore, additional research is required to identify the phenolic profile of each extract and thus clarify the possible mechanism.

This finding gives us more information and details about the relation between pollen types and phenolic components and anti-inflammatory activity of a bee pollen ethanolic extract. However, these results remain preliminary, whereby future investigations are needed to affirm them.

Several studies must be launched inside and outside Algeria on monofloral bee pollens to pinpoint the biological characteristics and the chemical composition of each pollen type, for better comprehension of their anti/pro-inflammatory activity mechanisms.

Conclusion

All bee pollen samples, intended for human consumption, collected from the northeast of Algeria, an area known for its flora diversity, are heterofloral. The ethanolic extracts of the studied samples are rich in total phenolic, flavonoid and flavonol contents. Most extracts exhibited a good anti-inflammatory activity. In this fact, Algerian bee pollen can be an important candidate which opens up new possibilities for developing many food supplements and pharmaceutical products. It is therefore advisable to give more attention and support more research on this bee hive product.

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Declaration of Interest:

The authors declare that they have no conflicts of interest, financial or otherwise. The authors alone are responsible for the content and writing of the paper.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

GREEN SYNTHESIS AND BIOCHEMICAL PROPERTIES OF PROPOLIS BASED SILVER NANOPARTICLES

Yeşil Sentez ile Propolis Temelli Gümüş Nanopartikül Sentezlenmesi ve Biyokimyasal Karakterizasyonu

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ABSTRACT

Propolis is a substance collected by honey bees from different parts of plants. Honey bees store it in their hives in order to defend against different threats. Propolis is a bee-product rich in plant waxes, esters, fatty acids, volatile components and phenolic compounds. It`s rich phenolic content makes propolis a potential reducer for Ag^+ to Ag. In this study, propolis-based silver nanoparticles were obtained using the green synthesis technique. It was determined that the obtained silver nanoparticles had a maximum absorbance at 425 nm and their sizes ranged from 67 to 75 nm. When the FT-IR data of propolis extract is examined, the band at 3200 cm^{-1} represents functional -OH groups, the band at 2919-2850 cm^{-1} represents C-H stretching, the band at 1634 cm^{-1} represents C=C, C=O or NH stretching, the band at 1508 cm^{-1} represents aromatic C=C stretching and the band at 1451 cm^{-1} originate from the C-H stretching of CH_3, CH_2, flavonoids and aromatic rings. Total phenolic content of propolis extract and silver nanoparticles was determined as 176.42±0.18 and 122.63±0.23 mg GAE/mL, respectively. IC_{50} value of P-AgNPs for α-amylase and α-glycosidase enzyme inhibition was defined as 47.08 ± 0.002 and 52.18 ± 0.001 µg/mL, respectively. Inhibition of α-Amylase and α-glycosidase is still a valid approach in the treatment of diabetes. The high inhibition effect of the obtained nanoparticles on the related enzymes shows that they have diabetes treatment potential. In addition, showing that cheap and abundant nanoparticles can be obtained by using propolis, this study may contribute to the development of new products containing nanoparticles that can be used in apitherapy applications.

Key words: Green synthesis, Eco-friendly, Diabetes mellitus, Enzyme inhibition

ÖZ

Propolis; bal arılarının kovanlarını farklı tehditlere karşı savunmak amacıyla bitkilerin farklı kısımlarından topladıkları ve kovanlarında depoladıkları bir maddedir. Propolis, bitkisel mumlar, esterler, yağ asitleri, uçucu bileşenler ve fenolik bileşiklerce zengin bir üründür. Zengin fenolik içeriği propolisi potansiyel bir indirgen kılmaktadır. Yapılan bu çalışmada propolis temelli gümüş nanopartiküller yeşil sentez tekniği kullanılarak elde edildi. Elde edilen gümüş nanopartiküllerin 425 nm'de maksimum absorbansa sahip olduğu ve boyutlarının 67 ile 75 nm arasında değiştiği tespit edildi.

Propolis ekstraktı FT-IR verileri incelendiğinde 3200 cm^{-1}'deki bant fonksiyonel -OH gruplarını temsil eder. 2919-2850 cm^{-1}'deki bant CH gerilmesinden, 1634 cm^{-1}'deki bant C=C, C=O veya NH gerilmesinden, 1508 cm^{-1}'deki bant aromatik C=C gerilmesinden ve 1451 cm^{-1} 'deki bant CH_3, CH_2, flavonoidler ve aromatik halkaların CH gerilmesinden kaynaklanır. Propolis ekstraktı ve gümüş nanopartiküllerin toplam fenolik madde miktarları sırasıyla 176,42±0,18 ve 122,63±0,23 mg GAE/mL olarak belirlenmiştir. P-AgNPs'lerin α-amilaz ve α-glukozidaz enzimleri IC_{50} değerleri sırasıyla 47,08 ± 0,002 ve 52,18 ± 0,001 µg/mL olarak tespit edildi. α-Amilaz ve α-glukozidaz inhibisyonu diyabet tedavisinde hala geçerli bir yaklaşımdır. Elde edilen nanopartiküllerin ilgili enzimler üzerine yüksek inhibisyon etkisi diyabet tedavisi potansiyelleri olduğunu göstermektedir. Ayrıca bu çalışmanın, propolis kullanılarak ucuz ve bol miktarda nanopartikül elde edilebildiğini göstermesi, apiterapi uygulamalarında kullanılabilecek nanopartikül içeren yeni ürünlerin geliştirilmesine de katkı sunacağı söylenebilir.

Anahtar Kelimeler: Yeşil sentez, Çevre dostu, Diabetes mellitus, Enzim inhibisyonu

GENİŞLETİLMİŞ ÖZET

Amaç: Nanoteknoloji, tıp, diş hekimliği, ilaç salınım sistemleri, eczacılık gibi birçok biyomedikal uygulamalar ile çevre ve mühendislik alanlarında yaygın olarak kullanılan gelişmiş bir teknoloji dalıdır. Nanopartiküller (1-100 nm boyutunda), nanoteknolojinin temel yapı taşları olarak görülmektedir. Gümüş, altın, çinko gibi metallerin kullanılmasıyla hazırlanan metalik nanopartiküller geniş kullanım alanına sahiptir. Özellikle gümüş nanopartiküller, göstermiş oldukları antimikrobiyal aktiviteleri nedeniyle sağlık uygulamalarında en çok tercih edilen nanopartiküllerden biridir. Nanopartiküllerin sentezinde farklı fiziksel ve kimyasal teknikler kullanılmaktadır. Kullanılan kimyasal teknikler çok miktarda toksik ürün oluşturmaktadır. Bu nedenle çevre dostu üretim tekniklerine ihtiyaç duyulmaktadır. Yeşil sentez çevre dostu ve biyouyumlu nanopartiküllerin elde edilmesini sağlayan hızlı ve düşük maliyetli bir yöntemdir. Genellikle funguslar, bakteriler, algler ve bitkiler yeşil sentezde kullanılan doğal ürünlerdir. Bitkilerin içerdiği fitokimyasalllar da yeşil sentez ile daha kararlı, ekonomik ve geniş uygulama alanına sahip nanopartiküllerin elde edilmesine olanak sağlamaktadır. Bal, polen, propolis ve arı sütü gibi fitokimyasallarca zengin arı ürünleri de yeşil sentez ile nanopartikül elde edilmesine olanak sağlamaktadır.

Gereç ve Yöntem: Çalışmada kullanılan ham propolis örneği 2021 yılında Bilecik ilinden hasat edildi. Dondurulmuş ve öğütülmüş ham propolis örneği %70'lik glikol kullanılarak ekstrakte edildi. Propolis temelli gümüş nanopartikülleri (P-AgNPs) elde etmek amacıyla 5mM $AgNO_3$ ve eşit hacimli propolis ekstraktı karıştırıldı ve reaksiyonun gerçekleşmesi beklendi. Propolis bazlı gümüş nanopartiküller UV-Vis spektrofotometre kullanılarak karakterize edildi. Nanopartiküllerin maksimum absorbans verdiği dalga boyu değeri kaydedildi. Propolis ekstraktının içerdiği fonksiyonel gruplar FT-IR kullanılarak belirlendi. 4500 rpm'de santrifüjlenerek elde edilen nanopartiküller 50°C'de kurutuldu ve partikül boyutları SEM ile belirlendi.

Daha sonra propolis ekstraktı ve P-AgNPs'lerin toplam fenolik madde miktarı tespit edildi. Ayrıca propolis ekstraktı ve P-AgNPs'lerin α-amilaz ve α-glukozidaz enzimleri üzerine inhibisyon etkileri de incelendi.

Bulgular: Yapılan bu çalışmada propolis temelli gümüş nanopartiküller yeşil sentez tekniği kullanılarak sentezlendi. Elde edilen gümüş nanopartiküllerin 425 nm'de maksimum absorbans verdiği ve boyutlarının 67 ile 75nm arasında değiştiği tespit edildi. Propolis ekstraktı FT-IR verileri incelendiğinde 3200 cm^{-1}'deki bant fonksiyonel -OH gruplarını temsil eder. 2919-2850 cm^{-1}'deki bant CH gerilmesinden, 1634 cm^{-1}'deki bant C=C, C=O veya NH gerilmesinden, 1508 cm^{-1}'deki bant aromatik C=C gerilmesinden ve 1451 cm^{-1}'deki bant CH_3, CH_2, flavonoidler ve aromatik halkaların CH gerilmesinden kaynaklanır. Propolis ekstraktı ve gümüş nanopartiküllerin toplam fenolik madde miktarları sırasıyla 176,42±0,18 ve 122,63±0,23 mg GAE/mL olarak belirlendi. P-AgNPs'lerin α-amilaz ve α-glukozidaz enzimleri için IC_{50} değerleri sırasıyla 47,08±0,002 ve 52,18±0,001 µg/mL olarak tespit edildi.

Sonuç: Yapılan bu çalışma ile Bilecik ilinde hasat edilen ham propolis glikol ile ekstrakte edildi ve yeşil sentez tekniği kullanılarak propolis temelli gümüş nanopartiküller elde edildi. Kullanılan teknik

nanopartiküllerin ekonomik, çevre dostu, kolay ve hızlı bir şekilde sentezlenebilmesine olanak sağlamaktadır. Elde edilen P-AgNPs'lerin α-amilaz ve α-glukozidaz enzimleri üzerine inhibisyon etkisi olması, partiküllerin Diabetes mellitus tedavisinde potansiyel bir ürün olabileceğini göstermektedir. Ayrıca elde edilen bulguların, nanopartikül temelli farklı ürünlerin geliştirilmesine imkân sağlayabileceği de ifade edilebilir.

INTRODUCTION

Nanotechnology is an innovative technology that is widely used in medicine, dentistry, drug delivery systems, many biomedical applications, environment and engineering. Nanoparticles (1-100 nm in size) are seen as the basics of the nanotechnology (Beykaya and Çağlar 2016). Metallic nanoparticles prepared by using metal ions such as silver, gold and zinc have a wide range of uses. Especially silver nanoparticles are one of the most preferred nanoparticles in health applications due to their antimicrobial activities (Rai et al. 2009). Different physical and chemical techniques are used in the synthesis of nanoparticles. The chemical techniques used create a large amount of toxic products. Therefore, environmentally friendly production techniques are needed (Ali et al. 2016). Green synthesis is a fast and low-cost method that provides environmentally friendly and biocompatible nanoparticles. Generally, fungi, bacteria, algae and plants are natural products used in green synthesis. The phytochemicals contained in plants enable the production of nanoparticles with green synthesis resulting more stable, economical and have a wide range of applications (Mohammadi et al. 2019). Bee products such as honey, pollen, propolis and royal jelly are rich in such phytochemicals and they also could be used to obtain nanoparticles by green synthesis.

Propolis is a resinous natural bee product that worker bees collect from various parts of plants and use to protect their hives against all kinds of dangers (Keskin and Kolaylı 2018). Although the phytochemical content of propolis varies depending on the flora of the region where it is collected, propolis is quite rich in these phytochemicals. Propolis has been found to contain more than 300 different components up to now and it is very rich in volatile (terpene, terpenoid, etc.) and phenolic components (Özkök et al. 2021). Therefore, propolis is a very good product that can be used to obtain nanoparticles.

In this study, silver nanoparticles were synthesized by green synthesis technique using raw propolis samples harvested in Bilecik province in 2021. The synthesized nanoparticles were characterized using UV-Vis spectrophotometer, FT-IR and SEM methods. In addition, inhibition effects of propolis-based nanoparticles on α-amylase and α-glycosidase enzymes, which are important to be inhibited in the treatment of Diabetes mellitus, were determined.

MATERIALS AND METHOD

Raw propolis sample was harvested from Bilecik province in 2021. Silver nitrate, α-amylase, α-glycosidase, glycol, Folin reagent and p-nitro phenyl-α-D-glucopyranoside were purchased from Sigma Aldrich. All other chemicals used were of analytical grade. FTIR (Perkin Elmer), UV spectrophotometer (GENESYS 150) and scanning electron microscope (SEM, EVO 40 LEQ) were used in the characterization processes.

Extraction of Raw Propolis

Propolis extraction was performed according to the method indicated by Yıldız (2020). The frozen and ground raw propolis sample was extracted by using 70% glycol. For this purpose, 20 g of crude propolis sample was mixed with 100 mL of 70% glycol. The mixture was stirred at room temperature under constant speed for 24 hours. At the end of the period mixture was filtered and the obtained extract was stored at +4°C until use.

Green Synthesis of Propolis-Based Silver Nanoparticles (P-AgNPs)

Propolis based nanoparticles were synthesized using the method described by Keskin (2022) with minor modifications. For this purpose, 500 mL of $AgNO_3$ (5 mM) solution and an equal volume of propolis extract were mixed. The mixture was stirred in the dark under constant speed for 24 hours. A color change from light yellow to dark brown was observed.

Characterization of P-AgNPs

Propolis based silver nanoparticles were characterized using UV-Vis spectrophotometer. The wavelength at which the nanoparticles gave maximum absorbance was recorded. The functional

groups contained in the propolis extract were determined using FT-IR. The nanoparticles obtained by centrifugation at 4500 rpm were dried at 50°C and particle sizes were determined by SEM.

Determination of Total Phenolic Content of Propolis extract and P-AgNPs

The total phenolic content of propolis extract and obtained nanoparticles were determined by the Folin-Ciocalteu method (Singleton and Rossi 1965, Singleton et al. 1999). The blue colored complex formed by the phenolic components with the Folin reagent gives maximum absorbance at 765 nm. The calibration curve was prepared using gallic acid. Solutions of gallic acid at varying concentrations (0.03125-1.0 mg/mL) were prepared and a graph of absorbance versus concentration was drawn. The total phenolic content of propolis extract and P-AgNPs was calculated using this graph and the results were expressed in mg GAE/mL

Inhibition Properties of P-AgNPs on α-Amylase

α-Amylase enzyme activity was determined by DNS method in the presence of soluble starch as substrate. 300 µL of 1% soluble starch and 300 µL of enzyme were incubated at 35ºC for 30 minutes. After adding an equal volume of DNS reagent, the mixture was kept in a boiling water bath. At the end of the reaction, tubes were cooled to room temperature and the absorbance values were recorded at 550 nm (Bernfeld 1955). The IC_{50} values of propolis extract and P-AgNPs were determined in triplicate with concentration between 10 to 200 µg/mL under the above-mentioned analysis conditions. Acarbose was used as a reference inhibitor.

Inhibition Properties of P-AgNPs on **α-Glycosidase**

α-glycosidase enzyme activity was determined by using the method specified in the Gholamhoseinian et al. (2008). *p*-nitro phenyl-α-D-glucopyranoside was used as substrate. 5 µL of substrate, enzyme solution (0.1 U) and 900 µL of phosphate buffer 6.8 (50 mM) were mixed. The mixture was incubated at 37 °C and absorbance values at 405 nm were recorded. The IC_{50} values of propolis extract and P-AgNPs were determined in triplicate with concentration between 10 to 200 µg/mL under the above-mentioned analysis conditions. Acarbose was used as a reference inhibitor.

RESULTS

In this study, propolis-based silver nanoparticles were synthesized using green synthesis technique. It was determined that the obtained silver nanoparticles gave maximum absorbance at 425 nm. The size of the beads varied between 67 and 75nm.

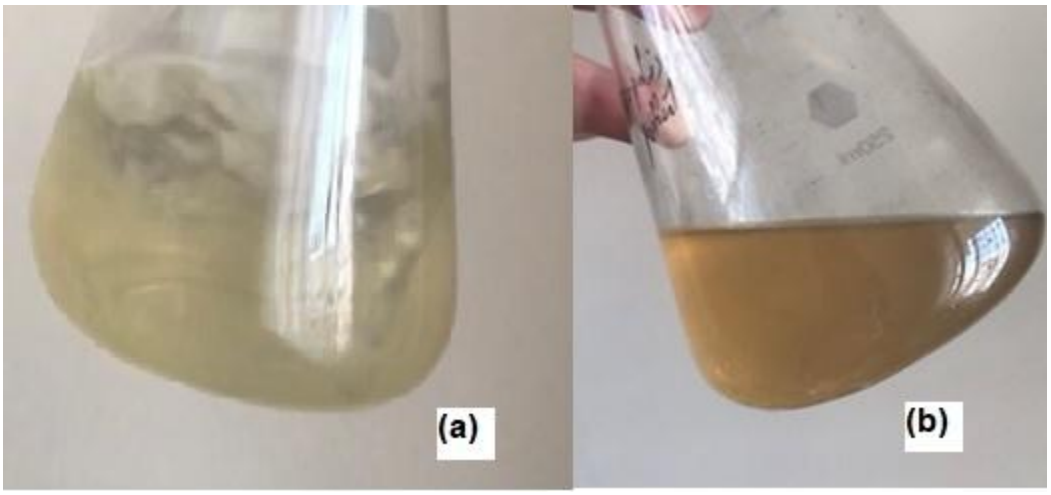

Figure 1. a) Propolis extract b) P-AgNPs synthesis

Şekil 1. a) Propolis ekstraktı b) P-AgNPs sentezi

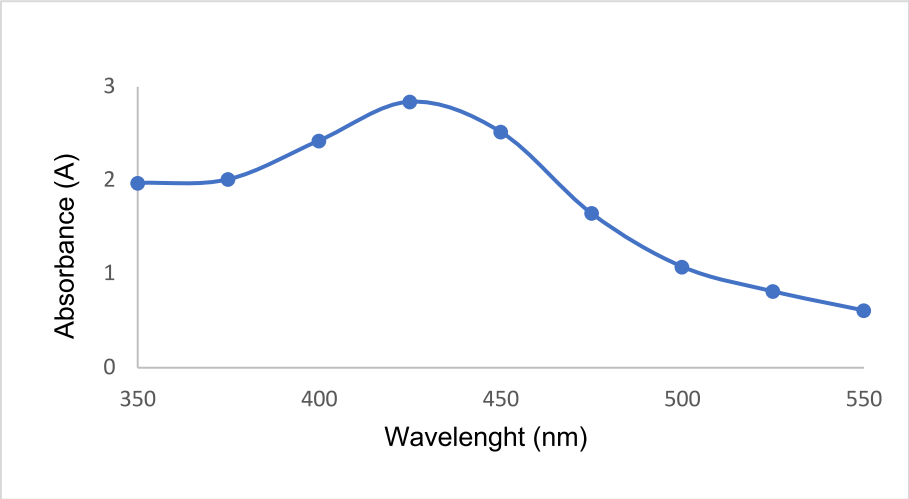

Figure 2. UV-VIS spectrum of P-AgNPs

Şekil 2. P-AgNPs UV-Vis spektrumu

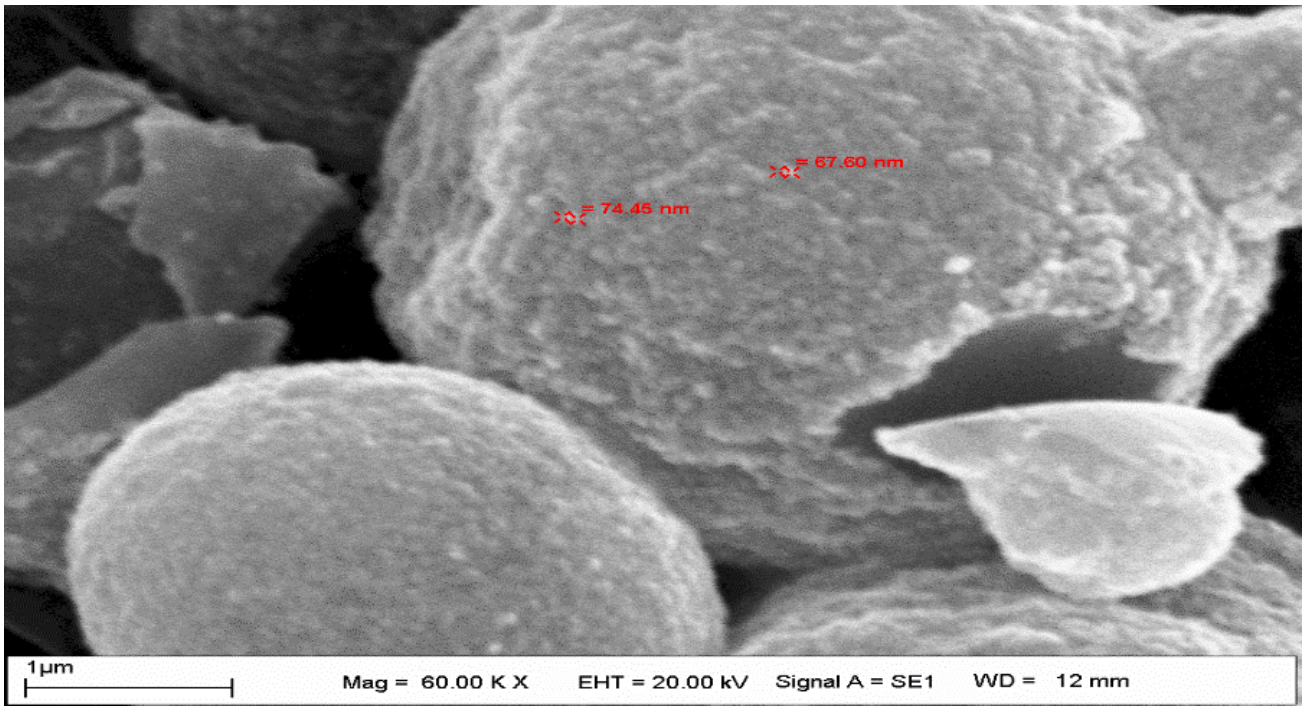

Figure 3. SEM data of P-AgNPs

Şekil 3. P-AgNPs SEM verisi

When the FT-IR data of propolis extract was examined, the bands could be explained with the presence of functional -OH groups at 3200 cm^{-1}, the C-H groups at 2919-2850 cm^{-1}, the C=C, C=O or NH groups at 1634 cm^{-1}, the aromatic C=C bonds at 1508 cm^{-1} and the CH_3, CH_2, flavonoids and aromatic ring C-H groups at 1451 cm^{-1} (Corciova et al. 2019).

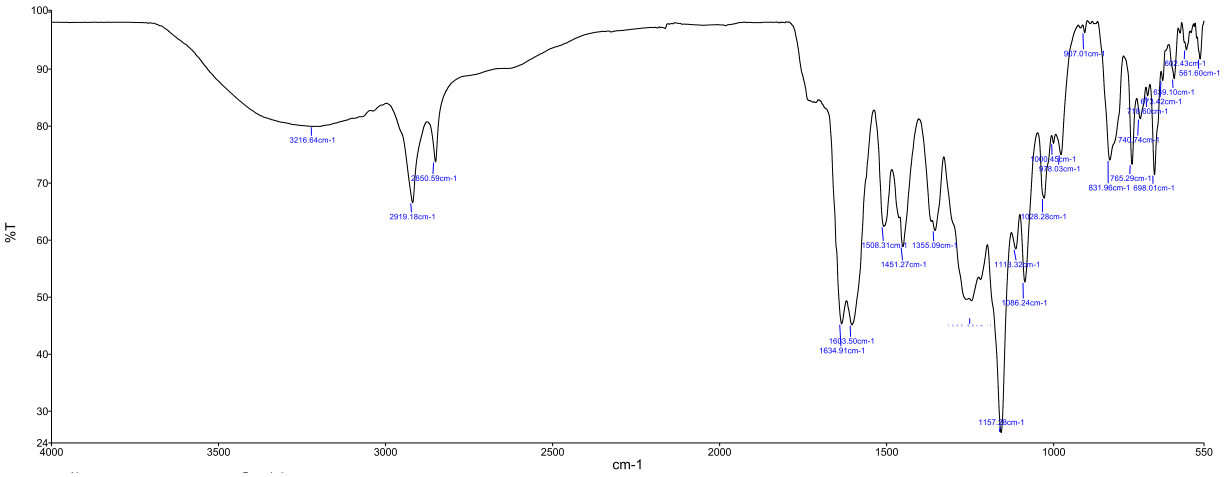

Figure 4. FT-IR data of propolis extract

Şekil 4. Propolis ekstraktı FT-IR verileri

Results for the inhibition effects of P-AgNPs on α-amylase and α-glycosidase enzymes and total phenolic content of propolis extract and P-AgNPs were summarized at Table 1. Lower IC_{50} values obtained for P-AgNPs indicating better inhibition activity than both propolis extract and acarbose, a standard inhibitor.

Table 1. Biochemical properties of propolis extract and P-AgNPs

Tablo 1. Propolis ekstraktı ve P-AgNPs'lerin biyokimyasal karakterizasyonu

	Total Phenolic Content mg GAE/mL	**α-amylase IC_{50}(µg/mL)**	**α-glucosidase IC_{50} (µg/mL)**
Propolis Extract	176.42±0.18	59.78±0.001	67.43±0.001
P-AgNPs	122.63±0.23	47.08±0.002	52.18±0.001
Acarbose		96.7±0.42	96.7±0.42

DISCUSSION

Propolis is a natural substance rich in phenolic compounds especially as flavonoids. Since phenolic compounds and flavonoids are high-capacity natural reducing agents, they play a role in AgNPs green synthesis ($Ag^+ \rightarrow Ag^0$). In this study, it was observed that the inhibition effect of P-AgNPs on α-amylase and α-glycosidase enzymes was achieved with a lower IC_{50} value than propolis extract. Two possible reasons for this situation can be expressed as the easy ability of silver to transfer electrons and the high abundance of biomolecules on the AgNPs surface (Corciova et al. 2019). Al-Fakeh et al. (2021)

synthesized silver nanoparticles with green synthesis from propolis sample harvested from Saudi Arabia. It was stated that the synthesized nanoparticles gave maximum absorbance at 437 nm and had a higher total phenolic substance content than raw propolis. Corciova et al. (2019) stated that the silver nanoparticles they synthesized gave a maximum absorbance of around 480 nm and the sizes of the nanoparticles ranged from 10 nm to 50 nm. A quite low total phenolic content of the supernatant remaining after the silver nanoparticle production was expressed by the authors because of the fact that the components in the propolis extract take part in the reduction of silver. Priyadarshini et al. (2018) obtained propolis-based silver nanoparticles by green synthesis. It was stated that the particle size of the obtained nanoparticles, which gave maximum absorbance at 420 nm, was smaller than 100 nm but highly variable. In a study, Al-Yousef et al. (2020) reported the synthesis of bee pollen-based silver nanoparticles. It was stated that the synthesized nanoparticles gave maximum absorbance at approximately 440 nm and the particle sizes vary between 10-30 nm on average. Debnath et al. (2019) synthesized silver nanoparticles using mushroom extract and investigated the effects of obtained particles on α-amylase. They stated that the obtained nanoparticles gave maximum absorbance at 420 nm and the particle sizes ranged between 2-20 nm. It was also stated that silver nanoparticles have an inhibitory effect on α-amylase enzyme and the percentage of α-amylase inhibition increases with the use of nanoparticles with increasing concentration. Ramkumar et al. (2010) synthesized Gymnema Montanum-based silver nanoparticles and investigated the effects of nanoparticles on α-amylase and α-glycosidase. It was declared by the authors that the obtained nanoparticles inhibited α-amylase and α-glycosidase enzymes with IC_{50} values as 5 µg/mL and 7 µg/mL, respectively. Johnson et al. (2018) obtained Bauhinia variegate-based silver nanoparticles and determined their inhibition properties on α-amylase enzyme. It was stated that the nanoparticles obtained in that study gave maximum absorbance at 430 nm and the particle size was reported to be ranged between 5-15 nm. The IC_{50} value for α-amylase enzyme was declared to be 4.64 µg/mL. Synthesis of silver nanoparticles based on *Enhalus acoroides* and effects of the particles on the α-glycosidase enzyme was reported by Senthilkumar et al. (2016). It was stated that the obtained particles gave maximum absorbance at 419 nm, their sizes varied between 2-100 nm, and the IC_{50} value for the α-glycosidase enzyme was 47 µg/mL. Variable IC_{50} values of AgNPs on α-amylase and α-glycosidase enzymes have been reported in literature. This variation might be the result of used enzyme type. It is clear that our results are compatible with the literature data's.

Conclusion

In this study, crude propolis harvested in Bilecik province was extracted with glycol and propolis-based silver nanoparticles were obtained by using green synthesis technique. The technique used enables nanoparticles to be synthesized economically, environmentally friendly, easily and quickly. The inhibition effect of the obtained P-AgNPs on α-amylase and α-glycosidase enzymes shows that the particles have a potential in the treatment of Diabetes mellitus. It can be concluded that synthesis of silver nanoparticles by using propolis extract is a suitable way for the development of different nanoparticle-based products.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

IMMUNOMODULATORY EFFECT OF INDONESIAN PROPOLIS IN PREGNANT MICE: A PRELIMINARY RESULT

Endonezya Propolis'in Hamile Farelerde İmmünomodülatör Etkisi: Bir Ön Sonuç

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ABSTRACT

Propolis has been widely accepted to exhibit immunomodulatory activity. However, the activity during pregnancy has not been investigated yet. This study was a preliminary study that aimed to analyze the effect of several types of Indonesian propolis on the histological changes of maternal mice spleen. A total of 25 pregnant mice were divided into 5 groups, control (1% Tween 80) group, low (380 mg/kg) and high dose (1400 mg/kg) ethanol extract of South Sulawesi propolis groups, and low (380 mg/kg) and high dose (1400 mg/kg) water extract of Banten propolis groups. Propolis samples were administered daily during pregnancy. At day 18 of gestation, the mice were sacrificed to obtain spleen which was used for histological evaluation using hematoxylin and eosin staining. The number and diameter of white pulp were observed under the 10x magnifying of microscope. The results showed that all propolis extracts at low dose significantly increased the number of white pulp ($p < 0.05$). However, an increase in the diameter was found not significant in all propolis administered groups. This study suggests that Indonesian propolis may modulate maternal immune system.

Keywords: Immunomodulatory, Histology, Pregnancy, Propolis

ÖZ

Propolisin immünomodülatör aktiviteye sahip olduğu yaygın olarak kabul edilmiştir. Bununla birlikte, hamilelik sırasındaki aktivite henüz araştırılmamıştır. Bu çalışma, hamilelik sırasında propolis uygulamasının anne fare dalağının histolojik değişiklikleri üzerindeki etkisini analiz etmeyi amaçladı. Toplam 25 hamile fare, Güney Sulawesi propolisinin kontrol (%1 Tween 80), düşük (380 mg/kg) ve yüksek doz (1400 mg/kg) etanol özütü ve düşük (380 mg/kg) ve yüksek dozlu (1400 mg/kg) Banten propolisinin su özütü olmak üzere 5 gruba ayrıldı. Propolis uygulaması gebelik boyunca günlük olarak yapıldı. Gebeliğin 18. gününde fareler, hematoksilen ve eozin boyaması kullanılarak histolojik

değerlendirme için kullanılan dalak elde etmek için sakrifiye edildi. Beyaz pulp sayısı ve çapı 10x büyütmeli mikroskop altında gözlendi. Sonuçlar, düşük dozdaki tüm propolis ekstraktlarının beyaz pulp sayısını önemli ölçüde arttırdığını gösterdi ($p < 0.05$). Bununla birlikte, propolis uygulanan tüm gruplarda çaptaki bir artış anlamlı bulunmadı. Bu çalışma, Endonezya propolisinin annenin bağışıklık sistemini modüle edebileceğini düşündürmektedir.

Anahtar kelimeler: Immunomodulatori, Histoloji, Hamilelik, Propolis

GENİŞLETİLMİŞ ÖZET

Amaç: Propolis, yoğun fitokimyasal bileşenleri nedeniyle çeşitli sağlık yararları olan doğal bir üründür. İmmünomodülatör aktivite, araştırmacılar tarafından geniş çapta rapor edilen propolisin ana aktivitelerinden biridir. Ancak gebelikte yapılan inceleme henüz değerlendirilmemiştir. Bu çalışma, günlük Endonezya propolis uygulamasının anne fare dalağının histolojik değişiklikleri üzerindeki etkisini değerlendirmeyi amaçladı.

Gereç ve Yöntem: Toplam 25 gebe fare kullanıldı ve kontrol grubu (%1 Tween 80), Güney Sulawesi'nin düşük (380 mg/kg) ve yüksek doz (1400 mg/kg) propolis etanol özü ve Banten'in düşük (380 mg/kg) ve yüksek doz (1400 mg/kg) propolisin su özütü grubu olmak üzere 5 gruba ayrıldı. Propolis, gebeliğin 0. gününden 18. gününe kadar günlük olarak uygulandı. Uygulamanın sonunda, fareler dalak elde etmek için sakrifiye edildi. Dalak, hematoksilen ve eozin boyama ile muamele edildi ve beyaz pulpanın sayısını ve çapını hesaplamak için mikroskop altında 10x büyütme ile gözlendi.

Bulgular: Bu çalışma, yalnızca düşük doz dışında tüm propolis ekstraktlarının beyaz pulp sayısını önemli ölçüde artırdığını gösterdi ($p < 0.05$). Ancak, propolis uygulanan tüm gruplarda beyaz pulp çapındaki artış anlamlı değildi ($p > 0.05$). Ayrıca, tüm gruplarda spesifik histopatolojik değişiklikler gözlenmedi.

Sonuç: Bu çalışma, düşük doz Endonezya propolisinin (380 mg/kg) annenin bağışıklık sistemini aktive edebileceği sonucuna varmıştır. Bununla birlikte, yüksek dozda (1400 mg/kg) alanlar daha az önemli değişiklikler göstermiştir. Bu çalışmada sadece histolojik değerlendirme kullanıldığı düşünülerek daha fazla çalışma yapılması gerektiği kanısına varılmıştır.

INTRODUCTION

Propolis is a bee product derived from resins of various plants and provides wide range of health benefits (Sforcin et al. 2017). Propolis is actually used by bees for self-defense and nest construction (Mohammadzadeh et al. 2007). Nonetheless, propolis has been used for centuries in the fields of medicine due to its pharmacological properties, such as anti-microbial, anti-ulcer, anti-inflammatory, antioxidant, anti-tumor, and cytotoxic activity (Fikri et al. 2019b, Król et al. 2013, Rao Muvva et al. 2021, Sevim et al. 2021). Moreover, several studies have reported propolis is safe for consumption (Burdock 1998).

The evaluation of propolis on pregnant conditions has attracted considerable attention since it has a great potential on supporting physiological well-being in pregnancy. Our previous study showed propolis has a potential to suppress emesis during early stage of pregnancy (Fikri et al. 2018). Also, propolis (380 mg/kg) has been reported to support fetal development and did not show maternal toxicity in mice (Fikri et al. 2019a, Fikri et al. 2021). In addition, propolis may improve the pregnancy outcomes and placental oxidative stress of diabetic rats (Usman et al. 2018).

However, the effect of propolis on the maternal immune system has limited evidence. On the other hand, the immunomodulatory property is one of the main activities of propolis (Sforcin, 2007). An adequate immune system is important to prevent maternal and fetal infection (Morelli et al. 2015). However, over-activation of the maternal immune system may lead to fetal rejection and other negative pregnancy outcomes (Burwick et al. 2021). Thus, this study aimed to evaluate histological changes in the spleen after the administration of Indonesian propolis during pregnancy.

MATERIALS AND METHODS

Propolis Preparation

Propolis samples originated from Banten Province, and South Sulawesi Province produced by *Tetragonula laeviceps* and *Tetragonula biroi*,

respectively. Ultrasound-assisted extraction was applied to obtain two types of propolis extracts, water extract of Banten propolis and ethanol extract of South Sulawesi propolis which were previously described to be potential in the treatment of emesis during pregnancy (Fikri et al. 2018). The procedure of extraction followed the method of Fikri et al. (2018). The propolis sample was cut into small 0.5 cm pieces and subsequently dissolved in a solvent at a ratio of 1:10 Ultrasound was applied for 3 hours and the samples were evaporated until dry. Water extract of Banten popolis contained phenolics (15.64 mg/g), flavonoids (1.80 mg/g), and IC_{50} of 503.93 mg/ L, whereas ethanol extract of South Sulawesi propolis found to be higher in total phenolics (22.30 mg/g), and total flavonoids (3.39 mg/g) content, but lower in antioxidant activity with IC_{50} of 543.93 mg/L (Fikri et al. 2019b).

Animals

The present study used mice (*Mus musculus*) weighed 25-20 g and aged 8-10 weeks. Female mice at proestrus and estrus were mated with male mice for a night and the vaginal plug was checked in the next morning. If the vaginal plug was found, day 0 of pregnancy was determined. A total of 25 pregnant mice were equally divided into 5 groups to receive 1% Tween 80, ethanol extract of South Sulawesi propolis at low dose (380 mg/kg) and high dose (1400 mg/kg), and water extract of Banten propolis at low dose (380) and high dose (1400 mg/kg), respectively. Dose of 380 mg/kg is an active dose that commonly used in biological activity studies, whereas dose of 1400 mg/kg is a non-observed adverse effect level (NOAEL) of propolis (Burdock, 1998; Eda et al. 2005). Propolis sample was dissolved in 1% Tween 80 and administered in 5 ml/kg daily during pregnancy. The mice were sacrificed at day 18 of gestation using 10% ketamine and 2% xylazine at a ratio of 20:1. Spleen was harvested after laparotomy and fixed with 10% neutral buffer formalin.

Histological Evaluation

Histological evaluation was performed using hematoxylin and eosin (HE) staining following the method of Pillai et al. (2011). The spleen was further dehydrated with alcohol and cleaned with xylene. It was then infiltrated with paraffin and cut using a microtome at a thickness of 4-6 µm. The number and diameter of white pulp were calculated under a microscope with a 10x magnify and Java Image J program.

Data Analysis

Data were reported as mean ± standard deviation. The differences between the groups were determined using ANOVA with Duncan's multiple range tests. A significant difference was determined at a p-value < 0.05.

RESULTS

Our result indicates that propolis administration during pregnancy could affect the immune system of mice. Low dose of both types of extract significantly increased the total number of white pulps ($p<0.05$). However, high dose extracts did not change the number. In addition, a non-significant increase of white pulp diameter was seen in all groups administered with propolis ($p>0.05$) (Table 1). According to univariate analysis of variance, the type and dosage of propolis had significantly effect on the number of white pulps ($p<0,05$). Regardless of the dosage, the number of white pulps were found to be higher in group administered with water extract of Banten propolis compared to ethanol extract of South Sulawesi propolis. Meanwhile, regardless of the type of propolis, higher number of white pulps were notably observed in low dose group. Both the type and dosage of propolis, and its interaction did not have significant effect on the diameter of white pulp (Table 2). Histopathologically, no specific changes in the spleen were observed in all groups. The histological section of the mice's spleens can be seen in Figure 1.

Table 1. Number and diameter of maternal white pulp after propolis administration

Groups	Number	Diameter (µm)
Control	9.40 ± 1.82^{c}	326.18 ± 51.12^{a}
Low dose EE	13.60 ± 2.61^{ab}	391.95 ± 31.36^{a}
High dose EE	9.33 ± 3.78^{c}	376.42 ± 68.85^{a}
Low dose WE	19.00 ± 2.83^{a}	348.19 ± 91.42^{a}
High dose WE	10.25 ± 2.75^{c}	395.13 ± 84.03^{a}

*Superscript with the different letter in the same column shows significant different at p-value < 0.05 using ANOVA with Duncan's multiple range test

Control : 1% Tween 80

Low dose EE : Ethanol extract of South Sulawesi propolis (380 mg/kg)

High dose EE : Ethanol extract of South Sulawesi propolis (1400 mg/kg BB)

Low dose WE : Water extract of Banten propolis (380 mg/kg BB)

High dose WE : Water extract of Banten propolis (1400 mg/kg BB)

Table 2. Univariate analysis of variance among factors

Factors	p-value	
	Number of white pulps	Diameter of white pulps
Type of propolis	0.046	0.757
Dosage	0.001	0.327
Interaction	0.143	0.551

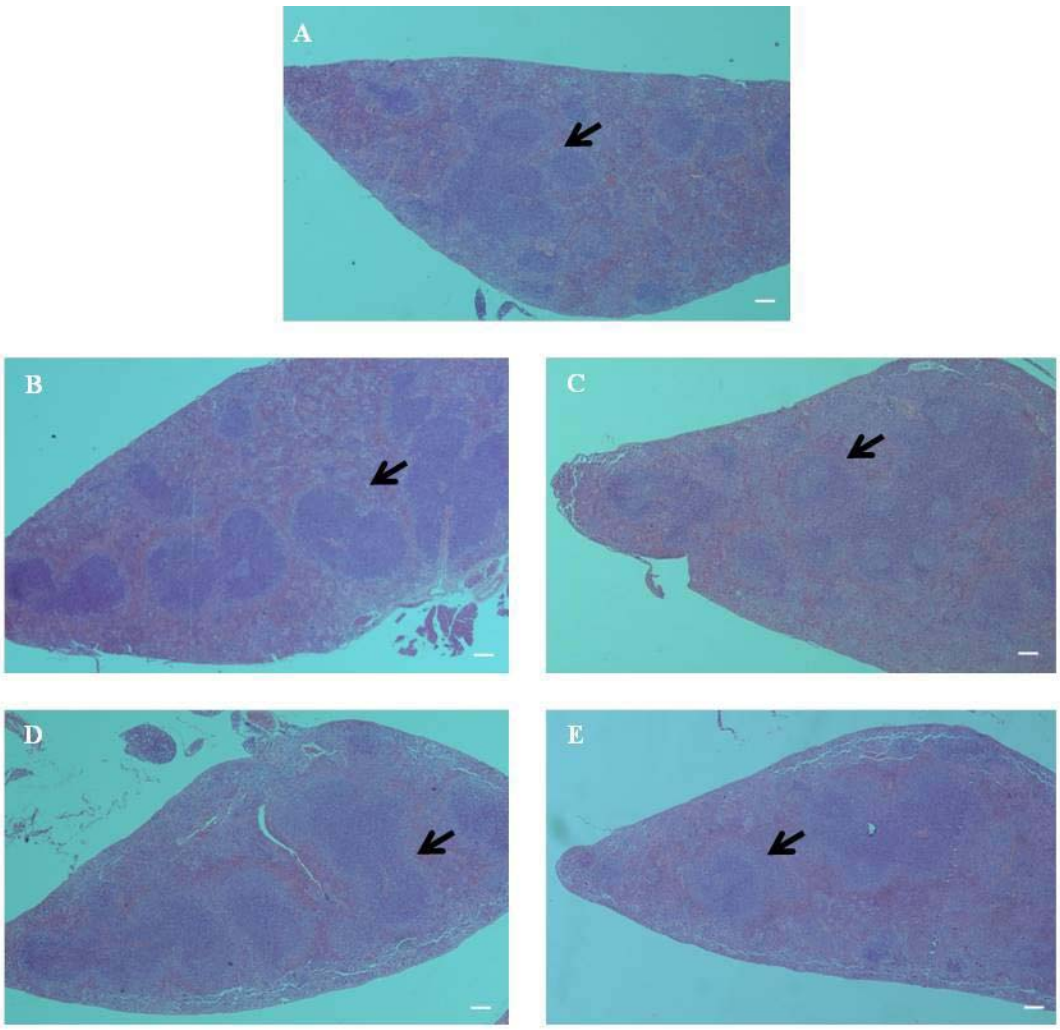

Figure 1. Histological section of maternal mice spleen at day 18 of gestation after propolis administration during pregnancy (hematoxylin and eosin staining; black arrow shows white pulp; 10x magnifying). A: control group (1% Tween 80); B: low-dose of ethanol extract of South Sulawesi propolis (380 mg/kg); C: high-dose of ethanol extract of South Sulawesi propolis (1400 mg/kg); D: low-dose of water extract of Banten propolis (380 mg/kg); E: high-dose of water extract of Banten propolis(1400 mg/kg).

DISCUSSION

Spleen is the largest immune organ in the body which responsible for immune system activation. The spleen is composed of two main compartments, the red pulp and the white pulp (Cesta 2006). The red pulp contains a large number of macrophages which efficiently remove foreign materials, cellular debris, and aging erythrocyte. Likewise, the white pulp contains a periarteriolar lymphatic sheath (PALS), lymphoid follicles, and a marginal zone, where the adaptive immune response can be initiated (Mebius & Kraal, 2005).

Our study suggests that propolis may activate the immune system during pregnancy. An increase in the number and diameter of the white pulp indicates the activation of the immune system (Horton & Manning 1974, Makiyah et al. 2014). This is actually not a surprising result because a lot of studies report the immunomodulatory action of propolis (Al-Hariri 2019, Conti et al. 2016, Dimov et al. 1991, Oršolić &

Bašić 2003). Using the same samples, Kalsum et al. (2017) described *Trigona* propolis at a low dose (0.16%) could regulate the nitric oxide, IgG antibody production, and phagocytic index of rats infected with *Staphylococcus aureus,* whereas high-dose (1.44%) did not make any changes. Moreover, propolis at high dose may act as an immunosuppressant where the results of the previous study analyzing the effect of propolis on the concentrations of CD4, CD8 T cells, and T cell memory (Rohmawati & Rifa'i 2014).

As the immune system highly affects the maternal and fetal health, the activation of maternal immune system by propolis should be further discussed. An inadequate maternal immune system may increase the risk of infection, whereas over-activation may cause fetal rejection (Burwick et al. 2021, Morelli et al. 2015). However, instead of causing negative outcomes, the activation of the immune system as the present study found might support fetal development. Our previous studies which used the same type and dose showed that propolis did not inhibit fetal development and did not show any indication of maternal toxicity (Fikri et al. 2019a; Fikri et al. 2021). Specifically, the present results are in line with our previously published study reporting that water extract of Banten propolis had better pregnancy outcomes than ethanol extract of South Sulawesi propolis (Fikri et al. 2019a). Using pure water in propolis extraction may produce an extract with less wax and resin content leading to less concentrated phytochemical compounds. In addition, propolis extracts prepared using water (100% and 80%) may not contain gallic acid and *p*-OH benzoic acid compared to ethanol extract (Kara et al. 2022). However, the extract may have more vitamins and minerals that extracted from bee pollen (Najafi et al. 2007). Nevertheless, water extract of Banten propolis was reported to possess higher antioxidant activity than the ethanol extract (Fikri et al. 2019b). Kara et al. (2022) found that caffeic acid phenethyl ester (CAPE) content was higher in the water extract than ethanol extract. Thus, water extract of Banten propolis might be more tolerated in pregnant condition which having favorable outcomes compared to the ethanol extract of South Sulawesi propolis. Moreover, propolis at high dose provides no beneficial effect. The previous study showed that propolis extracts at 1400 mg/kg disrupted placental development and caused intrauterine growth retardation (Fikri et al. 2019a). Therefore, consuming propolis at high dose are not recommended during pregnancy.

Conclusion

This preliminary study concludes that the maternal immune system may be modulated by all samples of Indonesian propolis. Moreover, remarkable activation was found in the group administered with propolis at low dose (380 mg/kg), whereas propolis at high dose (1400 mg/kg) did not provide beneficial effect. However, the results were only based on histological evaluation of the spleen, thus further investigation needs to be done.

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Ethics issue: All protocols have been approved by Animal Care and Use Committee, IPB University

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DERLEME / REVIEW

BAL ARISI (*Apis mellifera*) ZEHRİNİN ETLİK PİLİÇLERDE KULLANIMI

Using of Honeybee (*Apis mellifera*) Venom in Broiler Breeding

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ÖZ

Çalışmanın amacı, bal arısı (*Apis mellifera*) zehrinin etlik piliçlerde kullanılmasına yönelik çalışmaların sistematik derleme şeklinde incelenmesidir. Apiterapi, arı ürünleri ile alternatif tedavi yöntemi olarak tanımlanabilmektedir. Apiterapide bal, polen, propolis ve arı sütü gibi yaygın olarak bilinen ve besin olarak da tüketilen ürünlerin yanı sıra bal arısı zehri (venom) de kullanılmaktadır. Bal arısı zehri içerdiği peptidler, enzimler ve aktif aminler sayesinde özellikle kanser araştırmalarında sıklıkla incelenen bir madde olup bağışıklık sistemi üzerine önemli etkileri bulunan bir üründür. Bal arısı zehrinin antimikrobiyal ve antioksidan etkileri ile gelişim performansı ve bağışıklık sistemi üzerine etkilerini inceleyen çeşitli çalışmalar yapılmıştır. Çalışmalarda bal arısı zehri su ve yem katkı maddesi olarak veya kümes içerisine püskürtülerek kullanılmıştır. İçme suyuna eklenen bal arısı zehrinin, gelişim performansını önemli derecede etkilediği, yem katkı maddesi olarak kullanılmasının bağışıklık sistemi ve karaciğer fonksiyonlarına olumlu etkiler sağladığı ve dilüe edilmiş formunun kümes içerisine aerosol yol ile verilmesinin *Salmonella gallinorum'*a karşı etkili olduğu bildirilmiştir. Parenteral yollar ile uygulanan bal arısı zehri özütü, büyüme gelişme ve bağışıklık sistemi üzerinde olumlu etki göstermiştir. Bal arısı zehrinin çeşitli hayvan türlerinde yem katkı maddesi olarak kullanılmasına yönelik çalışmalar da devam etmektedir. Çalışmada, etlik piliç üretiminde bal arısı zehrinin çeşitli yaş dönemlerinde, farklı oranlarda ve farklı yöntemler ile verilmesinin etkileri incelenmiştir.

Anahtar kelimeler: Antimikrobiyal etki, Bal arısı zehri, Civciv gelişimi, Et kalitesi, *Salmonella*

ABSTRACT

The aim of the study is to examine the studies on the use of honey bee venom (*Apis mellifera*) in broiler chickens in the form of a systematic review. Apitherapy can be defined as an alternative treatment method using bee products. Bee venom (apitoxin) is used in apitherapy as well as commonly known and consumed products such as honey, pollen, propolis and royal jelly. Thanks to the peptides, enzymes and active amines it contains, bee venom is a substance that is frequently studied in cancer research and a product that has important effects on the immune system. Several studies have been conducted on the antimicrobial and antioxidant effects of bee venom, as well as its effects on developmental performance. In the studies, honey bee venom was used as a water and feed additive or by spraying in the hive. Honey bee venom added to drinking water was reported to significantly affect growth performance, its use as a feed additive had beneficial effects on immune system and liver functions, and its diluted form was effective against *Salmonella gallinorum* when sprayed into the pen by aerosol. Administration of honey bee venom extract to day-old chicks has a positive effect on

growth, development and immune system. Studies on the use of honey bee venom as a feed additive in different animal species are also continuing. In this study, the effects of adding honey bee venom at different ages, in different ratios and in different ways in broiler production were investigated.

Keywords: Antimicrobial effects, Honey bee venom, Chicken development, Meat quality, *Salmonella*

EXTENDED SUMMARY

Goals: The aim of this study is to investigate the use of honey bee venom (Apis mallifera) in chicken farming. For this purpose, studies in which bee venom was used in broiler farming between 2010-2021 were searched in the Google Scholar database using the terms apitherapy, bee venom, honey bee venom, bee venom and broiler, considering the PRISMA 2020 guideline. 23 of the 58 studies and publications found in the search were included in the scope of the study using the PRISMA 2020 chart guideline. Apitoxin (honey bee venom) is produced and stored in the venom sac in the abdominal cavity of female bees. Production reaches its maximum at 16-19 days of age, but according to some studies as early as 12 days. With age, a change in apitoxin content is observed, but production continues. (Selçuk et al. 2010; Bogdanov 2011; Aydın et al. 2017; Sorgucu 2019; Tanuğur Samanç and Kekeçoğlu, 2021). In the study, the chemical structure of bee venom (apitoxin) used in apitherapy was also investigated, and in this way, information about the composition of apitoxin was provided. Bee venom (apitoxin) is a yellowish, bitter-tasting substance that crystallizes on contact with air (Şahinler et al. 2019). More than half of its chemical structure consists of melittin, a peptide. Melittin has radioprotective, anti-inflammatory, antifungal, antiviral, antibacterial, and antitumor effects (Rady et al. 2017; Socarras et al. 2017; Bogdanov 2020). In addition to melittin, apitoxin also contains many organic compounds such as phospholipase A2 and other enzymes, active amines, volatile compounds, and sugars. Apamin, a peptide from the constituents, is reported to have an anti-inflammatory effect by stimulating the release of cortisone, and also act on cancer cells with its antiseratonic effect (Şahinler et al. 2019). Mast cell degranulation peptide (MCD) inhibits histamine release and has immunosuppressive and nociceptive effects (Bogdanov 2012; Bellik 2015). The enzyme hyalurinidase has detoxifying effects and increases capillary permeability (Bellik 2015; Şahinler et al. 2019).

Discussion: Since apitoxin has many such effects, it is successfully used against various cancers, joint and corneal diseases, and various tissue problems such as mastitis. Apitoxin is recommended as an alternative to the use of antibiotics, which are the main problem in animal products produced for food purposes, especially in broiler production. To this end, Han et al (2010) showed that the addition of apitoxin to drinking water resulted in better performance, antioxidant activity and liver functions in experimental groups. Jung et al. (2013) sprayed apitoxin to disinfect chicken houses to prevent Salmonella gallinarum infections and found an increase in antibody formation. Ali and Mohanny (2014) in their study investigated the effects of apitoxin extract injection on developmental performance and immune system of chicks. Significant differences were observed in the experimental groups in terms of feed intake, feed conversion ratio, live weight gain and blood parameters compared to the control group. Kim et al (2018) showed that the addition of purified apitoxin to the diet, its effect on various biological properties and its contribution to growth and animal health. Arteaga et al (2019) studied the effect of apitoxin on strains of Salmonella spp. isolated from chickens and found that the formation of biofilms was reduced, while biofilms already formed were highly destroyed. When frozen apitoxin was tested by inovo injection, Khalil et al (2021) obtained better results in hatching performance of hatching eggs compared to control groups. In conclusion, apitoxin can be used as an alternative to antimicrobial pharmacological agents and has positive effects on growth and development.

Conclusions: All studies have shown that the use of honey bee venom in broiler production has important benefits. The effects on growth, immune system, especially T-cells, and pathogenic microorganisms are increasing, and increasing antibiotic resistance necessitates the use of alternative antimicrobial agents, which threatens animal health and healthy food production. Thanks to the organic compounds of apitoxin, it seems possible to produce healthy broiler chickens and healthy food without residual or resistance risk. And also the use of apitoxin as a feed additive, aerosol spray, extracts and dried forms in broilers and as a hygienic agent in the food chain promise us a healthy and residue-free risk in the

production of broilers and other livestock animals.

GİRİŞ

Arı; ekosistemde pek çok işlevi olan ve ülkemizde kendisine geniş bir yayılım alanı bulan bir canlıdır. İnsan tüketiminin yanı sıra pek çok arı ürünü hayvan beslemede de aktif olarak kullanılmaktadır. Genellikle yem katkı maddesi olarak değerlendirilen arı ürünlerinin başında polen, propolis, arı sütü ve bal arısı zehri gelmektedir (Sur Arslan v.d. 2017; Topal v.d. 2015). Arı ürünlerinin besin maddesi olarak kullanılmasının yanında tedavi amaçlı kullanımı 6000 yıl öncesi Antik Mısır'a kadar uzanmaktadır (Altıntaş ve Bektaş 2019). Arı ürünleri çeşitli hastalıkların tedavisi amacıyla Romalılar ve Yunanlılar tarafından da yoğun olarak kullanılmıştır. Günümüzde artan antibiyotik kullanımı sonucunda alternatif çözümler aranmaya başlamış ve böylece apiterapi tekrar güncellik kazanmıştır (Çelik ve Aşgun 2016). Apiterapi, arı ürünleri ve başta bal arısı zehrinin çeşitli hastalıkların tedavilerinde kullanılması anlamına gelmekte (Bektaş v.d. 2016) ve temelinde homeopatik tedavi prensibine göre değerlendirilmektedir (Atalayoğlu ve Atalayoğlu 2015; Korkmaz ve Korkmaz 2015).

Hayvansal protein eldesi amacıyla hayvanların et verim yönlü yetiştirilmesi, pek çok ıslah çalışmasını da beraberinde getirmiştir. Son elli yılda hızla gelişen etlik piliç ıslahı sonucunda, hibrit hatlar ortaya çıkmış ve 42 günlük yaşta kesime hazır hale gelen piliçler elde edilmiştir. Tavuklarda nesil aralığının kısa olması, üretimlerinin hızlı olmasına, geliştirilen hatlarda ortaya çıkan hibrit vigor durumu da hatların hızlı gelişmesine imkan sağlamıştır. Antibiyotiklerin hayvansal üretimde kullanım amacı sadece tedavi olmayıp; koruyucu hekimlik uygulamaları ile bağışıklık sistemi ve büyüme gelişmenin uyarılmasını da sağlamaktır. Çok düşük dozlarda verilen antibiyotiklerin, büyüme gelişme üzerine etki mekanizmaları tam olarak açıklanamasa da bağırsak mikrobiatası üzerine olumlu etki sağladıkları bilinmektedir (Costa v.d. 2017). Ticari etlik piliç üretiminde sürenin kısa olması, hayvanlarda büyüme ve gelişmenin sağlıklı bir şekilde sağlanması ve çeşitli hastalıklara karşı önlem amacıyla uygulanan antiparaziter uygulamalarının veya antibiyotik ajan kalıntılarının gıda ile insana geçmesi ise çeşitli endişeleri meydana getirmektedir. Bu kaygılar, etlik piliç üretiminde antibiyotik yerine geçebilecek ürünler olan prebiyotikler, probiyotikler, sinbiyotikler gibi biyolojik ürünlerin kullanımını arttırmıştır. Apiterapi, diğer pek çok türün tedavisinde olduğu gibi antibiyotik alternatifi olarak etlik piliç üretiminde de kullanılmaktadır (Kim v.d. 2018). Bal arısı zehri, içerdiği çeşitli kimyasal bileşenler sayesinde gelişim üzerine de olumlu etkiler göstermektedir. Antibiyotik etkinliğini, melittin ve fosfolipaz A2'nin sinerjist çalışarak hücre zarı eritme özelliklerinden almaktadır (Marques Pereira v.d. 2020). Melittin, hücre zarına karşı düşük bir seçiciliğe sahiptir ve gözenek oluşumu süreci yoluyla lipidleri üzerinde güçlü bir şekilde etki eder. Bu süreç, hücre sitoplazmik içeriğinin salınmasına neden olarak hücre lizisine yol açmaktadır (Ostroumov v.d. 2015). Yakın zamanda yapılan bir çalışmada, bal arısı zehrinin bu özelliği kanser hücreleri üzerinde denenmiş ve bu hücrelere karşı seçici bir etki geliştirdiği gözlenmiştir. Ayrıca ilgili çalışma bal arısı zehrinin kanserde epigenetik tedavi amacı ile DNA demetilasyon aracı olarak kullanılabileceğini ortaya koyan ilk çalışma olarak görülebilir (Uzuner v.d. 2021). Bal arısı zehrinin hem Gram + hem de Gram – bakteriler üzerinde etkili olduğu, yapılan çeşitli çalışmalar ile ortaya konmuştur (Jenkins v.d. 2011). Gıda kaynaklı patojen mikroorganizmalardan Gram pozitif *L. monocytogenes'*e ait sekiz ve Gram negatif *S. enteritidis'*e ait elli suşta yapılan bir çalışmada beş farklı bölgeden toplanan bal arısı zehrinin kullanılması ile minimum inhibisyon konsantrasyonu önemli derecede etkilenmişir (Lamas v.d. 2020). Yapılan diğer çalışmalarda, kovan içerisindeki tüm alanlardan toplanan bal arısı zehir örnekleri benzer antimikrobiyal etki göstermiştir (Hegazi v.d. 2015). Bal arısı zehri ve melittin ilave edilmiş balarısı zehrinin ile birlikte kullanıldığı bir çalışmada Lyme hastalığının etkeni olan *Borrelia burgdorferi* bakterisinin tüm morfolojik formları üzerinde, hatta antibiyotiğe dirençli eklenmiş biyofilmler üzerinde bile önemli etkiler gösterdiği tespit edilmiştir. Farklı antibiyotiklerin tek başına veya birlikte uygulandığında sınırlı etki göstermeleri, bal arısı zehrinin kullanılabilirliği açısından büyük önem taşımaktadır (Socarras v.d. 2017). Metisiline dirençli *S. aureus*'un (MRSA) tedavisinde varolan antibiyotiklerin kullanılması sınırlı etki göstermektedir. Bal arısı zehri ve aktif bileşenlerinden melittinin antimikrobiyal etkisi, MRSA oluşturulmuş fareler üzerinde denenmiştir. Çalışmanın sonunda, özellikle melittin uygulanan gruplarda yüksek oranda bakterisidal etki gözlenmiştir (Choi v.d. 2015). MRSA tedavisinde bal arısı zehri ve melittin kullanılmasının incelediği bir başka çalışmada, oksasilin ile sinerjist etkileri değerlendirilmiştir. Çalışmada enterotoksijenik *S.*

aureus suşlarında enterotoksin oluşumunun azalmadığı ancak oksasilin ile kombine edilen bal arısı zehri ve mellitinin bakterisidal etkinliği arttırdığı saptanmıştır (Marques Pereira v.d. 2020).

Bal arısı zehri ve diğer apiterapi yöntemlerinin insanlarda kanser çalışmaları ile çeşitli hayvan türlerinde eklem yangıları, çeşitli kemik hastalıkları, sıcak stresi çalışmaları, mastitis gibi hastalıklar ve durumlarda kullanıldığı bilinmektedir (Topal v.d. 2015; Madras-Majewska v.d. 2015; Sur Arslan v.d. 2017). Bal arısı zehri ana bileşenlerinden biri olan melittinin antikanserojenik etkileri de preklinik çalışmalar ve hayvan deneyleri ile ispatlanmış ancak insanlar üzerinde sitotoksite değerlerinin değişkenliği ve hemolitik aktivitesi nedeniyle kullanımı sınırlanmıştır (Rady v.d. 2017). Prostat kanseri, meme kanseri, hapatoselüler karsinom, akciğer kanseri, melanom ve lösemi gibi pek çok karsinojenik hastalığın tedavisinde bal arısı zehri ve melittin çalışmaları devam etmektedir (Ağan ve Kekeçoğlu 2020). Melittinin antibiyotik etkisinin değerlendirildiği bir başka çalışmada ise yanık lezyonlarında sekonder enfeksiyon olarak gelişen ve antibiyotiğe dirençli *Acinobacter spp*. suşları üzerinde önemli bakteriyostatik ve bakterisidal etkileri gözlenmiştir (Pashei v.d. 2019). Bu çalışmada etlik piliç üretiminde bal arısı zehrinin kullanıldığı çalışmalar ve sonuçları ele alınmıştır.

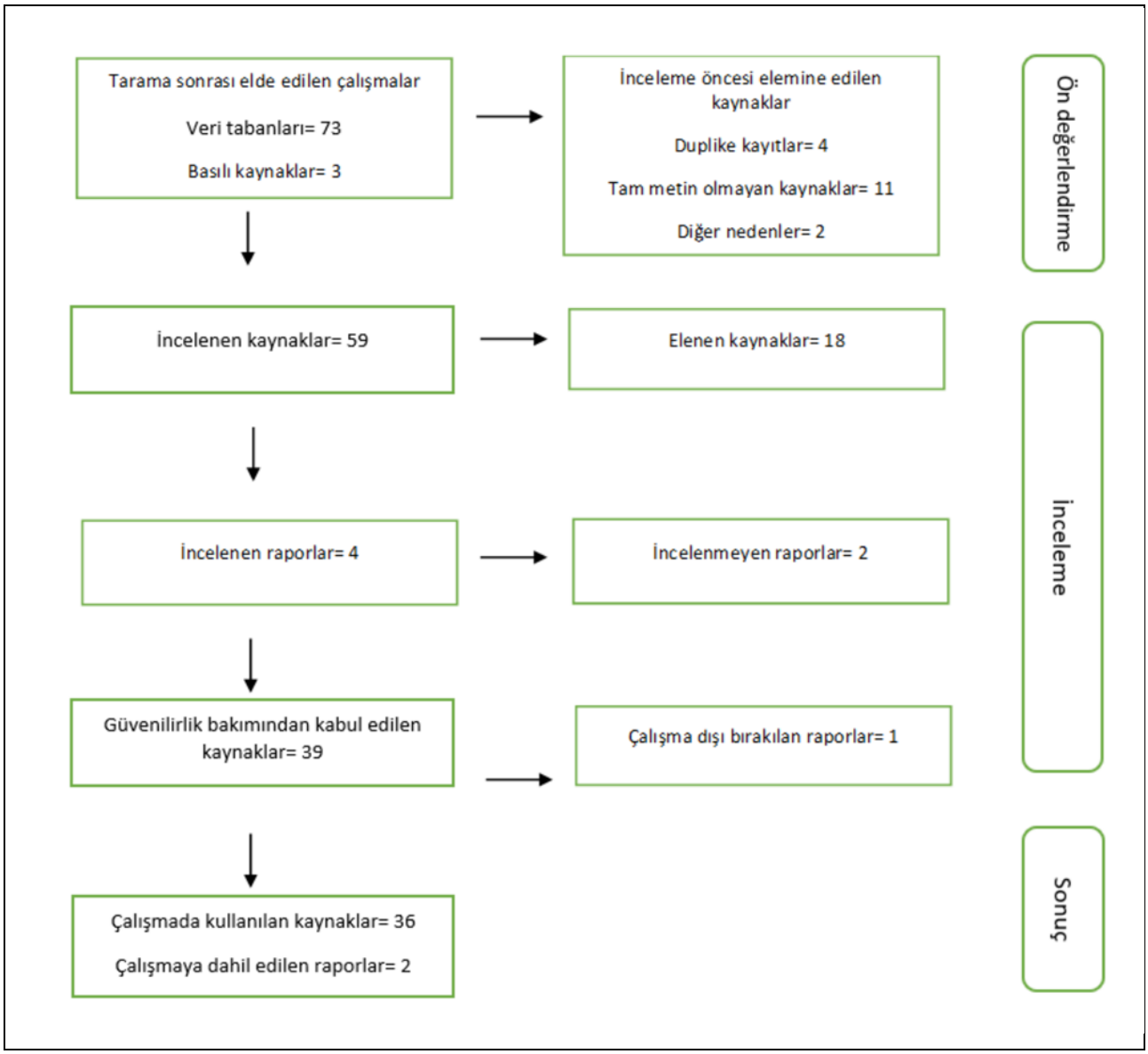
METOD

Çalışma, PRISMA 2020 Sistematik Derleme yönergesine göre yapılmış ve çalışmada izlenen yol, Şekil 1'de verilmiştir (Page v.d. 2020). Çalışmada Google Akademik veri tabanı kullanılarak *apiterapi, bal arısı zehri, etlik piliç, melittin, honeybee venom, bee venom and broiler*, *melittin* anahtar kelimeleri kullanılarak hem Türkçe hem de İngilizce makaleler taranmıştır. Çalışma verileri 2015-2021 yılları arasında taranmış ancak konu ile ilgili öncü çalışmalar olmaları nedeni ile 2010 yılına ait 1, 2011 yılına ait 2 ve 2014 yılına ait 2 çalışma da derlemeye dahil edilmiştir. Anahtar kelime ile aramada elde edilen 73 kaynaktan 4'ü aynı çalışma olmasından, 1'i tam metni olmamasından, 12'si Türkçe veya İngilizce'den başka bir dilde hazırlanmış olmasından kaynaklı tam incelemeye dahil edilmemiş; kalan kaynaklar detaylıca incelendikten sonra 11'i ilgisiz içerik içermesinden ve 7'si çeşitli sebeplerden inceleme dışı bırakılmıştır. Çalışmada ele alınan 38 kaynak 2 kitap bölümü, 3 kongre bildirisi, 2 birlik ve merkez bildirisi, 1 online makale ve 30 yayımlanmış makaleden oluşmaktadır.

BAL ARISI ZEHRİ

Bal arısı zehri (venom) apitoksin olarak tanımlanmakta ve dişi arıların (işçi ve kraliçe) karın boşluğundaki zehir bezlerinde üretilip zehir kesesinde depolanmaktadır. Toplam depolanabilen zehir miktarı ortalama 0,3 mg kadardır (Selçuk v.d. 2010; Bogdanov 2011). Bazı çalışmalarda balarısı zehrinin en yüksek değere 12 günlük yaşta eriştiği ve 20 günlük yaştan sonra üretiminin tamamen bittiği bildirilirken (Bogdanov 2011; Aydın v.d. 2017; Sorucu 2019), son dönem çalışmalarında üretimin 2 günlük yaşta başlayıp maksimum üretim değerine 16-19 günlük yaşta erişildiği ve yaşa bağlı olarak içerik ve miktarın azalarak üretiminin devam ettiği bildirilmektedir (Tanuğur Samanç ve Kekeçoğlu 2021; Çaprazlı ve Kekeçoğlu 2021). Bal arısı zehri fiziksel olarak keskin kokulu, sarımtırak renkte, acı tada sahip olup hava ile teması sonucunda kristalize olabilen bir maddedir (Şahinler v.d. 2019). Farmakolojik olarak aktif bileşikler içeren bal arısı zehrinin kimyasal yapısının yaklaşık %50'si melittindir. Melittin, polipeptit yapısında olup majör peptit olarak tanımlanmakta (Rady v.d. 2017) ve radyoprotektif, antienflamatuvar, antibakteriyel, antifungal ve antitümoral etkileri bulunmaktadır (Rady v.d. 2017; Socarras v.d. 2017). Son yapılan çalışmalarda bal arısı zehri içerisindeki melittin ve fosfolipaz A2 enziminin antviral etki de gösterdiği saptanmıştır (Bogdanov 2020). Bal arısı zehrinin kimyasal yapısında bulunan peptidler, enzimler, aktif aminler, şekerler, yağ, aminosit ve çeşitli uçucu bileşiklerden oluşup, genel içeriği Tablo 1'de verilmiştir.

Zehir kesesi, arı iğnesine bağlı olup; iğne yardımı ile enjekte edilebilmektedir. Enjekte edilen salgının %88'i su olup toplam salgıda 0,1 mikrogram venom bulunmaktadır (Şahinler v.d. 2019). Bal arısı zehri içerisinde yer alan her bileşiğin ayrı bir etkisi bulunmaktadır. Örneğin peptidlerden biri olan apamin, kortizon salınımını uyararak antienflamatuar etki göstermektedir. Antiseratonin etkisi de olduğu bilinen bu peptidin kanser hücreleri üzerine sitotoksik etki gösterdiği düşünülmektedir (Şahinler v.d. 2019). MCD (mast hücre degranülasyon) peptid, immunsupresif ve nosiseptif etki göstermesinin yanında histamin salınımını önlemektedir (Bogdanov 2012; Bellik 2015). Hyolurinidaz enzimi detoksik etki göstermekte ve kılcal damar geçirgenliğini arttırmaktadır. Fosfolipaz A2 enzimi ise öldürücü etki gösterebilmektedir (Bellik 2015; Şahinler v.d. 2019).



Şekil 1. Sistematik derlemeler için PRISMA 2020 hazırlık diyagramı (https://estech.shinyapps.io/prisma_flowdiagram/).

Figure 1. PRISMA 2020 flow diagram for systematic reviews (https://estech.shinyapps.io/prisma_flowdiagram/).

Tablo 1. Bal arısı zehri kimyasal bileşimi (Bogdanov 2011; Sorucu 2019).

Table 1. Chemical composition of bee venom (Bogdanov,2011; Sorucu 2019).

Molekül	Bileşen	% Oran
Peptid	Melittin ve türevleri	50
	Apamin	1-3
	MCD peptid	1-2
	Sekapin	0,5-2
	Tertiapin	0,1
	Adolapin	1
	Proteaz inhibitörleri	0,8
	Prokamin A-B	1-4
	Kardiyopeptin	-
Enzim	Fosfolipaz A2	10-12
	Hyalurolinaz	1-3
	Asit fosfomonesteraz	1
	Lizofosfolipaz	1
	Glikozidaz	0,6
Aktif amin	Histamin	0,6-1,6
	Dopamin	0,13-1
	Norepinefrin	0,1-0,5
Yağ	6-fosfolipidler	-
Aminoasit	Alfaaminoasitler	1
	Aminobutirik asit	0,4
Uçucu bileşikler	-	4-8

Bal arısı zehrinin çeşitli hayvan türlerinde kullanımı ile antimikrobial ve antiviral etkinlikleri kanıtlanmıştır. Lee v.d. (2014), domuzlarda yardımcı T lenfosit tip 1 spesifik immun yanıt üzerine bal arısı zehrinin etkisini inceledikleri çalışmada, bağışıklık sistemi bileşenlerinde ciddi bir olumlu etki elde etmiştir. Benzer şekilde Han v.d. (2009) ineklerde mastitise bal arısı zehrinin etkisini inceledikleri çalışmalarında 12 mg bal arısı zehri verilen ineklerde tedavinin 3. gününde somatik hücre sayısında %55 azalma tespit edilmiştir.

BAL ARISI ZEHRİNİN ETLİK PİLİÇ ÜRETİMİNDE KULLANILMASI

Gıda kaynaklı patojen mikroorganizmaların antibiyotik direnç geliştirmek ve biyofilm oluşturmak gibi riskleri bulunmaktadır. Benzer şekilde, antibiyotik kalıntıları, çeşitli ilaç alerjilerinin gelişmesine de neden olabilmektedir. Bu durum, halk sağlığını ciddi anlamda tehdit etmekte, gelişen çeşitli enfeksiyonların kötü sonuçları olabilmektedir. Bu nedenle etlik piliç üretimi gibi hayvansal gıda üretimlerinin tamamında antibiyotik yerine kullanılabilecek alternatif kaynak arayışları bulunmaktadır.

Han v.d. (2010) etlik piliç içme suyuna bal arısı zehrinin ilave edilmesinin performans, antioksidan aktivitesi ve karaciğer fonksiyonları üzerine etkisini incelemişlerdir. Denemede bal arısı zehrini 0,5 mg/L ve 1,0 mg/L oranlarında, iki ayrı deneme grubu olarak kullanmışlardır. Deneme toplamda 28 gün sürmüş ve günlük etlik civcivler kullanılmıştır. Denemenin sonucunda canlı ağırlık artışının bal arısı zehri ilave edilen gruplarda istatistiksel olarak önemli derecede yüksek olduğunu; 1,0 mg/L bal arısı zehri ilave edilen grupta ise en yüksek sonuçların alındığı bildirilmiştir. Karaciğer fonksiyonlarında, karaciğer enzimleri ile diğer biyokimyasal parametrelerde ise kontrol grubu ile

deneme grupları arasında herhangi bir fark bulunamamıştır. Antioksidan aktivitesinde ise, benzer şekilde, bal arısı zehri ilave edilen grupta daha yüksek değerler elde edilmiştir.

Jung v.d. (2013), etlik piliç üretiminde *Salmonella gallinarum*'a karşı bal arısı zehrinin bağışıklık sisteminin uyarılması amaçlı kullanımını araştırmıştır. Bu amaçla kümes içerisine %95,7 distile su, %3,5 etanol ve %0,8 proplan glikol ile hazırlanan, 2,1 mg/mL bal arısı zehri içeren karışım hazırlanmıştır. Salmonellosis geçmişi olmayan etlik civcivlerden oluşan denemede, kontrol grubuna sadece etanol ve proplan glikol içeren solüsyon; deneme grubuna ise bal arısı zehri içeren solüsyon aerosol olarak uygulanmıştır. Aerosol uygulanması 3, 9 ve 15. Günlerde 30 ml, 60 ml ve 90 ml olacak şekilde tekrarlanmıştır. Aerosol uygulamarı bittikten sonra civcivler üç alt gruba ayrılmış ve üç ayrı çalışma grubu oluşturulmuştur. İlk grupta formalinle öldürülebilen *S. gallinarum*'a karşı antikor üretiminin değerlendirilmesinde, ikinci grupta öldürücü olmayan bir *S. gallinarum* şusuna karşı geliştirilen bağışıklık tepkisi ve son grupta bakterinin minimum lethal dozuna (LD25,5 x 109 cfu) karşı hayatta kalma ve patolojik değişiklikler incelenmiştir. Çalışmanın sonucunda 3 günlük canlı ağırlık bakımından gruplar arasında bir farklılık gözlenmezken, 23 ve 33. günlerde bal arısı zehrinin aerosol olarak uygulandığı grupta kontrol grubuna oranla önemli derecede farklılık kaydedilmiştir. S. gallinorum'a karşı üretilen antikor miktarı önemli derecede artış göstermiştir. Benzer şekilde, bal arısı zehrinin uygulandığı grupta, öldürücü olmayan *S. gallinorum* suşuna karşı T lenfosit oranında tüm deneme boyunca artış gözlenmiştir. Relatif interlökin-18 (IL-18), interferon gama (INF- γ) mRNA ekspresyonunda, deneme grubunda artış tespit edilmiştir. Son deneme olan ve hayatta kalma değerleri değerlendirilen gruplarda kontrol grubu %75; deneme grubu ise %85 hayatta kalma oranı sergilemiştir. Patolojik değişiklikler ise deneme grubunda kontrol grubuna oranla daha az yoğunlukta gözlenmiştir.

Ali ve Mohanny (2014), bal arısı zehri özütünün günlük etlik piliç civcivlere enjeksiyonu ile gelişim performansı ve bağışılık sistemi gelişimine etkisini incelemiştir. Denemede biri kontrol olmak üzere toplam dört grup oluşturulmuştur. Denemede 0-3. haftalarda ve 4-6. haftalarda iki ayrı besleme programı uygulanmıştır. Kontrol grubunda yer alan civcivlere haftalık olarak kas içerisine tuzlu su çözeltisi enjekte edilmiş diğer gruplara ise sırası ile 0,5; 1,0 ve 1,5 mg bal arısı zehri özütü yine aynı yöntemle kas içerisine uygulanmıştır. Enjeksiyonlar haftalık yapılmış ve 5. haftada enjeksiyon sonlandırılmıştır. Çalışmanın sonucunda deneme gruplarında kontrol grubuna oranla 3 haftalık yaşta canlı ağırlığın; 0-3 haftalık dönemde günlük canlı ağırlık artışının ve 4-6 haftalar ile 0-6. haftalar arasında günlük yem tüketiminin önemli derecede artış gösterdiği saptanmıştır. Ancak 6. hafta canlı ağırlıkta, 4-6 ve 0-6 haftalarda günlük canlı ağırlık artışında ve 0-3 haftalarda yem tüketimi ve yemden yararlanma oranında; hemaglutinin antikor titresi (SRBC), aspartat amino transaminaz (AST) ve alanin amino transaminaz (ALT), serum total protein, albümin, globülin, albümin globülin oranı, kolesterol ile toplam lipidlerde deneme grupları ile kontrol grubu arasında önemli bir fark bulunamamıştır.

Han v.d. (2016), saflaştırılmış bal arısı zehrinin, ölü etlik piliçlerden izole ettikleri Cl. perfiringens, S. thyphimurium ve S. Montevideo üzerine etkilerini incelemiştir. Bu amaçla, agar kuyusu difüzyon metodu, minimum inhibitör konsantrasyon, minimum bakterisidal konsantrasyon ve postantibiyotik etkiler değerlendirilmiştir. Bal arısı zehrinin saflaştırılmış şekilde minimum inhibitör konsantrasyon miktarları, Cl. perfiringens, için 0,85 µg/mL S. thyphimurium için 0,68 µg/mL ve S. Montevideo için 0,69 µg/mL olup, bakteriyel büyümeyi önemli derecede baskıladığı bildirilmiştir. Minimum bakterisidal aktivite için değerler sırasıyla 3,33 µg/mL, 2,66 µg/mL ve 2,86 µg/mL olarak; postantibiyotik etki ise, sırasıyla, 3,5; 4,0 ve 3,5 saat olarak kaydedilmiştir. Saflaştırılmış arı zehri, 24 saat boyunca pH 1'den pH 8'e kadar arttırılan asidik ortama maruz bırakılmış; maruziyet sonrası melittin içeriği ve antibakteriyel aktivesinde herhangi bir değişiklik gözlenmemiştir. Benzer şekilde 15 dakika boyunca ısıtılan saflaştırılmış arı zehrinde 100°C'nin altındaki sıcaklık değerlerinde herhangi bir bozulma meydana gelmediği de bildirilmiştir.

Kim v.d. (2018), etlik piliç rasyonlarına saflaştırılmış bal arısı zehrini, sırasıyla; 0, 10, 50, 100 ve 500 µg/kg yem olacak şekilde ilave etmiştir. Rasyon içeriği, denemede oluşturulan 5 grupta da aynı olup mısır ve soya fasülyesi küspesinden oluşmuştur. Çalışmanın sonucunda rasyon bal arısı zehri içeriği arttıkça, yemden yararlanma oranı ve canlı ağırlık artışının ilk 21 günde kuadratik olarak önemli derecede arttığı gözlenmiştir. Ancak 21. günde rasyon bal arısı zehri arttıkça dalak (linear ve kuadritik), bursa fabricius (kuadratik) ve karaciğer (linear ve kuadratik) ağırlığının azaldığı bildirilmiştir. Göğüs eti ağırlığı 21.

gün ölçümlerinde rasyon bal arısı zehri miktarına paralel olarak kuadratik; 35. gün ölçümlerinde ise linner olarak artış gözlenmiştir. 21. günde et kalitesi üzerine yapılan çalışmalarda renk açıklığının lineer ve kuadratik olarak arttığı gözlenmiştir. İleum villus ölçümlerinde ise 21. günde villus yüksekliğinin lineer olarak azaldığı; villus genişliğinin ise kuadratik olarak daraldığı bildirilmiştir. İleum mukozası üzerindeki IgA sekresyon konsantrasyonunun lineeer olarak arttığı ve 31 ile 35. günlerde serum numulerindeki nitrik oksit içeriğini kuadratik olarak azalttığı gözlenmiştir. Kreatinin haricinde diğer serum parametreleri bal arısı zehri düzeyinden etkilenmemiştir. Rasyon zehir miktarı arttıkça sekumdaki kısa zincirli yağ asitlerinde de azalma gözlenmiştir.

Arteaga v.d. (2019), tavuklardan izole ettikleri on altı *Salmonella sp.* suşuna ait 11 serotip ve 2 alt türden toplam 16 tip üzerine bal arısı zehrinin antimikrobiyal ve antibiyofilm aktivite etkisini incelemiştir. Çalışmada ayrıca, bal arısı zehrinin *Salmonella* suşlarının motilitesi, biyofilm ve virülans ilişkili genlerin ifadesi üzerine etkileri de değerlendirilmiştir. Sonuçlarda, bal arısı zehrinin minimum etki dozu *S.enterica* subsp. *Salamae'*de 1024 µg/mL, S. thphimurium Lhica T21'de 256 µg/mL, incelenen diğer on dört tipte ise 512 µg/mL olarak belirlenmiştir. Mobilite üzerine etkiler incelendiğinde sadece *S. isangi IG1* ve *S. infantis I17* tiplerinin etkilenmediği; diğer on dört tipin mobilitesi üzerine bal arısı zehrinin önemli derecede etkili olduğu gözlenmiştir. Bal arısı zehri, on dört tipte biyofilm oluşumunu yavaşlatırken, *S. enteritidis Lhica ET2* ve *S. enterica subsp. Salamae Lhica SA3* tiplerinin biyofilm oluşumlarında herhangi bir değişiklik gözlenmemiştir. Biyofilm oluşumu ve virülans üzerine etkili genler olan csgD, sroC, csrB, rprA, oxyS ve dsrA genlerinde de bal arısı zehrinin etkisi sonucu çeşitli değişikliklerin gözlendiği, transkripyon sRNA'ları üzerinde de yeniden düzenlenmeye yol açtığı bildirilmiştir.

Khalil v.d. (2021) iki fazda liyofilize edilmiş bal arısı zehrinin in ovo enjeksiyon yöntemi ile çıkım oranı, yumurta çıkım ağırlığı ve civcivlerde bağışıklık sistemi gelişimi üzerine etkilerini incelemişlerdir. In ovo enjeksiyon 2 gruba ayrılıp; 10 µg/yumurta ve 20 µg /yumurta ve 3. grup kontrol olmak üzere toplam dokuz yüz döllü yumurtada kuluçkanın 18. gününde uygulanmıştır. Sonuçlar, 10 µg/yumurta bal arısı zehri ile in-ovo enjeksiyonun çıkım oranı (%89,45) kontrole (%87,5) ve 20 µg/yumurta bal arısı zehrine (%77,73) göre önemli ölçüde arttırdığını göstermiştir. En yüksek bağıl civciv ağırlığı, kontrol grubunun yumurtalarında gözlenmiş, bunu daha düşük (10 µg) ve daha yüksek (20 µg) dozda bal arısı zehri grupları izlemiştir. Doz artışının göreceli civciv ağırlığı üzerinde azaltıcı etkisi olduğu tespit edilmiştir. Toplam protein ve albümin değerleri 10 ve 20 µg bal arısı zehri/yumurta gruplarında kontrol grubuna göre önemli ölçüde azalmıştır ($P \leq 0.001$). Çıkımdaki civciv kanının triiyodotironin (T3) ve tiroksin (T4) konsantrasyonları için sonuçlar, in-ovo bal arısı zehri enjeksiyonunun çıkım civciv serumundaki hem T3 hem de T4 konsantrasyonlarını önemli ölçüde azalttığını göstermiştir. Ayrıca, 20 µg doz in-ovo grubunda dalak nispi ağırlığının kontrol grubuna göre önemli ölçüde arttığı gözlenmiştir. Kontrol grubuna kıyasla Bursa fabricius nispi ağırlığı ile de benzer sonuç alınmıştır. Sonuç olarak, 10 µg bal arısı zehri/yumurta ile in-ovo enjeksiyon, damızlık yumurtalarda daha iyi kuluçka yüzdesi ile sonuçlanmıştır.

SONUÇ ve ÖNERİLER

Çalışmada bal arısı zehrinin etlik piliç yetiştiriciliğinde kullanımı üzerine yapılan çalışmalar incelenmiştir. Bal arısı zehri ve bileşenlerinin (melittin, Fosfolipaz A2 gibi) pek çok alanda kullanılmaya başlaması ile apiterapiye olan ilgi giderek artmıştır. Bal arısı zehrinin, insanlarda özellikle kanser araştırmalarında ve pek çok hayvan türünde artritis, kornea hastalıkları, mastitis, çeşitli deri hastalıkları gibi problemlere olumlu etkileri kanıtlanmıştır. Bal arısı zehri, içerdiği kimyasal bileşikler sayesinde bağışıklık sistemi ve büyüme gelişme üzerine olumlu etkiler göstermektedir. Antibiyotik direnci gösteren bazı bakterilere karşı etkinliğinin antibiyotiklere oranla daha yüksek olduğu belirtilmiştir. Etlik piliç yetiştiriciliğinde Salmonella sp. başta olmak üzere zoonoz özellikte olan hem hayvanın hayat kalitesini hem de ürünün kalitesini düşüren mikroorganizmaları etkisiz hale getirdiği tespit edilmiştir. Etlik piliç üretiminde önemli olan yemden yararlanma oranı ve canlı ağırlık artışına olumlu etkileri, çeşitli çalışmalar ile kanıtlanmıştır. Bal arısı zehrinin kanatlı yetiştiriciliğinde etkinliğine dair yapılan çalışmaların az olması nedeni ile, ilgili çalışmaların tümünde daha fazla çalışma hazırlanması gerektiği vurgulanmıştır. Bal arısı zehri özütünün gelişim ve fizyolojik parametreler üzerinde herhangi bir istenmeyen etki göstermediği ancak bu konuda daha fazla çalışma yapılması gerektiği,

araştırıcılar tarafından bildirilmiştir. Gıda kaynaklı patojen mikroorganizmaların ortadan kaldırılması veya patojenite değerlerinin azaltılmasında sanitasyon amacıyla bal arısı zehrinin kullanılması önerilmektedir. Bal arısı zehri ana bileşenlerinden biri olan melittinin özellikle biyofilm oluşturan ve antibiyotiğe dirençli mikroorganizma türleri üzerinde önemli etkilerinin gözlenmesi, yem katkı maddesi olarak kullanılmasının gerek tedavi gerekse koruyucu hekimlik uygulamaları kapsamında değerlendirilmelidir. Bal arısı zehrinin, etlik piliç yetiştiriciliğinde, su ve yeme çeşitli oranlarda katılması, aerosol olarak uygulanması, canlı organizmaya ve in-ovo embriyoya enjekte edilmesi ile umut vadeden sonuçlar alınmıştır. Ancak, etlik piliç üretiminde çalışmaların az olması ve çalışmalarda benzer özelliklerin incelenmesi, alanda yapılacak pek çok çalışmayı gerekli kılmaktadır. İnsanlarda kanser araştırmalarında incelenen epigenetik mekanizma, çevresel mikroorganizmalar ve özellikle etlik piliç üretiminde refah sorunlarının başında gelen sıcaklık stresi, taban yanıkları gibi metabolik hastalıkların önlenmesi ile tedavisinde bal arısı zehrinin etkisi incelenmelidir. Sonuç olarak, antibiyotik kullanımı yerine bal arısı zehrinin etlik piliç yetiştiriciliğinde kullanılması ve yeni çalışma alanlarının geliştirilmesinin teşvik edilmesi önerilmektedir.

Yazar Katkıları:

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Denetleme/Danışmanlık: Serkan Erat

Veri Toplama ve/veya İşleme: Erva Eser

Analiz ve/veya Yorum: Erva Eser, Serkan Erat

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DERLEME / REVIEW

EFFECTS OF ROYAL JELLY ON OBESITY

Arı sütünün obezite üzerine etkileri

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ABSTRACT

Obesity is a disease that affects people's quality of life and is a risk factor for some fatal diseases. Its prevalence is increasing in the world and the biggest factor is nutritional problems and a sedentary lifestyle. Royal jelly (RJ), a natural product for obesity, a preventable disease, has been the subject of various studies. RJ secreted by young honey bees (worker bees) is the only food source for the queen bee and the first three days of food for immature bees. Several studies have shown RJ's anti-obesity, antioxidant, anticarcinogenic, antimicrobial, and anti-inflammatory activities. In addition, RJ is seen as a promising functional food in the prevention of obesity and protection from its negative effects. This study is an examination of studies on the effects of RJ on obesity and its accompanying problems.

Keywords: Obesity, Royal jelly, Bee

ÖZ

Obezite insanların yaşam kalitesini etkileyen ve bazı ölümcül hastalıklar için risk faktörü oluşturan bir hastalıktır. Dünyada prevalansı artmaktadır ve en büyük etken beslenme sorunları ve hareketsiz yaşam tarzıdır. Önlenebilir bir hastalık olan obezite için doğal bir ürün, arı sütü (RJ) çeşitli çalışmalara konu olmuştur. Genç bal arıları (işçi arılar) tarafından salgılanan RJ kraliçe arının tek besin kaynağı ve olgunlaşmamış arıların ilk üç günlük besinleridir. RJ'nin antioksidan, antikanserojen, antimikrobiyal, antienflamatuar ve antiobezite aktiviteleri çeşitli çalışmalarla gösterilmiştir. Bu çalışma RJ'nin obezite ve beraberinde getirdiği sorunlar üzerindeki etkilerini konu alan çalışmaların bir derlemesidir.

Anahtar Kelimeler: Obezite, Arı sütü, Arı

GENİŞLETİLMİŞ TÜKÇE ÖZET

Amaç: Arı ve ürünleri antik çağlardan beri insan sağlığını olumlu yönde etkilemesinden dolayı tüketilmektedir. Arı ürünlerinden RJ''nin antioksidan, antifungal, antibakteriyel, antiviral, antikanserojen, antienflamatuar ve antiobezite gibi biyoaktif özellikleri çeşitli in vitro ve in vivo çeşitli çalışmalarla gösterilmiştir. RJ genç işçi arıların yaşamlarının ilk üç günü beslendiği ve kraliçe arının ise hayat boyu beslendiği, arıların hipofarengeal ve mandibular bezlerinden salgılanan vizkoz, asidik, sarımsı renkte karbonhidrat, protein, lipit ve serbest aminoasitlerce zengin suda çözünür bir salgı maddesidir. Arı ürünlerinin bileşenleri, bulundukları ekosistem ve çevre şartlarına göre değişiklik göstermesi nedeniyle standardize edilememiştir. Fakat RJ içeriğinde bulunan ve diğer doğal ürünlerde bulunmayan 10-HDA molekülü kalite belirteci olarak kullanılıp miktarı %0.75 ile %3.39 arasında değişmektedir. RJ obezite

ve getirdiği sorunların çözümü konusunda birçok araştırmacı tarafından ilgi çekici bulunmuştur. Özellikle insülin benzeri aktivite gösteren biyoaktif maddeleri içeriğinde bulundurması, antioksidan ve antiinflamatuar aktiviteye sahip olması, östrojen sinyal yolağında rol oynaması gibi potansiyelleri nedeniyle yardımcı ve tamamlayıcı tedaviler için umut vadetmektedir. Bu çalışmada RJ ve 10-HDA'nın obezite ve beraberinde getirdiği sorunları çözmedeki potansiyelini konu alan çalışmalar incelenmiştir.

Tartışma: RJ içeriğinde genellikle %1,5 mineral, %7-21,2 karbonhidrat, %8-9 protein ve çeşitli karboksilik asitler, vitaminler ve serbest aminoasitler bulundurmaktadır. Bunlara ek olarak çeşitli polifenoller ve flavonoidler bulundurur. Polifenoller, flavonoidler ve serbest aminoasitler araştırmacılar tarafından antioksidan aktiviteyle ilişkili bulunmuştur. İçeriğindeki yağ asitlerinden 10-HDA antibakteriyel, antiinflamatuar, hücre immün aktivite düzenleyici role sahip olduğu bulunmuştur. RJ'nin hücre büyümesinde, farklılaşmasında, hayatta kalımında etkili ve insülin benzeri aktiviteye sahip olduğu çeşitli çalışmalarla gösterilmiştir. RJ, günlük toplam enerji alımını düşürmüştür, yaşa bağlı kas zayıflığını azaltmıştır. RJ KK-Ay (KK-Ay fareleri: diyabetik KK ve lethal yellow (Ay) farelerinin melezi. değiştirilmiş adipokin ekspresyonu, obezite, dislipidemi ve insülin direnci gösteren özel farelerdir.) farelerinde kısmen vücut ağırlığını düşürmüştür, hiperglisemiyi iyileştirmiştir. Yaşlı obez sıçanlarda RJ takviyesi kullanımı kas lipotoksisitesine karşı koruyucu olup, insülin direncini azaltmıştır. Ek olarak di yete bağlı obezitenin etkilerini ve kas iskelet fonksiyonlarını iyileştirdiği bildirilmiştir. 10-HDA, 3 T3-L1 adiposit hücrelerinde adipojenik aktivite göstermiştir. Asemptomatik aşırı kilolu yetişkinlerde RJ takviyesi lipit profili, tokluk, iltihaplanma ve antioksidan kapasitesi üzerindeki olumlu etkiler göstermiştir. RJ sıçanlarda oksidatif stresi inhibe etmiştir, serum lipit profilindeki olumsuzluklar, karaciğerdeki lipit birikimi, lipit peroksidasyonu ve sirkadyen gen ifadesindeki bozuklukları gibi olumsuzlukları iyileştirmiştir. Tüm bu bilgiler RJ'nin obezite ve beraberinde getirdiği metabolik problemlerin çözümünde etkili olabileceğini göstermektedir fakat RJ'nin standardizasyonu ile ilgili belirsizlikler ve örneklem sayısının azlığı daha çok araştırma sonuçlarıyla desteklenmesi gerektiğini göstermektedir.

Sonuç: Sonuç olarak RJ'nin obezite ve metabolik bozukluklarla mücadele için umut verici olduğu, obezite için terapötik ilaç olma potansiyelinin bulunduğunu, obeziteyi azaltmak için yeni bir seçenek olarak diyete eklenebileceğini, yağ dokusu disfonksiyonuna ve inflamasyona karşı koruyucu etkilerinin bulunduğunu fakat daha çok çalışmayla desteklenmesi gerektiğini, obeziteyle ilgili komplikasyonları önleme ve olumsuz etkilerden koruma potansiyelinin bulunduğunu göstermektedir. Tüm bu nedenlerden dolayı terapötik olarak ve bu hastalığı önleme için umut vadeden fonksiyonel bir gıda olduğu düşünülmektedir. Daha geniş örneklem kitlesiyle sonuçlar desteklenmelidir.

INTRODUCTION

This review is about the studies on obesity and the possible prevention and improvement of obesity with royal jelly (RJ). Bee products and the RJ in these products have been a source of healing for people since ancient times (Pasupuleti et al., 2017). Nowadays, bee products and other similar natural foods are used as auxiliary, complementary, and supportive products in the prevention of various diseases and the treatment of diseases. Various studies have shown that people improve their survival and quality of life. Longevity and procreation, which are thought to be provided by RJ to the queen bee, are the subjects of much research (Kunugi & Mohammed Ali, 2019). Apart from these, one of the benefits that should be taken into account is the prevention of obesity and increasing the quality of life by mitigating the consequences of obesity. This study, in which current publications are compiled to draw attention to this issue, emphasizes that RJ is very important and remarkable in obesity.

Obesity

Obesity, which causes various health problems and reduces the quality of life; is one of the leading problems in the world. Obesity is defined as excessive fat accumulation or abnormal distribution of fat in the body (Li et al., 2020). If an adult's body mass index is more than 30, it is considered obese, and increasing obesity is a risk factor for many fatal diseases (Thomas, 2020). According to WHO data, approximately 13% of the world's population was obese in 2016, and the prevalence has tripled compared to 1975. Obesity is caused by both genetic and environmental factors, and WHO states that obesity can be prevented. To prevent it, easy regulations such as limiting energy intake from fat and sugar, increasing fruit and vegetable

consumption, increasing physical activity, and choosing healthy diets are sufficient.

Many physiological problems, such as obesity, impaired glucose tolerance, and hyperglycemia, are metabolic syndromes resulting from disturbances in the maintenance of glucose homeostasis (Pandey & Rizvi, 2009). These syndromes are caused by disorders in diet and sedentary lifestyle, genetic and environmental factors and insulin resistance develops (Manach et al., 2004; Sun et al., 2015). This situation, which develops with the deterioration of glucose metabolism and insulin resistance, causes increased oxidative stress and inflammation in the body, and then muscle and adipose tissue, liver, etc. causes dysfunction of many organs (Knekt et al., 2002). An important reason for the disorder in glucose metabolism is the consumption of a high glycemic diet, which causes rapid absorption of glucose, which increases the risk of obesity (Yarmolinsky et al., 2015). In addition, inflammation throughout the body is effective in the development of obesity by increasing the development of free oxygen species and oxidative stress (Alvehus et al.). The antioxidant activity ability, which is generally attributed to polyphenols and flavonoids, has been proven in various studies to be protective from the damage of free oxygen species and free radicals (Lin et al., 2002).

Royal Jelly (RJ)

Named by the Swiss botanist Francois Hubber in 1788, RJ is secreted from the hypopharyngeal and mandibular glands of young bees and is a white/yellow viscous, acidic substance (Crane, 1990; Willson, 1955). The secretion secreted by young worker bees to feed the queen for life and the immature females for the first 3 days of their lives is considered responsible for the differentiation of the queen bee (Kanelis et al., 2018).

RJ is an aqueous emulsion of lipid, protein, sugar, free amino acids, and vitamins. It contains about 1.5% mineral salts (Fe, Zn, Na, K, Ca, Mn, Cu) and small amounts of vitamins (B1, B2, B3, B5, B7, folic acid, inositol, and vitamin E), polyphenols, flavonoids contains. The flavonides reported to be present in RJ are: herperetin, isosacuranetin and naringenin flavanones; acetin, luteolin, apigenin, and crisis glucoside flavones; kaempferol and isorhamnetin are the glucoside flavonols, and coumestrol and genistein are isoflavonoids. It has been demonstrated that the water content of RJ varies between 50% and 70%. It has been shown that the sugar composition, mainly consisting of fructose and glucose, varies between 7% and 21.2%. Apart from this, there are also studies reporting the presence of sucrose, trehalose, maltose, gentiobiose, isomaltose, raffinose, erlos, and hybridize oligosaccharides. Major RJ proteins (MRJP) make up 90% of the protein content of RJ. The free amino acid composition, which is also associated with antioxidant activity, has been reported as alanine, aspartic acid, phenylalanine, glycine, glutamic acid, glutamine, hydroxyproline, isoleucine, lysine, leucine, proline, serine, cysteine, cystine, tyrosine, threonine, valine(Isidorov et al., 2012). It is estimated that the total content of fat and fatty acids in RJ is in the range of 7-18%. 80-85% of RJ fatty acids and the main component of the sieve residue are lipids. The most important of the fatty acids contained in RJ and what makes it special is 10-hydroxydecanoic acid (10-HDA), which is not found in any other natural raw material, and its content varies between 0.75% and 3.39%. In addition, 10-HDA is used to determine the product quality index of RJ (Ramadan & Al-Ghamdi, 2012).

RJ fatty acids exist in solid crystal form at room temperature, the melting point of 10-HDA is 52ºC (Barker et al., 1959). 10-HDA is antibacterial (Fratini et al., 2016), anti-inflammatory (Chen et al., 2018), and cell immune activity regulator (Mihajlovic et al., 2013). Apart from 10-HDA, there are short hydroxy fatty acids and dicarboxylic acids with 8-12 carbon atoms, and 10-hydroxy-2-decenoic acid ($10H_2DA$) and sebacic acid (Isidorov et al., 2012).

RJ; It contains bioactive substances that are effective in cell growth, differentiation, survival and show insulin-like activity proven by various *in vivo* and *in vitro* studies (Dixit & Patel, 1964; Kramer et al., 1977). RJ can activate the estrogen receptor (ER) and play an active role in the estrogen signaling pathway (Moutsatsou et al., 2010; Suzuki et al., 2008). Royalactin, one of the proteins in the RJ content, was found to be effective in the differentiation of the queen bee (Kamakura, 2011).

Table-1. Ingredients in royal jelly

Tablo-1. Arı sütü içeriğindeki bileşenler

Fat and fatty acids	10-hydroxy decanoic acid (10-HDA) 10-hydroxy-2-decenoic acid (10-H_2DA) Sebacic acid (SA)
Free amino acids	Alanine, aspartic acid, phenylalanine, glycine, glutamic acid, glutamine, hydroxyproline, isoleucine, lysine, leucine, proline, serine, cysteine, cystine, tyrosine, threonine, valine
Saccharides	Fructose, glucose, sucrose, trehalose, maltose, gentiobiose, isomaltose, raffinose, erlose and hybridose
Proteins	Major royal jelly proteins (MRJP), royalsin
Vitamins	B1, B2, B3, B5, B7, folic acid, inositol and vitamin E
Mineral salts	Salts of iron, zinc, copper, sodium, potassium, calcium, manganese
Polyphenols	Herperetin, isosacuranetin, naringenin, akacetin, apigenin, crisis, luteolin, isorhamnetin, kaempferol, coumestrol, genistein

Investigation of the Effects of Royal Jelly on Obesity

In an eight-week study investigating the effect of RJ (1000 mg/day) on body weight and daily energy and macronutrient intake in fifty women (25 RJ, 25 placeboes) volunteers with type 2 diabetes, the body mass index of the volunteers was calculated before and after the administration. As a result, it has been reported that RJ application reduces average body weight and average daily total energy intake. It can prevent age-related muscle wasting (Ali & Kunugi, 2020) and researchers state that RJ has the potential to be beneficial in the weight management of diabetic patients (Pourmoradian et al., 2012).

Administration of 10 mg/kg RJ for 4 weeks in KK-Ay mice with type 2 diabetes has been shown to improve hyperglycemia. The results of the study; hyperglycemia was improved, insulin resistance did not develop, and glucose 6-phosphatase mRNA expression was suppressed in the liver. Induced expression of adiponectin (AdipoQ) in abdominal fat. Expression of pAMPK, which suppresses glucose 6-phosphatase level, and adiponectin receptor-1 expression were induced in the liver of KK-Ay mice. As a result, the researchers reported that they improved hyperglycemia and partially reduced body weight in obese and diabetic KK-Ay mice. In addition, it has been suggested that activation of AMPK after activation of AdipoQ and AdioR1 expressions in the liver results in suppression of glucose 6-phosphatase expression (Yoshida et al., 2017).

In a study investigating the protective effects of RJ on skeletal muscle and adipose tissue metabolism and inflammation as a result of a high-fat diet in aged obese rats, the researchers concluded that the use of RJ suphybridizemay be protective against muscle lipotoxicity and reduces insulin resistance. In the study, 40 male rats were divided into 5 groups. The first group consisted of young rats and was fed a standard diet (Grup A). The other 4 groups consist of old rats. The first of these 4 groups were fed a standard diet (Grup B). The second group was fed with RJ only (Grup C). The third group was exposed to a high-fat diet (Grup D). Finally, the fourth group was both exposed to a high-fat diet and fed with RJ (Grup E). All diets lasted 8 weeks. From the results of the study, the order of body weight gain is A>D>E>B>C and the order of abdominal fat weight is D>E>B>C>A. The order of increase in tibialis anterior muscle weight and hind limb muscles weight is B>E>D in line with only the statistically significant data reported. Serum triglycerides and total cholesterol levels were higher in the old group of normally fed rats (B>A). Serum triglycerides and total cholesterol levels, which were found to be high with a high-fat diet in old rats, decreased in those receiving RJ supplementation (D>E>C). While the level of muscle triglycerides was higher in the old and high-fat diet group compared to the young group

or the group fed with a normal diet, it was found to be lower in the RJ-fed groups (D>E>B>C>A). In the evaluation of the amount of insulin in the serum, it was highest in the old and high-fat diet group, while it was almost as low in the old fed both high-fat diet and RJ group as in normally fed rats (D>E). TNFR1 (Tumor necrosis factor-alpha receptor 1) expression is found quite high in serum and adipose tissue in rats fed a high-fat diet (D) (respectively 1952.7±106.4; 3385.94±140.61 pg/ml). In young normally fed rats (A), 1558.7±75.01; 2117.41±140.94 pg/ml. In the old group fed with both a high-fat diet and RJ, 1615.12±123.5; 2809.41±132.1pg/ml. As a result, serum and adipose TNFR1 levels were found to be lower as a result of diets in which RJ was added. Therefore, researchers report that feeding with RJ reduced adiposity, decreased TNFR1 expression, improved lipid profile, and insulin resistance. In addition, musculoskeletal functions improved in older rats (Metwally Ibrahim & Kosba, 2018).

RJ has been the subject of research on the prevention of obesity and metabolic disorders because it increases insulin sensitivity. About this, in mouse models investigating the anti-obesity effect of RJ, C57BL/6J mice were exposed to four different feeding conditions for 17 weeks. The first of these is a normal diet, the second is a high-fat diet, the third is a 5% RJ and high-fat diet, and finally the fourth is a 5% honey bee larva powder. The data obtained by the researchers as a result of the study: RJ suppressed the formation of white adipose tissue and hepatic triglyceride accumulation caused by a high-fat diet, and improved hyperglycemia and insulin resistance. In the group with RJ in their diet, thermogenic uncoupling protein 1 (UCP1) and mitochondrial cytochrome c oxidase 4 subunit (COX-4) gene and protein expressions were increased in brown adipose tissue (BAT). It has been demonstrated that the use of RJ and honey bee larva powder does not brown the white adipose tissue. As a result, it has been reported that RJ is promising for combating obesity and metabolic disorders (Yoneshiro et al., 2018).

In the study investigating the antiadipogenic activity of RJ, researchers demonstrated that adipogenic transcription factors and leptin were decreased by 10-HDA, triacylglycerol accumulation, and reactive oxygen species were suppressed in 3 T3-L1 adipocyte cells. 10-HDA, one of the most important compounds of RJ, inhibits the cyclic adenosine monophosphate/ Protein kinase A (cAMP/PKA) pathway and activates insulin-dependent mitogen with phosphorylated Akt (p-Akt). inhibited the protein kinase (MAPK) pathway. This adipogenic activity provides the potential to be a therapeutic drug for obesity (Pandeya et al., 2019).

In a study investigating the lipid profile, satiety, inflammation, and antioxidant capacity of RJ in asymptomatic overweight adults, thirty humans were given lyophilized RJ (330 mg/capsule, two capsules daily before breakfast) supplementation for eight weeks and thirty humans were exposed to placebo. According to the results compared with placebo, RJ supplementation provided a significant decrease in cholesterol and C-reactive protein (CRP) levels. Serum total antioxidant capacity, adiponectin, bilirubin, and uric acid showed a significant increase. As a result, researchers state that RJ has been beneficial in improving human health with its positive effects on the lipid profile, satiety, inflammation, and antioxidant capacity of overweight adults (Petelin et al., 2019).

RJ inhibited oxidative stress in obese and ovariectomized rats with non-alcoholic fatty liver disease. In addition, it was concluded that Per1, Per2 (Period circadian clock 1, 2) modulate the expression of periodic circadian genes and alleviates non-alcoholic fatty liver disease. Nonalcoholic fatty liver disease is common in postmenopausal women. In this study, a non-alcoholic fatty liver disease model was created in ovariectomized rats and 150, 300, and 450 mg/kg RJ was administered intragastrically daily for 8 weeks. After the administration, weekly weight weighing and elevated plus maze (EPM) test were applied, together with the examination of blood, liver, brain, and uterus samples. As a result, researchers report that RJ can ameliorate the negative effects on serum lipid profile caused by estrogen deficiency, lipid accumulation in the liver, lipid peroxidation, and disturbances in circadian gene expression (You et al., 2020).

There is a study in which RJ and tocotrienol-rich fractions were applied in the obesity treatment of 50 obese Wistar rats with calorie restriction. In this study, BAT activation, browning, and thermogenic capacity were investigated. Rats were divided into five groups and exposed to one of the diet types of a high-fat diet, calorie restriction, RJ + calorie restriction, tocotrienol + calorie restriction, RJ + tocotrienol + calorie restriction for 8 weeks.

Table-2 Results table

Tablo-2 Sonuçlar tablosu

Results	References
RJ inhibited oxidative stress It attenuated nonalcoholic fatty liver disease by regulating Per1 and Per2 expression.	You et al., 2020.
RJ plays a regulatory role in metabolism through the irisin protein. RJ and tocotrienol consumption decreased developmental and inflammatory parameters	Irandoost et al., 2020
The daily energy intake to the environment has been reduced. Contributed to the reduction of body weight. It prevents age-related muscle weakening.	Ali & Kunugi, 2020 Pourmoradian et al., 2012
Diets with RJ and RJ and tocotrienol significantly reduced weight gain compared to the calorie-restricted diet alone. UCP1, PRDM16, CREB1, P38MAPK, and BMP8B expressions were increased.	Mesri Alamdari et al., 2020
RJ reduced ER stress. RJ reduced the expression of GRP-78 in the hypothalamus and white adipose tissue compared to calorie restriction, and the expression of inflammatory markers in white adipose tissue.	Irandoost et al., 2020
A significant decrease was observed in cholesterol and CRP levels. A significant increase was observed in adiponectin, bilirubin, uric acid, and total antioxidant capacity in serum.	Petelin et al., 2019
It has been shown that adipogenic transcription factors and leptin are decreased by 10-HDA in 3 T3-L1 cells. 10-HDA suppressed Triacylglycerol accumulation and reactive oxygen species. 10-HDA inhibited the cAMP/PKA pathway. 10-HDA inhibited p-Akt and insulin-dependent MAPK pathway.	Pandeya et al., 2019
It has been reported to be protective against muscle lipotoxicity. Muscle fat is reduced TNFR1 expression decreased It has improved the lipid profile. Improvement was observed in insulin resistance.	Metwally Ibrahim & Kosba, 2018
White adipose tissue formation is reduced. Hepatic triglyceride accumulation is suppressed. UCP1 expression is increased in brown adipose tissue. Mitochondrial COX-4 expression is increased.	Yoneshiro et al., 2018
It showed a positive effect on hyperglycemia. Induced AdipoQ expression in abdominal fat In the liver of KK-Ay mice, pAMPK expression, which suppresses glucose 6-phosphatase level, and adiponectin receptor-1 expression were induced. It has been reported to partially reduce body weight.	Yoshida et al., 2017

Researchers have stated that diets containing RJ and RJ and tocotrienol provide a significant reduction in weight gain compared to a diet with only calorie restriction. As a result of the study, it was observed that the expression of uncoupling protein 1 (UCP1) gene, PR domain genes 16 (PRDM16), cAMP response element-binding protein1 (CREB1), P38 mitogen-activated protein kinases (P38MAPK), and Bone morphogenetic protein8B (BMP8B) increased significantly. In addition, it was stated that no significant changes were observed in CCAAT/enhancer-binding protein beta (CEBPβ) and Bone morphogenetic protein7 (BMP7) gene expressions. When all the results were evaluated, it was reported that RJ supports white adipose tissue thermogenesis and browning. In addition, it has been stated that it can be added to the diet as a new option to reduce obesity (Mesri Alamdari et al., 2020).

Irisin protein in RJ may be effective on obesity-associated inflammation and glucose intolerance. Forty-five male Wistar rats exposed to 40 high-fat diets and 5 normal diets were used in the study. In the high-fat-fed group, the obese model was provided at the end of 17 weeks, followed by exposure to RJ and tocotrienol for eight weeks. In this group, rats were randomly divided into 4 groups. These are the group fed with 100mg/kg RJ, the group fed with 85mg/kg tocotrienol, the group fed with 100mg/kg RJ and 85mg/kg tocotrienol, and the last group is the group that continues only the high-fat diet as a control. As a result of the examinations, the researchers stated that there was no change in body weight in the group fed with RJ and tocotrienol, but developmental and inflammatory parameters decreased. This shows that it can prevent metabolic disorders caused by obesity. Researchers state that RJ and tocotrienol consumption can promote healthy obesity (Irandoost, Mesri Alamdari, Saidpour, Shidfar, Roshanravan, et al., 2020).

Similarly, as a result of investigating the curative effect of RJ with a tocotrienol-rich fraction on inflammation caused by endoplasmic reticulum stress, it is stated that RJ can prevent some obesity-related disorders by reducing endoplasmic reticulum stress. Then, adipose tissues and the hypothalamus were examined for inflammation. RJ has been shown to reduce the expression of glucose-regulated protein-78 (GRP-78) and inflammation markers in white adipose tissue compared to calorie restriction in the hypothalamus and white adipose tissue. Tocotrienol-rich fractions reduced serum inflammatory markers without significant effect on endoplasmic reticulum stress. Researchers state that RJ has protective effects against adipose tissue dysfunction and inflammation, prevents some obesity-related complications, and therefore is promising therapeutically (Irandoost, Mesri Alamdari, Saidpour, Shidfar, Farsi, et al., 2020).

RJ has been successful in dealing with obesity and its various problems. But the sample should be expanded. In addition to these studies, the results should be strengthened with animal and human experiments. Although the mechanisms of success in preventing obesity and various metabolic disorders are tried to be revealed in all aspects, there are many aspects to be clarified (Table-2).

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DERLEME / REVIEW

SIGNIFICANCE OF HYDROXYMETHYLFURFURAL AND MELANOIDINS AS PRODUCTS OF MAILLARD REACTIONS IN HONEY

Baldaki Maillard Reaksiyonlarının Ürünleri Olarak Hidroksimetilfurfural ve Melanoidinlerin Önemi

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ABSTRACT

Honey presents exceptionally favorable conditions for a non-enzymatic glycation of proteins or Maillard reaction (MR), which is a complex network of chemical reactions which is favored during processing and storage and that often influence the quality and acceptability of honey. One of the organic compounds produced in the intermediate stages of MR that has been the subject of several investigations and controversies, due to its relationship with adverse effects on human health, is 5-hydroxymethylfurfural (HMF), which has become an indicator of honey quality. Conversely melanoidins, polymeric molecules responsible for non-enzymatic browning and which have been related to beneficial effects due to the antioxidant and antibacterial properties of honey, are produced in the final stages of MR. The aim of this article is to provide a review on the formation as well as the positive and negative effects associated with the formation of HMF and melanoidins as MR products in honey.

Keywords: Honey, Maillard reaction, Hydroxymethylfurfural, Melanoidins

ÖZ

Bal, proteinlerin enzimatik olmayan glikasyonu veya işleme ve depolama sırasında tercih edilen ve genellikle balın kalitesini ve kabul edilebilirliğini etkileyen karmaşık bir kimyasal reaksiyonlar ağı olan Maillard reaksiyonu (MR) için son derece uygun koşullar sunar. İnsan sağlığı üzerindeki olumsuz etkileri nedeniyle birçok araştırma ve tartışmaya konu olan MR'ın ara aşamalarında üretilen organik bileşiklerden biri de bal kalitesinin bir göstergesi haline gelen 5-hidroksimetilfurfural (5-HMF)'dir. Tersine, enzimatik olmayan esmerleşmeden sorumlu olan ve balın antioksidan ve antibakteriyel özelliklerinden dolayı faydalı etkileri ile ilişkilendirilen polimerik moleküller olan melanoidinler, MR'ın son aşamalarında üretilir. Bu makalenin amacı, balda MR ürünleri olarak HMF ve melanoidinlerin oluşumu ile ilgili olumlu ve olumsuz etkilerinin yanı sıra oluşumu hakkında bir inceleme sunmaktır.

Anahtar Kelimeler: Bal, Maillard reaksiyonu, Hidroksimetilfurfural, Melanoidinle

GENIŞLETILMIŞ ÖZET

Amaç: Bu makalenin amacı, balda MR ürünleri olarak HMF ve melanoidinlerin oluşumu ile ilgili olumlu ve olumsuz etkilerinin yanı sıra oluşumu hakkında bir inceleme sunmaktır.

Google Scholar, PubMed ve ScienceDirect gibi sunuculardaki tam erişim sağlanan bilimsel makalelerde bu bilgilere başvurulmuştur. Dahil edilen anahtar kelimeler şunlardır: tatlı, Maillard reaksiyonu; hidroksimetilfurfural; melanoidinler.

Giriş: Gıda endüstrisi, balın hidrasyon, viskozite, aroma, renk, higroskopiklik, karışabilirlik ve yayılabilirlik gibi bazı özellikleri desteklediği gözlemlendiğinden birçok farklı gıda ürününde bal kullanmaktadır (Otles, 2006). Ancak bal, Maillard reaksiyonu (MR) için son derece elverişli koşullar sunar. MR, genellikle gıda işleme veya depolama sırasında meydana gelen karmaşık bir kimyasal reaksiyonlar ağıdır (Martins ve diğerleri 2001). MR sırasında oluşan geniş ürün yelpazesi, gıdanın kalitesini ve tüketiciler tarafından kabulünü etkileyen bir öneme sahiptir (Martins ve diğerleri 2001, Bertrand ve diğerleri 2018). Organoleptik özelliklerde (aroma ve pigment oluşumu), protein işlevselliğinde ve sindirilebilirlikteki (Machiels & Istasse 2002, Lund & Ray 2017) değişikliklerin yanı sıra, MR ürünlerinin anti/pro-oksidan potansiyeli açısından sağlık üzerinde olumlu veya olumsuz etkileri olabilir. İmmünojenisite, alerjenite ve kanserojenite (Bertrand ve ark. 2018). Bu nedenle, bu reaksiyon gıda kimyasında en önemli olarak kabul edilir (Machiels & Istasse, 2002).

Tartışma: Balda yüksek konsantrasyonda indirgeyici şekerler, glukoz ve fruktoz ve proteinlerin ve serbest amino asitlerin (özellikle lizin) varlığı, depolama sırasında ve bazı bal işleme adımlarında meydana gelen MR (Türkmen ve ark. 2006) için uygun koşullardır (Türkmen ve ark. 2006). Isıya maruz kalmayı gerektiren ve nihai ürünün daha homojen bir sunumunu sağlamak (Blidi ve ark. 2017), viskoziteyi azaltmak ve paketleme sürecini kolaylaştırmak (Chua ve ark. 2014), kristalleşmeyi önlemek (Turhan ve ark. 2008) için tanıtılmıştır Escriche ve diğerleri 2009). Ayrıca ozmofilik mayaların yok edilmesi yoluyla fermantasyonun engellenmesi ve dolayısı ile (Subramanian ve diğerleri. 2007) raf ömrünü uzatılır (Guo ve diğerleri 2011). Ancak ısıtma, HMF ve diğer bileşiklerin konsantrasyonlarını artırarak bal kalitesini doğrudan etkileyebilecek (Chua ve ark. 2014) bir işlemdir (Annapoorani ve ark. 2010).

Sonuç: Çok sayıda reaksiyon yolu ve ürünü ile MR hala araştırma gerektirmektedir. Balın üretiminde ve sanayileşmesinde arılarda gözlemlenenin aksine balın insan sağlığına potansiyel olarak zararlı bir gıda haline getiren HMF konsantrasyonları hakkında bilgi bulunmamakla birlikte, balın kalitesini mümkün olduğu kadar uzun süre korumak amaçlanmaktadır. Toksik maddelerin üretimini önlemek ve balın antioksidan ve antibakteriyel özelliklerini güçlendiren arzu edilen bileşenleri oluşturmak yerine MR'nin oluşmasını önlemek veya yavaşlatmak. Melanoidinlerle ilgili olarak baldaki oluşumları hakkında çok az bilgi vardır. Bu nedenle hasat sonrası ısıl işlemlerin ve depolamanın bu bileşiklerin bileşimi üzerindeki etkilerini ve bunların balın besin değeri ve fonksiyonel özellikleri üzerindeki etkilerini belirlemek için daha fazla araştırmaya ihtiyaç vardır.

INTRODUCTION

Maillard reaction: The origin

MR is a non-enzymatic browning reaction involving proteins and reducing sugars (Bertrand et al. 2018); where temperature, reaction time, water activity (Aw), pH, concentration and nature of the reagents in the food are important factors to consider (van Boekel, 2001).

MR can be divided into three main stages. The first corresponds to the reversible formation of a glucosylamine and its Amadori or Heyns rearrangements. In the second, degradation of the Amadori and Heyns rearrangement products occurs, leading to the formation of aromatic heterocyclic compounds. In the third, polymerization of the reactive intermediates and production of melanoidins is observed (Machiels & Istasse 2002, Silvan et al. 2011). All stages are interrelated, can occur simultaneously and are affected by reaction conditions (Silvan et al., 2011).

Although MR was first described by Louis Maillard in 1912, a first coherent scheme was presented by Hodge in 1953 (Echavarría et al. 2012, De Oliveira et al. 2016). However, to facilitate its understanding, a modification of the scheme proposed by Zamora & Hidalgo (2005) is presented here in Figure 1.

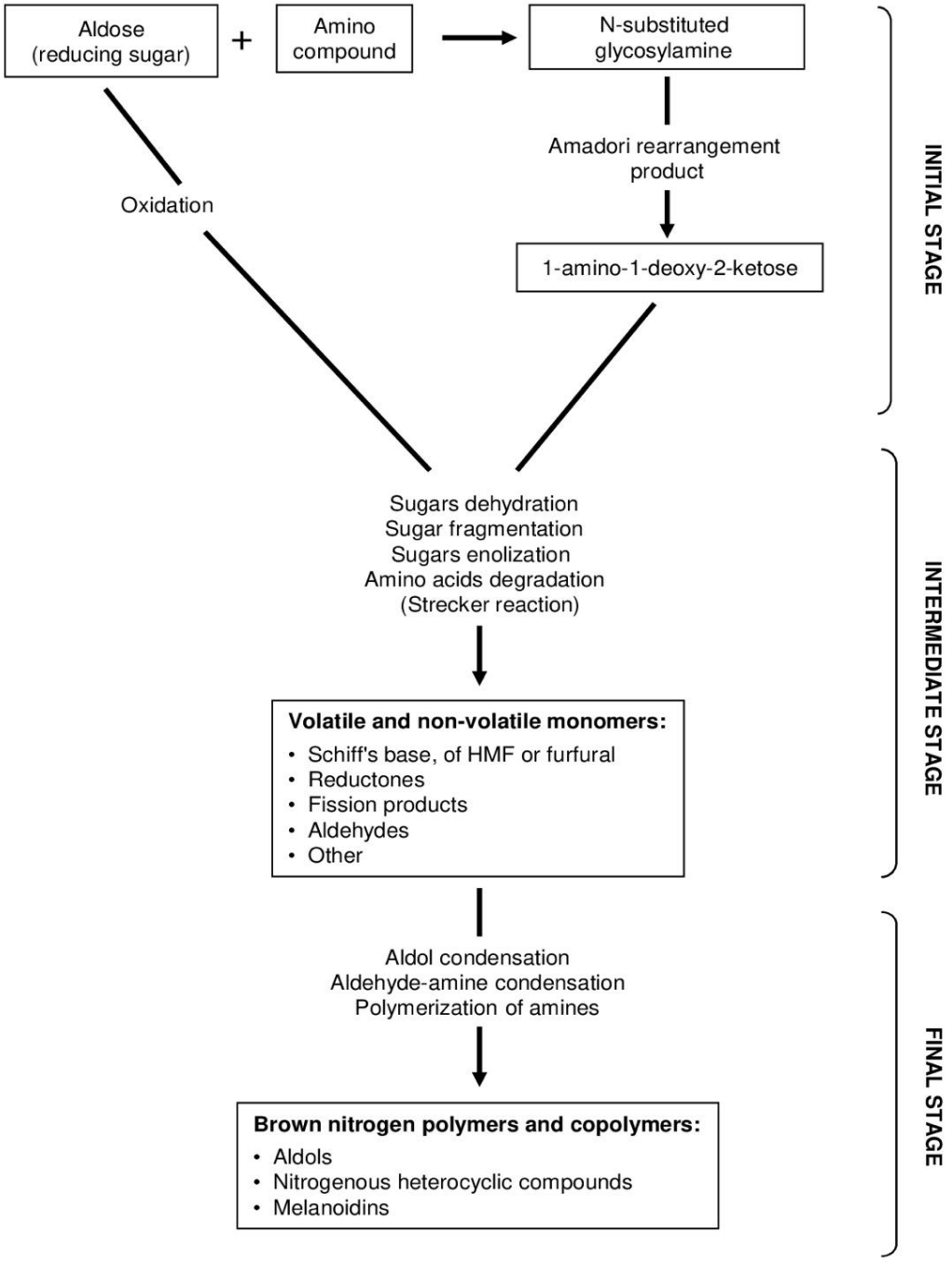

Figure. 1. General mechanism of Maillard reaction

First stage of MR is characterized by an initial glycosylation reaction (De Oliveira et al. 2016), a reaction that occurs when functional amino groups of free amino acids or amino groups of side chains of peptides and proteins condense with carbonyl groups of reducing sugars to form a Schiff base (Bertrand et al. 2018), with the release of a water molecule (De Oliveira et al. 2016). Schiff base obtained is in equilibrium with an N-glycosylamine, which will undergo a different rearrangement depending on the nature of the initial reducing sugar involved. If this is an aldose it will give rise to an aldosamine and then to the Amadori compound, a 1-amino-1-deoxyketose; whereas a ketose will give rise to a ketosamine and then to the Heyns compound, a 2-amino-2-deoxyketose (Echavarría et al. 2012, Bertrand et al. 2018). This second reaction is irreversible and obtaining the Amadori and Heyns intermediates marks the end of the first MR stage (Bertrand et al. 2018), where no color changes are observed (Martins et al. 2001, Echavarría et al. 2012;) and the nutritional value may be reduced due to decreased amino acid availability (De Oliveira et al. 2016).

Second MR or propagation stage depends on the reaction conditions, such as pH, temperature and Aw (Bertrand et al. 2018). With respect to a system such as honey, the Amadori product degrades producing mainly a 1, 2-enolysis with the formation of furfural (when dealing with pentoses) or hydroxymethylfurfural (HMF) (when dealing with hexoses) (Martins et al. 2001). All these compounds are highly reactive, resulting in the carbonyl groups being able to condense with free amino groups, leading to the incorporation of nitrogen into the reaction products, while the dicarbonyl compounds react with the amino acids to form aldehydes and aminoketones. This reaction is known as Strecker degradation (Martins et al. 2001). Bertrand et al. (2018) concluded that Strecker degradation plays an important role not only in the generation of aromatic properties of the product but also in the browning itself.

Finally, in the final stage of MR, condensation occurs between some of the formed products or with amino compounds to form brown pigments and polymers (Zamora & Hidalgo 2005). Meanwhile, amino acids could react with the unsaturated carbonyl molecules resulting in the formation of melanoidins (Bertrand et al. 2018).

Hydroxymethylfurfural, really harmful?

5-Hydroxymethyl-2-furaldehyde or, more commonly named, 5-hydroxymethylfurfural, consists of a furan ring containing functional groups: aldehyde and alcohol (Zirbes et al. 2013) and is formed at room temperature by dehydration of fructose in acidic media (Huidobro & Simal 1984).

Since the 1950s, the presence of HMF has been identified in a wide variety of thermally processed foods, and depending on the production technology and storage, levels in foods vary considerably (Abraham et al. 2011).

HMF formation in honey occurs naturally (Sanz & Sanz 1994) because the pH is set between 3.2 and 4.5 for *Apis mellifera* (Karabagias et al. 2014) and from 2.5 to 3.8 for stingless bee honeys (Nordin et al. 2018), which is accelerated if the honey has been heated or stored at high temperatures (Sanz & Sanz 1994). Thus, the concentration of HMF is in direct relation to the degree of heat or aging of honey (Khalil et al. 2010; Turkut et al. 2018).

From harvesting to packaging, honey can be exposed to a series of conditions that cause, to a greater or lesser extent, the deterioration of its intrinsic qualities (Visquert 2015). HMF concentration is one of the parameters that can be controlled with good practices mainly during storage, where several factors influence its formation such as: the use of metallic containers, the environmental humidity, thermal and/or photochemical stress (Spano et al. 2006).

European Union (EU 2002) and *Codex Alimentarius* have established that the HMF content of *Apis mellifera* honey after harvesting and/or blending should not exceed 40 mg/kg. However, in case of honey of declared origin from tropical countries or regions, as well as blends of these honeys, a maximum of 80 mg/kg is accepted (CXS 12 1981). Although stingless bee honeys are obtained in conditions of high humidity and temperature (Chuttong et al. 2016), Vit et al. (2004) suggested that for these honeys the maximum should be 40 mg/kg, while the Malaysian Standardization Department has established that the maximum HMF content should be 30 mg/kg (Suntiparapop et al. 2012). This is supported by researchers such as Sousa et al. (2016) and Biluca et al. (2016), who referred not to have detected quantifiable HMF concentrations in honeys of the *Melipona* genus from southern Brazil. De Almeida et al. (2013)

reported that low content of HMF in *M. subnitida* honey indicate that the samples were collected and stored under adequate conditions.

This possible resistance to HMF formation in stingless bee honey can be explained by several factors, for example, the type of carbohydrates, higher glucose content instead of fructose, and higher Aw and acidity which slows down MR (Biluca et al. 2014).

Considering the properties of honey, it is evident the need to prolong its shelf life without altering its nutritional and medicinal properties (Biluca et al. 2014). For this purpose, some applied techniques such as dehumidification, heat treatment and refrigeration (Turhan et al. 2008), have been used, being demonstrated that heat treatment has been the simplest and most effective (Biluca et al. 2014), and although the content of high concentrations of HMF in honey is more related to prolonged storage in inadequate conditions than to its heating (Turhan et al. 2008), it is important to establish time and temperature parameters to avoid loss of honey quality not only related to HMF production, but also to the loss of its nutritional (Chua et al. 2014) and bioactive properties such as antioxidant (Šarić et al. 2013) and antimicrobial (Libonnatti et al. 2014).

Souza et al. (2010) reported that *Melipona subnitida* honey subjected to heat treatment at 70°C for 4, 8, 16 and 24 hours caused a reduction in moisture content and total acidity, but increased the content of HMF and reducing sugars. Contrarily, Biluca et al. (2014), reported that HMF concentrations were not detected in 13 honey samples from stingless bees subjected to heat treatment of 75, 85 and 95° C for 20, 40 and 60 seconds, oppositely to what was observed in *A. mellifera* honey when subjected to the same conditions. These results suggest that high temperature associated with a short heat treatment could be an effective way to prolong shelf life without affecting the HMF content in stingless bee honey.

Effect of HMF on honey bee hives

Honey production depends to a large extent on the health, survival and quality of honey bees. Naturally, the main source of carbohydrates for honey bees is the nectar flower collected. However, according to the apibotanical cycle of each region, food and nutrients decrease at certain times of the year. Therefore, beekeepers must use alternative sources to replace the carbohydrate supply of bees, such as sucrose, high fructose corn syrup, fruit sugars or invert sugars (Neupane & Thapa 2005).

In any case, these sources are not safe due to the formation of HMF and its by-products, being a potential threat to bees (Neupane & Thapa 2005). As an example, in Belgium during 2009-2010 abnormal losses of bee colonies were observed and upon further analysis it was found that some of these colonies had been fed over winter with beet invert sugar syrup, which had a HMF concentration of up to 475 mg/kg (Zee & Pisa 2010). In fact, HMF present in bee feeding syrups during critical times could be a new factor involved in bee mortality, coupled with the invasive mite *Varroa destructor* and pathologies caused by the microsporidium *Nosema* spp and viruses (Zirbes et al. 2013).

In the case of *A. mellifera* honey used as prop food, mainly in stingless bee colonies, when this is fresh HMF may be absent or in very low concentrations (Bogdanov et al. 1999) However, temperatures inside a hive normally exceed 20°C and can reach up to more than 40°C, at which time HMF concentration can reach 10 mg/kg honey (Gregorc et al. 2020). And while HMF concentrations <10-15 mg/kg in honey pose little risk to bees, concentrations above 150 mg/kg can cause 50% colony mortality within 16 days to 19 days (Jachimowicz & El Sherbiny 1975) due to induction of lethal ulceration of the intestinal tract (Le Blanc et al., 2009).

And although several studies confirm a toxic effect of HMF on bee health, more research is needed to evaluate the involvement of HMF in their mortality to determine the maximum concentration of HMF in their sustaining food (Zirbes et al. 2013).

Negative effects of HMF on human health

The detection of HMF in several food products prompted the evaluation of potential health risks taking into account dietary intake (Abraham et al. 2011). *In vitro* studies indicate that HMF can be cytotoxic, mutagenic, carcinogenic and genotoxic (Capuano & Fogliano 2011) and thus the importance of controlling its concentrations in foods such as honey. However, most studies report this toxicity only at the preclinical level (Shapla et al. 2018), as presented in **Table 1.**

Table 1. Studies demonstrating the carcinogenic-mutagenic effect of HMF

Associated metabolite	Observed effect	Reference
5-hydroxymethylfurfural	Development of lipomatous renal tumors in rats.	Schoental et al. (1971)
5-hydroxymethylfurfural	Chromosomal aberrations in a Chinese hamster V79-derived cell line constitutively expressing human sulfotransferase SULT1A1 and CYP2E1.	Nishi, Miyakawa & Kato (1989)
5-hydroxymethylfurfural	Induction and promotion of foci of aberrant colon crypts (preneoplastic lesion) as a marker of colon cancer in rats.	Archer et al. (1992) Zhang et al. (1993) Bruce et al. (1993)
5-chloromethyfural	Induction of hepatocarcinoma in male B6C3F1 rats.	Surh et al. (1994)
5-sulfo-oximethylfurfural	Induction of cutaneous papillomas in mice.	Surh & Tannenbaum (1994)
5-sulfo-oximethylfurfural	Mutagenicidad en *Salmonella typhimurium* TA 104.	Lee et al. (1995)
5-hydroxymethylfurfural	DNA damage in five cell lines possessing different levels of SULT1A1 activity (mouse L5178Y, no activity; Chinese hamster: V79-Hp-PST, high activity; V79, negligible activity; human: HEK293, highest activity; and Caco-2, low activity).	Durling, Busk & Hellman (2009)
5-hydroxymethylfurfural 5-sulfo-oximethylfurfural	Increased number of small intestinal adenomas and flat dysplastic lesions (flat ACF) in the large intestine of mice.	Svendsen et al. (2009)
2,5 bishidroximethylfuran	Mutagenicity towards *S. typhimurium* TA100 expressing human SULT1C2	Glatt et al. (2011)
2,5 dimethylfuran	Genotoxicity in rat hematopoietic cells.	Fromowitz et al. (2012)
5- hydroxymethylfurfural	Genotoxicity in hepatic and renal cells of FVB/N (FVB) mice expressing hSULT1A1/1A2.	Høie et. al. (2015)

Although information obtained from bioassays have deduced the toxicity potential of HMF, epidemiological studies or case reports on the possible association of HMF and cancer in humans is insufficient, so in addition is required data to corroborate this activity also *in vivo* (Morales 2008).

As HMF is a product of the non-enzymatic reaction, there is no set concentration in foods, and due to the various factors involved, its content varies even among foods of the same type. Nevertheless, honey is a safe food with respect to HMF concentration when compared to other processed food products that require higher processing temperatures as well as longer times and different additives (Shapla et al. 2018).

As an example, Turhan et al. (2008) demonstrated that nectar honey processed at 95°C for 90 min and at 90°C for 75 min showed HMF levels below 40 mg/kg, while foods such as cookies baked at 300° C can contain up to 1100.1 mg/kg (Ameur et al. 2007); coffee up to 900 mg/kg roasted at 240°C (Murkovic & Bornik 2007); bread between 3.4 to 176.1 mg/kg,

depending on fermentation conditions, leavening agents added, crust and crumb thickness and type of bread (Ramírez et al. 2000).

In addition, it should be considered that, except for countries such as Turkey and Germany with a consumption 1.246 and 1.034 kg *per capita* of honey, the world consumption does not exceed one kilogram (Sanchez et al. 2018), so it is unlikely that honey is a high risk factor to be considered.

Positive effects of HMF on human health

Although the adverse effects of HMF have been studied, some research indicates that, as an antioxidant, the HMF exhibits free radical scavenging capacity as well as significant protective effects on erythrocytes (Zhao et al. 2013) and hepatocytes (Wang et al. 2010) against reactive oxygen species (ROS) induced damage.

Li et al. (2010) demonstrated that HMF could be a potent therapeutic agent against acute mountain sickness, high-altitude cerebral and pulmonary edema (HAPE) by observing that HMF increased mitochondrial membrane potential and decreased phosphorylated ERK levels in human umbilical cord cells. While in a murine model, pre-exposure to HMF significantly attenuated the extent of hypoxia-induced blood-brain barrier (BBB) permeability and decreased the extent of neuronal damage in the CA1 region of the hippocampus.

Yamada et al. (2011) demonstrated that HMF acts as an inhibitor of allergic reactions at different stages, to inhibit basophil and mast cell degranulation, interfering with antigen-antibody cross-linking, antibody-receptor binding and blocking calcium influx in IgE-sensitized cells.

Contrarily to studies demonstrating the carcinogenic-mutagenic effect of HMF, a study by Zhao et al. (2014) using the A375 cell line, indicated that HMF can induce apoptosis and G0/G1 arrest in DNA-damaged cells through the ROS mediated signal transduction pathway.

Regarding the use of HMF for other pathological conditions, it has been observed that HMF exerts an anti-inflammatory effect by decreasing nuclear factor kappa B NFκB activator (Kitts et al. 2012), inhibiting xanthine oxidase activity (Li et al. 2010) thereby decreasing purine catabolism and uric acid production, and thus the risk of hyperuricemia (Hayden & Tyagi 2004).

Tolerable daily intake (TDI) of HMF

HMF is found in many foods, with the estimated intake ranging from 4 to 30 mg per person per day (Abraham et al. 2011).

Many researches have revealed that the susceptibility of cells to HMF depends on the presence and expression levels of receptors, metabolism, structure and enzymatic activity (Shapla et al. 2018). At the preclinical level, no toxic effects have been observed at daily doses ranging from 80 to 100 mg/kg body weight (Zhao et al. 2014). Although the TDI for HMF has been established at 132 mg/day using a 40-fold safety margin and Janzowski et al. (2000) 30-150 mg per person, in a study carried out in Spain with 268 students, a statistically significant level of SMF in plasma was found although the daily intake of HMF of the students throughout the day was 10-70 mg (Pastoriza et al. 2016).

Currently, the European Food Safety Authority (EFSA) has set a threshold of 0.54 mg/day for the intake of furan derivatives used as flavoring agents in Europe (EFSA 2005). However, it should be noted that most of the experiments concerning the health effects of HMF have been conducted *in vitro* and in experimental animals. Therefore, it is not possible to determine a TDI based on the data available to date, which is why further research, especially at the clinical level, needs to be considered and assessed to update the TDI for HMF (Shapla et al. 2018).

HMF mitigation in the honey industry

There is no concrete strategy to mitigate HMF formation in honey due to numerous precursor and types of reaction orders involved. In addition to the fact that HMF is formed following a zero-order kinetic process in an exponential manner (Capuano et al. 2008). However, knowing the positive correlation between elevated temperature-time and acidic pH parameters for HMF formation is that several strategies can be adopted to mitigate its formation (Gökmen et al. 2007). Indeed, since long storage periods lead to elevated HMF formation in honey (Khalil et al. 2010) shortening the storage period as well as decreasing the processing temperature in case honey is subjected to heat treatment can significantly decrease HMF formation (Al-Diab & Jarkas 2015).

Even though more developed methods have been developed in recent years to mitigate HMF formation or to remove it from foods, including ultraviolet

irradiation, addition of phytochemicals, yeast fermentation, vacuum treatment microwave heating, non-thermal processing and formula adjustment (Lee et al. 2019), so far, there are few studies analyzing the effects of these technologies on physicochemical properties and sensory attributes in honey.

What about melanoidins?

Melanoidins are heterogeneous polymers of high molecular weight (5 kDa) that are formed in the final stage of MR (Tagliazucchi & Verzenolli 2014), absorb light at a wavelength of about 420 nm (Lindenmeier et al. 2002), and are largely responsible for the characteristic brown color of foods such as coffee, cocoa, bread or honey (Lindenmeier et al. 2002), which among other physical properties, make foods more palatable to consumers (Friedman 1996).

A critical step for the formation of melanoidins appears to be the degradation of Amadori products, in which several highly reactive intermediate propagators are produced (furans, pyrroles, pyrazines, dicarbonyl compounds) which, through reactions with each other and with free amino groups of amino acids or proteins, ultimately lead to the formation of melanoidin polymers (Van Boekel 1998).

Although at present, researchers have not been able to fully describe the structure of melanoidins (Liu et al. 2020), three main types have been described: the first consisting of furan or pyrrole repeat units (Tressl et al. 1998); the second based on cross-linking of proteins with low molecular weight color compounds (Hofmann 1998); and the third based on sugar degradation products forming polymers by aldol condensation and/or intact carbohydrates (Cämmerer et al. 2002).

Little information has been described on the occurrence and biological activities of melanoidins in honey; however, it appears to be an ideal natural system to study melanoidin formation and its influence on antioxidant activity by containing the main substrates for MR to occur (Brudzynski 2012).

Identification of melanoidins and methods for their quantification are usually based on the following criteria: the degree of browning and color formation after heat treatment, the molecular size of the pigments and their antioxidant activity (Manzocco et al. 2000), observing extremely significant correlations between these parameters in honey (Brudzinsky & Miotto 2011a, Brudzynski & Miotto 2011b, Brudzynski & Miotto 2011c).

Positive effects of melanoidins on human health

In the past, melanoidins were mainly perceived to cause a decrease in the nutritional value of foods, mainly due to the inactivation or destruction of amino acids or proteins or to the reduction of their absorption in the intestine following the inactivation of proteolytic enzymes (Martins et al. 2001, Silván et al. 2006), including trypsin (Ibarz et al. 2009).

Although the undesirable influence of MR end products on food quality, melanoidins show a number of beneficial effects, acting as antioxidant, antimicrobial, antihypertensive, antiallergic and prebiotic agents (Silván et al. 2006, Rufián-Henares & Morales 2007a). Melanoidins also demonstrate the ability to bind metal ions (Rufián-Henares & Pastoriza 2009, Tagliazucchi & Verzenolli 2014) and are considered antimutagenic compounds and tumor growth inhibitors (Langner & Rzeski 2013 as is presented in **Table 2.**

With respect to honey, in several of their investigations Brudzynski & Miotto (2011a, b, c) have demonstrated strong correlations between the concentrations of melanoidins and phenolic compounds and thus with their antioxidant and antibacterial capacity.

To date, the literature is contradictory on the levels of antibacterial activity of honey during storage, as some researchers have reported that exposure of honey to heat or prolonged storage resulted in a loss of antibacterial activity (Soliman et al. 2019), others found no correlation between time and antibacterial activity (Ríos et al. 2001). Therefore, Brudzynski (2012), concludes that the antibacterial and antioxidant activities of honey are influenced by the stages of MR (Brudzynski, 2012), since in the intermediate stage, where the generation of dicarbonyl compounds such as methylglyoxal, an important component in Manuka honey from *Leptospermum* spp. increases the antibacterial activity of honey (Adams et al. 2009, Mavric et al. 2008), in the advanced stage, a decrease in antibacterial activity occurs, associated with increased protein cross-linking, the formation of polyphenol-protein complexes and their incorporation into melanoidins (Brudzynski 2012).

Negative effects of melanoidins on human health

Even though most research has shown that

melanoidins play a crucial role in different biological activities, and have diverse functional properties with potential benefits on human health (Diaz et al. 2020), recent studies have shown that melanoidins may exert a pro-oxidant activity, which may be related to the formation of radicals by a Fenton mechanism due to the presence of iron or copper cations (Ibarz et al. 2009).

Wen *et al.* (2005) observed that the decrease of the antioxidant action based on the metal chelating activity of melanoidin is lost at high temperatures resulting in a gain of the cytotoxic pro-oxidant function.

Table 2. Studies demonstrating some functional properties of melanoidins

Effect	Melanoidins source	Results	Reference
Antimicrobial	Reaction between food protein or glycine and lactose or glucose.	Reduced adhesion and cell density of *H. pylori* in the gastric mucosa of mice.	Hiramoto et al. (2004)
	Red wine	Inhibition of in vitro growth of *L. monocytogenes, Salmonella* Enteritidis and *E. coli.*	Goulas et al. (2018)
Antioxidant	Bread crust	Activity as monofunctional glutathione-S-tranasferase inducers of "pronylated" proteins that are part of melanoidins.	Lindenmeier, Faist & Hofmann (2002)
	Malt barley	Radical scavenging capacity by metamyoglobin assay.	Carvalho et al. (2014)
Antihypertensive	Coffe	Inhibitory activity of angiotensin-I-converting enzyme (ACE) in vitro	Rufián-Henares & Morales (2007b)
	Red wine	Inhibitory activity of angiotensin-I-converting enzyme (ACE) in vitro.	Goulas et al. (2018)
Anti-allergenic	Pigments derived from a reaction between xylose and glycine	Induction of IFN-γ expression in mouse spleen cells and IL-12 in macrophages exposed with ovalbumin as allergen.	Hayase et al. (2005)
Antitumoral	Glucose and L-proline purified	Decreased organization and loss of microtubule integrity in MCF-7 human mammary carcinoma cells.	Marko et al. (2002)
	Soy sauce extract	Significant suppression of cell growth in human colon carcinoma-derived HCT-15 and human gastric carcinoma-derived AGS.	Kamei et al. (1997)
Prebiotic	Bread crust	Selective growth of bifidobacteria from bread crust.	Borrelli & Fogliano (2005)
	Malt barley	Significant divergence in gut microbiota profiles and sustained short-chain fatty acid production in barley malt-fed rats.	Aljahdali et al. (2020)

Also, it has been observed that together with the chelation of Fe and Mg metal ions interfering with bacterial growth and survival (Rufián-Henares & Pastoriza 2009), the antibacterial action of melanoidins may be the result of the inactivation of bacterial proteins due to their binding to semiquinones/quinones, leading to a permanent destruction of cell membranes (Rufián-Henares & Morales 2008). However, it should be noted that these effects have also been observed during plant and seed development affecting the viability of the latter during storage (Narayana Murthy & Sun 2000, Rawel & Rohn 2010). Thus, although this antibacterial effect could be considered beneficial during the therapeutic application of honey in the healing of wounds, the same cytotoxic characteristics towards human cells would pose a potential risk (Majtan 2011).

Therefore, although melanoidins have currently attracted much attention as a functional food ingredient, like polyphenols and other RM-derived products, they could also have dual (beneficial and potentially harmful) functions, depending on the balance (or lack thereof) between prooxidant and antioxidant activities (Brudzynski 2012).

CONCLUSION

MR with its multitude of reaction pathways and products still requires research. Although there is no information on HMF concentrations in honey that make it a potentially harmful food for human health, contrary to what has been observed in bees, in the production and industrialization of honey, the aim is to maintain its quality as long as possible, preventing MR from occurring or slowing it down to avoid the production of toxic substances and instead forming desirable components that potentiate the antioxidant and antibacterial properties of honey. With respect to melanoidins, there is little information on their formation in honey, so more research is needed to determine the effects of post-harvest heat treatments and storage on the composition of these compounds and their impact on the nutritional value and functional properties of honey.

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Conflicting interest

There are no conflicting interests in the realization of the present work.

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