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ULUDAĞ Arıcılık DERGİSİ

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

TÜRKİYE'DE İLLERE GÖRE ARICILIK ETKİNLİĞİNİN VERİ ZARFLAMA ANALİZİ İLE BELİRLENMESİ

The Determination of the Efficiency of Beekeeping by Provinces in Turkey via Data Envelopment Analysis

Duran GÜLER

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ÖZ

Ekolojik dengeye sağladığı katkının yanı sıra kırsal alanda alternatif gelir kaynağı olması arıcılığın önemini artırmaktadır. Bu çalışmanın amacı, Türkiye'deki illerin arıcılık faaliyetindeki etkinliklerinin belirlenmesidir. Çalışmanın ana materyalini 81 ildeki bal ve bal mumu üretimi, arıcılık faaliyeti yapan işletme sayısı ve kovan sayısı verileri oluşturmaktadır. İllerin arıcılık faaliyetindeki etkinliklerini belirlemek amacıyla veri zarflama analizinden yararlanılmıştır. Analizde girdi değişkenleri olarak arıcılık faaliyeti yapan işletme sayısı ve kovan sayısı değerlendirilmiştir. Çıktı değişkenleri ise ilk modelde bal üretimi iken, ikinci modelde bal ve bal mumu üretimi olarak belirlenmiştir. Ordu ili her iki modelde de tam etkinliğe sahip olan iller arasında yer almaktadır. Toplam etkinlik değerleri birinci ve ikinci modelde sırasıyla 0,19 ve 0,30 olarak hesaplanmış olup, bu değerler Türkiye'de arıcılıktaki etkinliğin düşük olduğunu göstermektedir. Bununla birlikte işletme ölçeği büyük ve bal verimi yüksek illerde etkinlik değerinin yüksek olduğu saptanmıştır. Bu nedenle, arıcılıkta üreticilerin kovan sayılarını artırmalarına yönelik teşvik edici politikaların yürütülmesi önem arz etmektedir.

Anahtar Kelimeler: Etkinlik, arıcılık, veri zarflama analizi

ABSTRACT

Besides its contribution to ecological balance, being an alternative source of income in rural areas increases the importance of beekeeping. The aim of this study is the determination of the efficiency of beekeeping by provinces in Turkey. The research data consists of honey production, beeswax production, the number of beekeeping businesses, and the number of hives in 81 provinces. Data envelopment analysis was used to determine the efficiency of beekeeping by provinces. The number of beekeeping businesses and the number of hives were defined as the input variables for analysis. Honey production was defined as the output variable in the first model, and honey production and wax production were defined as the output variables in the second model. Ordu is among the provinces that have full efficiency in both models. Total efficiency values were calculated as 0.19 and 0.30 in the first and second models, respectively. These values show that the efficiency in beekeeping in Turkey is low. However, it has been determined that the efficiency value is high in provinces with large-scale beekeeping businesses and high honey yield. Therefore, it is important to implement incentive policies for beekeepers to increase the number of hives.

Keywords: Efficiency, beekeeping, data envelopment analysis

EXTENDED ABSTRACT

Aim: Beekeeping is very important in terms of contributing to ecological balance, and being an alternative source of income in rural areas. In 2019, Turkey's honey production and beeswax production constitute, respectively, 5.90% and 7.17% of global production. Turkey is the second leading producer of honey in the world. However, the average honey yield in Turkey (13.5 kg) is well below the world average of 20.6 kg. The aim of this study is the determination of the efficiency of beekeeping by provinces in Turkey via data envelopment analysis. The study is expected to benefit policymakers in establishing support models for beekeeping by provinces and regions.

Material and Method: The study used the data of 2020, obtained from the TURKSTAT, on honey production, beeswax production, the number of beekeeping businesses, and the number of hives in 81 provinces. Data envelopment analysis was used to determine of the efficiency of beekeeping by provinces. The number of beekeeping businesses and the number of hives were defined as the input variables for analysis. Honey production was defined as the output variable in the first model, and honey production and wax production were defined as the output variables in the second model.

Results and Discussion: It has been determined that the total efficiency values of 75 provinces in the first model and 69 provinces in the second model are below 0.50. Ordu is among the provinces that have full efficiency in both models. Aydın has full efficiency in the first model, and Bilecik, Kars, Kırşehir, Siirt, Sivas, Şanlıurfa, and Zonguldak have full efficiency in the second model. In the first model, 88.89% of the provinces have increasing returns to scale. In the second model, on the other hand, 80.25% of the provinces have decreasing returns to scale. Total efficiency values were calculated as 0.19 and 0.30 in the first and second models, respectively. These values show that the efficiency in beekeeping in Turkey is low. However, it has been determined that the efficiency value is high in provinces with large-scale beekeeping businesses and high honey yield.

Conclusion: The results show that the scale of beekeeping businesses and honey yield are related to efficiency, and large-scale beekeeping businesses are advantageous in terms of efficiency. Therefore, it is important to implement incentive policies for beekeepers to increase the number of hives. In addition to this, studies on the efficiency of beekeeping businesses in Ordu which is among the provinces that have full efficiency in both models should be carried out, and the results of these studies should be evaluated in terms of increasing the efficiency of other provinces.

GİRİŞ

Arıcılık; bal, bal mumu, arı sütü, polen, propolis gibi arı ürünlerinin üretilmesi amacıyla arıyı, bitkisel kaynakları, işgücünü ve teknik bilgiyi bir arada kullanan sosyo-ekonomik bir faaliyet olarak tanımlanmaktadır (Burucu ve Gülse Bal 2018). Bu faaliyet kırsal alanda alternatif gelir kaynağı olması ve ekolojik dengeye sağladığı katkı bakımından oldukça önemlidir (Güler v.d. 2018).

Arıcılık açısından önemli bir potansiyele sahip olan Türkiye'nin coğrafi yapısı, bitki florası, ekolojisi, nektar kaynakları ve koloni varlığı bal üretimi açısından oldukça uygundur (Borum 2014). Bununla birlikte arıcılıktan yüksek verim elde edilebilmesi koloni verimliliğinin yanı sıra nektar ve polen kaynaklarının çeşidine ve miktarına bağlıdır (Behçet ve Yapar 2019).

Dünyada 2019 yılında üretilen 1,85 milyon ton balın %5,90'ı, 66 bin ton bal mumunun ise %7,17'si Türkiye'de üretilmiştir. Bu üretim oranlarıyla Türkiye bal üretiminde dünyada ikinci, bal mumu üretiminde ise dördüncü sırada yer almaktadır. Ancak verim açısından değerlendirildiğinde 2019 yılında Türkiye'nin kovan başına bal verimi 13,5 kilogram (kg) olup, dünya ortalamasının (20,6 kg) altındadır (FAO 2021). İllere göre değerlendirildiğinde bal veriminde önde gelen iller sırasıyla Ordu (30,0 kg), Adana (25,3 kg), Sivas (21,3 kg), Çanakkale (20,5 kg) ve Kars'tır (20,0 kg) (TÜİK 2021). Sahip olunan bitki örtüsü ve iklim tipi göz önüne alındığında Türkiye'nin bal verim ortalamasının artırılabilmesi mümkün görünmektedir (Onuç v.d. 2019). Bal üretiminde önde gelen iller sırasıyla Ordu (%16,54), Adana (%11,69), Muğla (%5,86) ve Sivas'tır (%5,26). Bal mumu üretiminde de yine Adana (%13,11), Muğla (%10,74), Sivas (%9,69) ve Ordu (%4,33) önde gelen iller arasında yer almaktadır. Muğla ise hem işletme sayısı (%5,72) hem de kovan sayısı (%11,01) bakımından iller arasında ilk sırada bulunmaktadır (TÜİK 2021).

Türkiye'de kovan başına bal verimi, bal çeşidi, üretilen arı ürünlerinin çeşitliliği, işletme sayısı ve üretim masrafları gibi değişkenler önemli ölçüde farklılık göstermektedir (Saner v.d. 2004, Saner v.d.

2011, Kekeçoğlu ve Göç Rasgele 2013, Çelik ve Turhan 2014, Çukur 2014, Öztürk v.d. 2014, Demen v.d. 2015, Kutlu v.d. 2016, Aksoy v.d. 2017, Öztürk 2017, Çevrimli ve Sakarya 2018). Bu değişkenlere ilişkin farklılıkların ortaya konulduğu literatürdeki çalışmalarda üreticilerin eğitim düzeyi, yaşı, yetiştiricilik deneyimi, arıcılıkta ihtisaslaşma oranı ve arıcılığın temel sorunları tespit edilmiştir. Buna göre arıcılıkta üreticilerin eğitim düzeyi ve arıcılıkta ihtisaslaşma oranı düşük, ortalama yaşı yüksek ve yetiştiricilik deneyimi fazladır. Bununla birlikte pazarlama ve konaklama arıcılığın en temel sorunları arasında yer almaktadır. Türkiye'de arıcılık etkinliğinin işletmeler bazında incelendiği çalışmalar (Ören v.d. 2010, Ceyhan 2017, Sert 2017, Aydın v.d. 2020, Kaya 2020) bulunmaktadır. Bu çalışmalardan biri Ören v.d. (2010) tarafından Adana ilinde yapılmış olup, çalışma kapsamındaki işletmelerde teknik etkinlik düzeyi %85 olarak saptanmıştır. Çalışmada teknik etkinsizliğin en büyük bileşenlerinden birini ölçek etkinsizliğinin oluşturduğu belirlenmiştir. Isparta ilinde yapılan çalışmada ise (Sert, 2017) teknik etkinlik düzeyi %86 olarak belirlenmiştir. Bu iki çalışmada teknik etkinliklerin belirlenmesi amacıyla girdiye yönelik olarak veri zarflama analizinden yararlanılmıştır. Ayrıca her iki çalışmada da çıktı olarak bal üretimi (kg/koloni); girdi olarak arı yemi (kg/koloni), işgücü (saat/koloni), diğer değişken masraflar ve diğer sabit masraflar (TL/koloni) değerlendirilmiştir. Ayrıca literatürde 81 ilin toplam kovan sayısı, bal üretimi ve bal mumu üretimi açısından benzerliklerinin ve farklılıklarının ortaya konulduğu bir çalışma (Güler v.d. 2018) da mevcuttur. Bu çalışmanın sonucuna göre arıcılığa sağlanan katkıların iller bazında farklılık gösterdiği, Muğla ve Adana illerinin incelenen değişkenler açısından diğer illerden ayrıştıkları ve arıcılığa en fazla katkı sağlayan il oldukları saptanmıştır. Ancak literatürde arıcılık açısından illere göre etkinliğin incelendiği herhangi bir çalışma bulunmamaktadır ve bu çalışma arıcılıkta etkinliği illere göre incelemesi bakımından önceki çalışmalardan farklılık göstermektedir.

Oysa literatürde iller/bölgeler arası sınıflandırma ve sıralama amacıyla veri zarflama analizinin (VZA) kullanıldığı çalışmalar mevcuttur. Deliktaş (2002) çalışmasında özel sektör imalât sanayi alt sektörlerinde teknik etkinlik ölçümü için VZA kullanmış olup hem iller arası hem de sektörler arası performans karşılaştırması yapmıştır. Örkcü ve Kardiyen (2006) çalışmalarında Türkiye'nin 81 ilinin gelişmişlik düzeylerini sınıflama ve sıralama amacıyla VZA'dan yararlanmış olup, sonuçları çok değişkenli istatistiksel yöntemlerle karşılaştırmışlardır. Bu çalışmada, hem gerçek veri hem de simülasyon çalışması sonuçlarına dayanılarak VZA'nın çok değişkenli istatistiksel yöntemlerin yerine kullanılabilecek bir yöntem olduğu sonucuna varılmıştır. Yılmazer v.d. (2006) VZA'dan yararlanarak Türkiye'nin 73 ilinde kamu yatırım harcamalarının illerin ekonomik ve sosyal göstergeleri üzerindeki etkilerini ölçmeyi amaçlamışlardır. Türkiye'de 81 ilin etkinliğinin incelendiği diğer çalışma örneklerinde ise Gülsevin (2014), sosyokültürel bir gösterge olan eğitim açısından, Çakmak (2016) sağlık, eğitim, ekonomi ve banka alanında sosyo-ekonomik göstergeler açısından, Koçak (2018) ise tüketici türü bazında elektrik tüketimi açısından illeri karşılaştırmış, bu illere ait etkinlik değerlerini VZA ile ölçmüşlerdir.

Bu çalışmanın amacı, bal ve bal mumu üretimi, arıcılık faaliyeti yapan işletme sayısı ve kovan sayısı değişkenleri çerçevesinde Türkiye'deki illerin arıcılık faaliyetindeki etkinliklerinin VZA ile belirlenmesidir. Çalışmanın arıcılıkta illere ve bölgelere yönelik destekleme modellerinin oluşturulmasında politika yapıcılara fayda sağlaması beklenmektedir.

MATERYAL VE YÖNTEM

Çalışmanın ana materyalini Türkiye İstatistik Kurumu'ndan elde edilen 2020 yılına ait 81 ildeki bal ve bal mumu üretimi, arıcılık faaliyeti yapan işletme sayısı ve kovan sayısı verileri oluşturmaktadır. Çalışmada illerin arıcılık faaliyetindeki etkinliklerini belirlemek amacıyla veri zarflama analizinden yararlanılmıştır. Analizde girdi değişkenleri olarak arıcılık faaliyeti yapan işletme sayısı ve kovan sayısı değerlendirilmiştir. Çıktı değişkenleri ise ilk modelde bal üretim verisi iken, ikinci modelde bal ve bal mumu üretim verileri olarak belirlenmiştir.

Etkinlik analizinde parametrik ve non-parametrik olmak üzere iki yöntem bulunmaktadır. Veri zarflama analizi çoklu girdi ve çıktılara sahip durumların incelenebildiği non-parametrik bir yöntemdir (Gul v.d. 2009, Koc v.d. 2011). İlk kez Farrell tarafından 1957 yılında önerilen Sınır Üretim Fonksiyonu ile şekillenmiş olan VZA; Charnes, Cooper, Banker ve Rhodes'in çalışmalarıyla bugünkü durumunu almıştır (Güler ve Saner 2020).

VZA'da kullanılan CCR (Charnes-Cooper-Rhodes) yöntemi ölçeğe göre sabit getiri varsayımına dayanırken, BCC (Banker-Charnes-Cooper)

yöntemi ölçeğe göre değişken (artan veya azalan) getiri varsayımına dayanmaktadır. Toplamsal yöntem ise bir modelde her iki yöntemin birlikte değerlendirilmesidir. Veri zarflama analizinde hesaplanan etkinlik değerleri toplam etkinlik, teknik etkinlik ve ölçek etkinliğidir (Charnes v.d. 1978, Banker v.d. 1984).

$$Toplam\ Etkinlik = Teknik\ Etkinlik\ x\ Ölçek\ Etkinliği \quad (1)$$

$$E = \frac{k_1Y_1+k_2Y_2+\cdots+k_nY_n}{z_1X_1+z_2X_2+\cdots+z_mX_m} \quad (2)$$

VZA doğrusal programlama yöntemine dayanmaktadır. Bu yöntemde her birimin etkinliği girdi ve çıktıların ağırlıklı toplamları arasındaki oran olarak tanımlanmakta olup birimin etkinliği (i. birim için) (Özden 2016): olacaktır. Burada n adet çıktı ve m adet girdi bulunmaktadır. Denklemde Y çıktı, X ise girdi miktarlarını belirtirken; z girdi ağırlıklarını, k ise çıktı ağırlıklarını temsil etmektedir

İncelenen illerde gruplar arasındaki farklılıklar etkinlik değerleri açısından istatistiksel olarak test edilmiştir. Buna göre illerde işletme büyüklüğüne ve bal verimine ilişkin ikili grupları karşılaştırmak amacıyla Mann-Whitney U testi uygulanmıştır.

ARAŞTIRMA BULGULARI

Etkinlik analizi için kullanılan değişkenler bal üretimi, bal mumu üretimi, işletme sayısı ve kovan sayısıdır. Buna göre iki farklı modelde etkinlik ölçümü yapılmıştır. Birinci modelde çıktıyı sadece bal üretimi oluştururken, ikincisinde çıktıları bal üretimi ve bal mumu üretimi oluşturmuştur. İllerde bulunan arıcılık işletme sayısı ve kovan sayısı ise girdiler olarak etkinlik analizi modelinde yer almıştır. Değişkenleri oluşturan bal üretimi 1285 ton, bal mumu üretimi 46 ton, işletme sayısı 1023 ve kovan sayısı 100976 olarak hesaplanmıştır (Tablo 1).

Tablo 1. Değişkenlere ilişkin tanımlayıcı istatistikler

Table 1. Descriptive statistics for variables

Çıktılar/Girdiler	Değişkenler	Birim	Minimum	Maksimum	Ortalama	Std. Sapma
Çıktılar	Bal üretimi	Ton	27	17213	1285	2427
	Bal mumu üretimi	Ton	0	493	46	82
Girdiler	İşletme sayısı	Adet	92	4741	1023	806
	Kovan sayısı	Adet	6198	900583	100976	129903

İllere göre etkinlik değerleri ölçeğe göre sabit getiri ve ölçeğe göre değişken getiri varsayımları altında çıktıya yönelik olarak hesaplanmıştır. Çıktıya yönelik hesaplamalarda amaç mevcut girdi miktarı değişmeden maksimum çıktı miktarına oransal olarak ne ölçüde ulaşılabileceğinin belirlenmesidir.

İllere ait çıktıya yönelik etkinlik değerlerinin dağılımı Tablo 2'de verilmiştir. Çıktı olarak bal üretim miktarının değerlendirmeye alındığı ilk modelde tam etkinliğe sahip olan il sayısı 2 iken, bal ve bal mumu üretim miktarının değerlendirmeye alındığı ikinci modelde tam etkinliğe sahip il sayısı 8 olmuştur. İlk modelde 75 ilin, ikinci modelde ise 69 ilin etkinlik değerinin %50'nin altında olduğu saptanmıştır ve bu sonuçlar Türkiye'de illerin önemli bir kısmında arıcılıktaki etkinliğin düşük olduğunu göstermektedir.

Tablo 2. İllere göre toplam etkinlik, teknik etkinlik ve ölçek etkinliği.

Table 2. Total efficiency, technical efficiency, and scale efficiency by provinces

Çıktı(lar)	Etkinlik Değeri	Toplam Etkinlik		Teknik Etkinlik		Ölçek Etkinliği	
		Sayı	%	Sayı	%	Sayı	%
Bal Üretimi	1	2	2,47	7	8,64	2	2,47
	0.901-0.999	0	0,00	0	0,00	38	46,91
	0.801-0.900	1	1,23	0	0,00	15	18,52
	0.701-0.800	2	2,47	2	2,47	11	13,58
	0.601-0.700	0	0,00	2	2,47	4	4,94
	0.501-0.600	1	1,23	1	1,23	4	4,94
	0.401-0.500	2	2,47	3	3,70	1	1.,3
	0.301-0.400	4	4,94	5	6,17	1	1,23
	0.201-0.300	5	6,17	8	9,88	1	1,23
	0.101-0.200	34	41,98	30	37,04	2	2,47
	0.000-0.100	30	37,04	23	28,40	2	2,47
	Ortalama	0,18675		0,26363		0,80870	
	Std. Sapma	0,20644		0,27859		0,22179	
	Minimum	0,003		0,008		0,045	
	Maksimum	1,000		1,000		1,000	
Bal ve Bal mumu Üretimi	1	8	9,88	16	19,75	9	11,11
	0.901-0.999	1	1,23	4	4,94	11	13,58
	0.801-0.900	2	2,47	8	9,88	2	2,47
	0.701-0.800	0	0,00	7	8,64	6	7,41
	0.601-0.700	0	0,00	5	6,17	9	11,11
	0.501-0.600	1	1,23	4	4,94	6	7,41
	0.401-0.500	3	3,70	6	7,41	6	7,41
	0.301-0.400	6	7,41	6	7,41	11	13,58
	0.201-0.300	16	19,75	8	9,88	12	14,81
	0.101-0.200	32	39,51	10	12,35	9	11,11
	0.000-0.100	12	14,81	7	8,64	0	0,00
	Ortalama	0,29809		0,56864		0,56783	
	Std. Sapma	0,2857		0,33787		0,30278	
	Minimum	0,002		0,008		0,116	
	Maksimum	1,000		1,000		1,000	

Türkiye'de en yüksek bal verimine sahip Ordu ili her iki modelde de tam etkinliğe sahip olan iller arasında yer almaktadır. Aydın ili birinci modelde tam etkinliğe sahip iken; Bilecik, Kars, Kırşehir, Siirt, Sivas, Şanlıurfa ve Zonguldak illeri ikinci modelde tam etkinliğe sahip iller arasındadırlar (Tablo 3). Bu illerden Aydın, Ordu, Kars, Sivas ve Şanlıurfa IPARD (Katılım Öncesi Yardım Aracı Kırsal Kalkınma Bileşeni) Programı uygulama illeri arasında yer almakta olup, bunlar Çiftlik Faaliyetlerinin Çeşitlendirilmesi ve İş Geliştirme (302) tedbiri kapsamında arıcılık ve arı ürünlerinin üretimi, işlenmesi ve paketlenmesine yönelik desteklenmektedirler (TKDK 2021).

Tablo 3. Çıktılara göre etkin olan iller

Table 3. The efficient provinces by outputs

Çıktı(lar)	Etkin Olan İller
Bal Üretimi	Aydın, Ordu
Bal ve Bal mumu Üretimi	Bilecik, Kars, Kırşehir, Ordu, Siirt, Sivas, Şanlıurfa, Zonguldak

Ölçeğe getiri durumlarına göre incelendiğinde birinci modelde illerin %88,89'u (72 il) ölçeğe göre artan getiri altında faaliyet göstermekte olup bu modelde illerin çoğunda girdi miktarında belli orandaki artış çıktı miktarında daha fazla oranda artış sağlamaktadır. İkinci modelde ise illerin %80,25'i (65 il) ölçeğe göre azalan getiri, %11,11'i (9 il) ölçeğe göre sabit getiri ve %8,64'ü (7 il) ölçeğe göre artan getiri altında faaliyet göstermektedirler (Tablo 4).

Tablo 4. İllerde ölçeğe göre getiri durumu

Table 4. Returns to scale for provinces

Çıktı(lar)	Etkinlik	İl Sayısı	Grup İçinde (%)
Bal Üretimi	ÖG Sabit Getiri (CRS)	4	4,94
	ÖG Azalan Getiri (DRS)	5	6,17
	ÖG Artan Getiri (IRS)	72	88,89
Bal ve Bal mumu Üretimi	ÖG Sabit Getiri (CRS)	9	11,11
	ÖG Azalan Getiri (DRS)	65	80,25
	ÖG Artan Getiri (IRS)	7	8,64

TÜİK (2021) verilerinden yararlanılarak illerde ortalama kovan sayısı 95 olarak hesaplanmıştır. Buna göre 95 ve 95'in altında kovana sahip işletmelerin bulunduğu illerle 95'in üzerinde kovana sahip işletmelerin bulunduğu illerin etkinlik değerleri arasında istatistiksel açıdan farklılık olup olmadığı Mann-Whitney U testi ile test edilmiştir. Elde edilen sonuca göre iki grup arasında istatistiksel olarak farklılık olduğu saptanmıştır. Buna göre birinci modelde 95'in üzerinde kovana sahip işletmelerin bulunduğu illerde toplam etkinlik (0,336) ve teknik etkinlik (0,409) değerlerinin daha yüksek olduğu saptanmıştır. Bal ve bal mumu üretiminin çıktı olarak değerlendirildiği ikinci modelde de gruplar arasında istatistiksel olarak farklılık olduğu saptanmış olup, 95'in üzerinde kovana sahip işletmelerin bulunduğu illerin teknik etkinlik değeri (0,716) 95 ve 95'in altında kovana sahip işletmelerin bulunduğu illerden daha yüksektir (Tablo 5).

Türkiye'de kovan başına bal verimi TÜİK verilerine göre 2020 yılında yaklaşık 13 kg'dır. Buna göre bal üretiminin çıktı olarak değerlendirildiği birinci model için bal verimi 13 kg ve altında bal verimine sahip iller ile 13 kg üzerinde bal verimine sahip illerin etkinlik değerleri karşılaştırılmıştır. Elde edilen sonuçlar, Mann-Whitney U testine göre iki grup arasında toplam etkinlik ve teknik etkinlik değerleri açısıdan istatistiksel olarak farklılık olduğunu ve bal verimi 13 kg üzerinde olan illerde toplam etkinlik (0,383) ve teknik etkinlik (0,436) değerlerinin daha yüksek olduğunu göstermektedir (Tablo 6).

Tablo 5. İşletme büyüklüğüne göre etkinlik değerleri

Table 5. Efficiency values by scale of beekeeping businesses

Çıktı(lar)	Etkinlik	İşletme büyüklüğü	Ort.	Std. Sapma	Min.	Maks.	z	p
Bal üretimi	Toplam Etkinlik*	95 kovan ve altı	0,099	0,055	0,003	0,273	-5,712	0,000
		95 kovan üzeri	0,336	0,275	0,073	1,000		
	Teknik Etkinlik*	95 kovan ve altı	0,178	0,226	0,008	1,000	-4,554	0,000
		95 kovan üzeri	0,409	0,303	0,089	1,000		
	Ölçek Etkinliği	95 kovan ve altı	0,788	0,246	0,045	0,998	-0,562	0,574
		95 kovan üzeri	0,843	0,171	0,176	1,000		
Bal ve Bal mumu Üretimi	Toplam Etkinlik	95 kovan ve altı	0,257	0,249	0,002	1,000	-1,424	0,155
		95 kovan üzeri	0,368	0,332	0,060	1,000		
	Teknik Etkinlik*	95 kovan ve altı	0,482	0,323	0,008	1,000	-3,088	0,002
		95 kovan üzeri	0,716	0,316	0,092	1,000		
	Ölçek Etkinliği	95 kovan ve altı	0.,585	0,285	0,164	1,000	-0,822	0,411
		95 kovan üzeri	0,539	0,334	0,116	1,000		

Mann-Whitney U testine göre gruplar arasındaki fark * p<0,01 düzeyinde anlamlıdır.

Tablo 6. Bal verimine göre etkinlik değerleri

Table 6. Efficiency values by honey yield

Etkinlik	Bal verimi	Ortalama	Std. Sapma	Min.	Maks.	z	p
Toplam Etkinlik*	13 kg ve altı	0,113	0,075	0,025	0,427	-4,949	0,000
	13 kg üzeri	0,383	0,302	0,003	1,000		
Teknik Etkinlik*	13 kg ve altı	0,199	0,228	0,032	1,000	-4,010	0,000
	13 kg üzeri	0,436	0,331	0,008	1,000		
Ölçek Etkinliği	13 kg ve altı	0,789	0,243	0,045	0,998	-1,035	0,301
	13 kg üzeri	0,861	0,144	0,380	1,000		

Mann-Whitney U testine göre gruplar arasındaki fark * p<0,01 düzeyinde anlamlıdır.

İllerde yer alan işletmelerin ortalama büyüklüğüne göre bal verimi farklılığı incelendiğinde, işletme büyüklüğü 95 kovan üzerinde olan işletmelerin yer aldığı illerde bal veriminin 95 ve altında kovana sahip işletmelerin yer aldığı illerden daha fazla olduğu (z = -1,780, p = 0,075) tespit edilmiştir (Tablo 7).

Tablo 7. İşletme büyüklüğüne göre bal verimi

Table 7. Honey yield by scale of beekeeping businesses

Değişken	İşletme büyüklüğü	Ortalama	Std. Sapma	Min.	Maks.	z	p
Bal Verimi (kg)*	95 kovan ve altı	9,77	3,92	3,64	21,34	-1,780	0,075
	95 kovan üstü	12,14	5,94	3,62	30,02		

Mann-Whitney U testine göre gruplar arasındaki fark * p<0,1 düzeyinde anlamlıdır.

TARTIŞMA

Araştırma sonucunda bal üretiminin çıktı olarak değerlendirildiği birinci modelde Ordu ve Aydın illerinin tam etkinliğe sahip oldukları saptanmıştır. Ordu ilindeki arıcılar gezginci arıcılık sayesinde mevcut floranın yanı sıra farklı yörelerdeki florayı da değerlendirerek ilin bal üretimini Türkiye'de ilk sıraya taşımışlardır (Anonim 2001, Sıralı 2015). Gezginci arıcılıkta mevcut bitki türlerinin ilin arı popülâsyonlarının ihtiyaçlarını karşılamada yetersiz kalması önemli bir etkendir (Sıralı 2017). Bununla birlikte Aksoy ve Öztürk (2012) tarafından yapılan çalışmada, Ordu ilinde arıcılar tarafından en fazla önem verilen faktörün 'modern tarım' olduğu tespit edilmiştir. Modern tarım uygulamaları ise eğitim programlarıyla ilişkilendirilebilir. Nitekim Kekeçoğlu ve Göç Rasgele (2013) teknik arıcılık eğitimi alan ve almayan üreticilerin işletmelerini bal verimi açısından karşılaştırdıkları çalışmalarında arıcılık eğitimi alan üreticilerin işletmelerinde işletme başına ortalama bal veriminin daha yüksek olduğunu belirtmişlerdir. Ayrıca Makri v.d. (2015), Olohungbebe ve Daniel (2015), Legesse v.d. (2020) ve Tarekegn ve Ayele (2020) çalışmalarında arıcılık eğitim programlarına yönelik ilgileri daha fazla olan üreticilerin işletmelerinde etkinlik değerinin daha yüksek olduğunu ortaya koymuşlardır. Bununla birlikte arıcılıkta modern tarım uygulamaları bal verimini ve kalitesini artırmaktadır (Fadare v.d. 2008, Affognon 2015, Cabrera v.d. 2019).

Aydın ili ise Türkiye bal üretiminden %3,5 oranında (3643 ton) pay almakta olup, bal üretim miktarı açısından iller arasında beşinci sırada yer almaktadır. Güler v.d. (2018), arıcılık açısından Türkiye'deki illerin benzerlik ve farklılıklarını inceledikleri çalışmalarında Aydın ile Ordu ilinin arıcılığa yaptıkları katkı bakımından birbirine benzerlik gösterdiklerini saptamışlardır. Literatürde Özbilgin v.d. (1999) ve Çevrimli ve Sakarya (2018) tarafından yapılan ve sonuçları Ege Bölgesi genelinde değerlendirilen çalışmalar dışında Aydın'daki arıcılık faaliyetinin özelliklerini inceleyen bir çalışmaya rastlanmamıştır.

Elde edilen sonuçlar işletme ölçeği büyük olan arıcılık işletmelerinin yer aldığı illerin etkinlik değerinin yüksek olduğunu göstermektedir. Arıcılık işletmelerinin etkinliğini inceleyen çalışmalarda da benzer sonuçlara ulaşılmıştır. Abdul-Malik ve Mohammed (2012) tarafından Gana'da yapılan çalışmada kovan sayısı ile işletmelerin etkinliği arasında pozitif yönlü bir ilişki olduğu ve bu ilişkinin istatistiksel açıdan anlamlı olduğu saptanmıştır. Makri v.d. (2015) tarafından Yunanistan'da yapılan çalışmada etkin olan arıcılık işletmelerinin ortalama kovan sayısının 130 olduğu belirlenmiştir. Türkiye'nin 37 ilinde 455 arıcılık işletmesini kapsayan bir başka çalışmada (Ceyhan 2017) orta ve büyük ölçekli işletmelerin etkinlik değerinin kovan sayısı az olan küçük ölçekli işletmelere göre daha yüksek olduğu ortaya konulmuştur. Aydın v.d. (2020) tarafından Çanakkale ilinde yapılan çalışmada ise işletmeler kovan sayısına göre 1-75 kovan (1. grup), 76-150 kovan (2. grup) ve 150 üzeri kovan (3. grup) olmak üzere üç gruba ayrılmış olup, gruplar arasında etkinlik değerleri bakımından farklılık olup olmadığı incelenmiştir. Buna göre kovan sayısı fazla olan arıcılık işletmelerinde etkin işletme oranının daha fazla olduğu ve bu farklılığın istatistiksel açıdan anlamlı olduğu tespit edilmiştir. Kaya (2020) tarafından Hatay ilinde yapılan çalışmada ise etkin bir arıcılık işletmesinin 180 ile 200 adet arasında kovana sahip olması gerektiği belirlenmiştir. Ayrıca Aksoy v.d. (2017) tarafından Erzurum ilinde yapılan çalışmada kovan sayısı fazla olan işletmelerde bal veriminin daha yüksek olduğu tespit edilmiştir. Keleş v.d. (2019) tarafından Trabzon ilinde yapılan çalışmada ise 145-300 arasında kovana sahip olan işletmelerin kovan başına verim yönünden en ideal grup olduğu saptanmıştır. Bunun yanı sıra bal veriminde ana arıyı değiştirme süresi de önem arz etmektedir. Özmen Özbakır v.d. (2016) Adıyaman ilinde yaptıkları çalışmada ana arının iki yılda bir değiştirildiği işletmelerde bal veriminin en yüksek miktarda olduğunu saptamışlardır.

Sonuç

Araştırmada illerin toplam etkinlik değeri incelendiğinde, çıktı olarak bal üretim miktarı dikkate alınan birinci modelde tam etkinliğe sahip olan il sayısının 2 (Aydın, Ordu) ve toplam etkinlik değerinin 0,19 olduğu belirlenmiştir. Bal ve bal mumu üretim miktarının çıktı olarak değerlendirildiği ikinci modelde ise tam etkin il sayısı 8 (Bilecik, Kars, Kırşehir, Ordu, Siirt, Sivas, Şanlıurfa, Zonguldak) ve toplam etkinlik değeri 0,30'dur. Elde edilen sonuçlar, her iki modelde de Türkiye'de arıcılıktaki etkinliğin düşük olduğunu göstermektedir. Bal üretiminde dünyada ikinci, bal mumu üretiminde ise dördüncü sırada yer almasına rağmen bal veriminin dünya ortalamasının %34 altında olan Türkiye'de, arıcılıktaki etkinliğin düşük olması önemli bir sorundur. Araştırma sonucunda, Türkiye'de

ortalama kovan sayısının üzerinde (95 kovan üstü) kovana sahip işletmelerin yer aldığı illerde etkinlik değerinin ve bal veriminin yüksek olduğu saptanmıştır. Bununla birlikte, Türkiye bal verim ortalamasının (13 kg) üzerindeki illerde de etkinlik değeri yüksektir. Bu sonuçlar etkinlik değerlerinin işletme ölçeği ve bal verimine göre farklılık gösterdiğini ve büyük ölçekli arıcılık işletmelerinin etkinlik açısından avantajlı olduklarını göstermektedir. Bu nedenle, arıcılıkta üreticilerin kovan sayılarını artırmalarına yönelik teşvik edici politikaların yürütülmesi önem arz etmektedir. Ayrıca, her iki modelde etkin olan iller arasında yer alan Ordu ilinde arıcılık işletmelerinin etkinliğine yönelik çalışmalar yapılmalı ve bu çalışmalardan elde edilecek sonuçlar diğer illerin etkinliğinin artırılması yönünde değerlendirilmelidir. Bununla birlikte modern arıcılık uygulamalarına yönelik yayım faaliyetlerinin sürdürülmesi işletmelerde bal veriminin ve kalitesinin artırılmasının yanı sıra etkinliğin artırılması açısından da önem arz etmektedir.

Mali Kaynak: Bu çalışma için sağlanmış mali kaynak bulunmamaktadır.

Etik Belgesi: Bu çalışma için etik belgesi gerekli değildir.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

DETERMINATION OF HONEY BEE (*Apis mellifera*) BACTERIAL FLORA, *CRY* GENE ANALYSIS AND HONEY BEE HEALTH

Bal Arısı (*Apis mellifera*) Bakteri Florasının Belirlenmesi, *Cry* Geni Analizi ve Bal Arısı Sağlığı

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ABSTRACT

Beekeeping provides important contributions to the agricultural economy and crop production through pollination both in Turkey and the world. It is evaluated that without bees, the plant production can decrease by 47%. Many factors affect honey production negatively. Among these reasons, besides diseases caused by microorganisms, diseases and dangers originating from organisms are at the forefront. Today, many methods are used in the control these pests and yet they are still unavoidable. Among these methods, the biological control method is not used commonly. The aim of the study is to create a basis for the development of biopesticides to control bee diseases. In this context, as a result of the study, 16 bacteria were isolated from honey bees. While, 12 bacteria belonging to the genus *Bacillus*, two bacteria belonging to the genus *Lysinibacillus*, one bacterium belonging to the genus *Paenibacillus* and one bacterium belonging to the genus *Pantoea* were obtained. Molecular and biochemical identifications of these bacteria were done and registered in GenBank and their accession numbers were obtained. *cry* gene analyzes of 15 bacteria belonging to the genus *Bacillus* were performed. As it is known, *cry* genes have the potential to be used against pests. In the future, these bacteria and their genes will have the potential to be used as biopesticides. According to these results, the *cry1* gene was observed in 8 bacteria and the *cry3* gene was observed in 3 bacteria. *cry2* and *cry4* genes could not be detected in these bacteria. Bacteria that including *cry* genes are of great importance for honey bee health. Bacteria have the potential to be developed as internal biopesticides and used against different bee diseases to improve honey bee health.

Keywords: *Apis mellifera*, Bacteria, Microbiology, Honey bee health, *cry* genes

ÖZ

Arıcılık gerek Türkiye'de gerekse dünyada tarım ekonomisine ve tozlaşma yoluyla bitkisel üretime önemli katkılar sağlar. Arıların olmadığı bir ortamda bitkisel üretimin %47 oranında azalabileceği değerlendirilmektedir. Arıcılık sektöründe birçok etken de bal üretimini olumsuz yönde etkilemektedir. Bu sebepler arasında mikroorganizma sebepli hastalıkların yanı sıra, organizma kaynaklı hastalıklar ve tehlikeler de ön sıralarda yer almaktadır. Günümüzde bu zararlılarla mücadelede birçok yöntem kullanılmakta olup halen önüne geçilememiş durumdadır. Bu yöntemler arasında biyolojik mücadele yöntemi kullanılmamaktadır. Buradan yola çıkarak, çalışmanın amacı, bal arılarının sağlığını korumak için biyolojik bir etmen kullanılarak hastalık ve zararlı organizmalarla mücadele konusunda biyopestisit geliştirilmesinde taban oluşturmaktır. Bu bağlamda çalışma sonucunda bal arılarından 16 adet bakteri izolasyonu gerçekleştirilmiştir. Elde edilen bakterilerden 12 tanesi *Bacillus* cinsine, iki tanesi *Lysinibacillus* cinsine, bir tanesi *Paenibacillus* cinsine ait iken bir tanesi de *Pantoea* cinsine aittir. Bu bakterilerin moleküler ve biyokimyasal tanımlamaları yapılarak GenBank a kayıt yaptırılmış ve kayıt

numaraları alınmıştır. On beş adet *Bacillus, Paenibacillus ve Lysinibacillus* cinslerine ait bakterinin *cry* gen analizleri yapılmıştır. Bilindiği üzere *cry* genleri hem zararlılara karşı kullanılma potansiyeline sahiptir hem de ileride bu bakteri ve genleri geliştirilerek biyopestisit kullanılma potansiyeli olabilecektir. Bu sonuçlara göre *cry1* geni 8 bakteride ve *cry3* geni de 3 bakteride gözlemlenmiştir. *cry2* ve *cry4* genleri bu bakterilerde tespit edilememiştir. Bu genleri taşıyan bakteriler bal arısı sağlığı açısından büyük önem taşımaktadır. Bakteriler biyopestisit olarak geliştirilerek başta organizma gibi zaralılar olmak üzere farklı arı hastalıklarına karşı kullanılma potansiyeline sahiptirler.

Anahtar Kelimeler: *Apis mellifera*, Bakteri, Mikrobiyoloji, Bal arısı sağlığı, *cry* genleri

GENİŞLETİLMİŞ ÖZET

Amaç: Bal arıları ekonomik ve biyolojik yönden oldukça önemlidir. Bal arıları bal, propolis, arı sütü, polen, bal mumu, arı zehri gibi arı ürünleri sayesinde dünya pazarında önemli yer almaktadır. Arıcılık, Dünya'nın hemen hemen her yerinde yapılan tarımsal bir faaliyettir. Arıcılık faaliyetlerini engelleyen en önemli nedenlerden biri de arı zararlı ve hastalıklarıdır. Bu nedenle de arı sağlığını korumak büyük önem arz etmektedir. Günümüzde bu zararlılarla mücadelede birçok yöntem kullanılmakta olup halen önüne geçilememiş durumdadır. Bu yöntemler arasında biyolojik mücadele yöntemi kullanılmamaktadır. Çalışmanın amacı, bal arılarının sağlığını korumak için biyolojik bir etmen kullanılarak hastalık ve zararlı organizmalarla mücadele konusunda biyopestisit geliştirilmesinde taban oluşturmaktır.

Yöntem: Çalışma için gerekli olan bal arısı örnekleri Gümüşhane ili Kürtün ilçesinden elde edilmiştir. İlçedeki belirlenen sağlıklı arılık ve kovanlardan ortalama 20 şer arı toplanmıştır. Bu arılar steril taşıma kaplarına alınarak laboratuvar ortamına getirilmiştir. Laboratuvara getirilen arı örnekleri öncelikle %70'lik alkol ile yüzey strelizasyonu sağlanmıştır. Yüzey sterilizasyonunun ardından 500µl Nutrient broth besiyeri içerisinde arıların ezilerek parçalanması sağlanmıştır. Artık parçaları ortamdan uzaklaştırmak için steril filtre ile süzme işlemi gerçekleştirilip, geri kalan sıvı Nutrient agar besiyerine ekim yapılarak 30°C' de 2-3 gün inkübasyona bırakılmıştır. Bu süre sonunda besiyerinde oluşan bakterilerin saflaştırılması için ayrı ayrı besiyerilerine ekimleri yapılmıştır. Bu bakterilerin tanımlanması için moleküler karakterizayonu, biyokimyasal testleri, fiziksel ve morfolojik analizleri yapılarak belirlenmiştir ve bakteriler GenBank'a kayıt ettirilmiştir.

Sonuç: Çalışma sonucunda bal arılarından 16 adet bakteri izolasyonu gerçekleştirilmiştir. Elde edilen bakterilerden 12 tanesi *Bacillus* cinsine, iki tanesi *Lysinibacillus* cinsine, bir tanesi *Paenibacillus* cinsine ait iken bir tanesi de *Pantoea* cinsine aittir. Bu bakterilerin moleküler ve biyokimyasal tanımlamaları yapılarak GenBank a kayıt yaptırılmış ve kayıt numaraları alınmıştır. On beş adet *Bacillus, Paenibacillus ve Lysinibacillus* cinslerine ait bakterinin *cry* gen analizleri yapılmıştır. Bilindiği üzere *cry* genleri hem zararlılara karşı kullanılma potansiyeline sahiptir hem de ileride bu bakteri ve genleri geliştirilerek biyopestisit kullanılma potansiyeli olabilecektir. Bu sonuçlara göre *cry1* geni 8 bakteride ve *cry3* geni de 3 bakteride gözlemlenmiştir. *cry2* ve *cry4* genleri bu bakterilerde tespit edilememiştir.

INTRODUCTION

According to the 2020 data of the Ministry of Food, Agriculture and Livestock, Directorate of Beekeeping Research Institute, Turkey ranks at the forefront of the beekeeping sector in the world with an average of 8 million 128 thousand hives and 109,330 tons of honey production. Beekeeping provides important contributions to the agricultural economy and crop production through pollination both in Turkey and in the world. So honey bees (*Apis mellifera*) play a very important role in pollination in natural ecosystem and agricultural field (Evans et al. 2010). In recent years, bee population and colony losses have increased in the world. Pathogens (parasites, fungi, viruses and bacteria) and abiotic stress factors can adversely affect colony health. All these factors are affecting the bee ecosystem (Brutscher et al. 2015, Li et al. 2018, Larsen et al. 2019). Over time, insects have developed a strong and effective immune system. The immune system of insects combats various pathogens and conseuqently has become one of the most diverse and successful immune systems in the world. Insects defense mechanisms include cellular and humoral immunity (Evans and Armstrong 2005, Schmid et al. 2008, Wilson-Rich et al. 2008).

Epidemic infection risks in honey bee colonies is reduced by individual and social immunity (Ilyasov et al. 2012). Both the type of immunities together at various levels protects the bees from diseases and ectoparasites (DeGrandi-Hoffman and Chen 2015, Larsen et al. 2019).

Pathogens, acaricides, fungicides, herbicides and other insecticides affect the bee immune system and thus affects bee health (Ilyasov et al. 2021). The social immune defense strategy, individual bees, reduces the pressure on the immune system (Larsen et al. 2019).

Like every living creature, honey bees are also have a microflora (Gilliam 1997, Olofsson and Vásquez 2008). These bacteria can play a positive or negative role on honey bee health and it is also important of rbutrient aquisition from a healthy diet (Evans and Spivak 2010). Bacterial microflora in bees, can inhibit lime disease (Reynaldi et al. 2004) and American foulbrood (Evans and Armstrong 2005, Evans et al. 2006). With a healthy microbiome, the reproduction of beneficial microorganisms is supported and the reproduction of pathogens is prevented. (Evans et al. 2006). Honey bees both at the individual and colony level is capable of immunity defense. Beekeepers use antibiotics to control pathogens and parasites, so pesticide applications are done frequently. This situation results in residues in hive products and hive equipment leading to an increased antibiotic resistance problem.

One of the biggest problems in beekeeping is attempting to treat honeybee diseases with chemical treatments. Limited success is achieved after chemical treatment, and there are problems, such as a danger to human health with chemical residues in the honey (Barganska et al. 2011). Problems are experienced in export markets for honey from treated bees, so attempts are made to sell this honey in the domestic market. As a result, the products cannot be sold easily and at their proper value. For this reason, biological control methods gain importance. Generally, for biological control *Bacillus* genus bacteria are used. *B. thuringiensis*, which was first isolated from diseased silkworm larvae by Japanese researcher Ishiwata (Ishiwata, 1901) in 1901, is the most widely used microbial control agent (Lacey et al. 2001). The insecticidal activity of *B. thuringiensis* is carried out by toxins in protein structure called insecticidal crystal proteins (ICP, cry proteins). These proteins are encoded by the genes (*cry*) located on the plasmids. These insecticidal proteins produced during spore formation constitute approximately 30% of the total protein content of the bacteria (Höfte and Whiteley, 1989; Aronson, 1993). ICPs are found undissolved under normal conditions. Therefore, they do not pose a risk to humans and other vertebrate organisms. On the other hand, their soluble properties at pH 9.5. Thus, the ICPs in the structure of this protein dissolve in the insect gut and turn into protoxin. Then, protoxins are broken down by intestinal enzymes and active toxins are formed. Active toxins cling to the receptors of intestinal epithelial cells, paralyze the intestinal wall of the insect and destroy it, forming pores. The poisoned insect can die immediately due to toxin activity and die as a result of blood poisoning within 2-3 days (Knowles, 1994). In this context, cry gene analyses were performed because 15 of the bacteria obtained belonged to the genus *Bacillus*. These bacteria will pave the way for developing biopesticides for organisms and microorganisms that affect bee health. The ground will be prepared for development for different agricultural or forest pests. In this context, it is aimed to obtain bacterial flora by collecting healthy honey bees. Bacteria obtained from bacterial flora will have the potential to be used against organisms that cause harm to bees.

MATERIAL AND METHODS

Sampling

Honey bee samples used in this study were collected from hives where are located in Gümüşhane province, distinct from Kürtün (It is located at latitude 40.748302 and longitude 38.984703). No chemical application has been made on the hives that samples were taken. The collected *Apis mellifera* were placed in sterile tubes and brought to the laboratory. The samples were collected twice a year, in the spring (June) and autumn (September) of 2020.

Isolation of Bacteria

Before bacterial isolation, honey bees were surface sterilized with 70% alcohol to remove possible contamination and then washed in sterile distilled water. The bee bodies were homogenized in 0.5 ml of sterilized Nutrient Broth (NB) using a glass tissue grinder and filtered. After preparing the homogenate for bacterial isolation, suspensions were diluted to 1×10-5 (Thiery and Frachon 1997) and 0.1 ml were spread on nutrient agar (Thiery and Frachon 1997). Plates were incubated at 30°C for 2-3 days. Isolates

were determined based on the color and morphology of the colonies. Individual colonies were isolated, sub-cultured twice to ensure purity and then stored in 15% sterilized glycerol at -80°C for further studies. Pure cultures of bacterial colonies were identified by their morphology, biochemical, physiological and molecular characteristics (16S rRNA).

Morphological, Physiological and Biochemical Characterisations of Isolates

Bacterial strains were selected based on morphological biochemical, physiological features according to Bergey's Manual of Systematic Bacteriology (Sneath et al. 1986). Phenotypic characteristics of the strains include cell and colony shape on NA. Optimum pH was determined, after 16 h incubation at 30°C by measuring the densities using a spectrophotometer (Spectramax M2) at OD600 (Ben-Dov et al. 1995). Biochemical panel test system API 20E (bioMerieux, France) was handled according to the manufacturer's instructions. Then the panels were incubated for 18-24 h at 30°C. The results of the tests were performed by referring to the API 20E reading table.

16S rRNA Gene Sequence Analysis

Genomic DNA from all samples was extracted by phenol/chloroform procedures (Sambrook et al. 1989). PCR amplification of 16S rRNA genes of bacterial isolates was performed with the following universal primers (William et al. 1991); UNI 16S-L: 5_-ATTCTAGAGTTTGATCATGGCTCA-3_ as forward and UNI 16S-R: 5_ATGGTACCGTGTGTGACGGGCGGTG TGTA-3_ as the reverse. PCR conditions were adjusted according to William et al. (1991). Reactions were totally in 50 µl; 1 µl of template DNA was mixed with 5 µl reaction buffer, 0.2 mM of each deoxynucleoside triphosphate (dNTP), 0.5 µM (each) with primer and 0.5 U with Taq DNA polymerase. Amplification was performed with 30 cycle program (each cycle consisting of denaturation at 94°C for 3 min, annealing at 55°C for 60 s and extension at 72°C for 3 min), followed by a final extension step at 72°C for 5 min, by using a thermal cycler (BioRad). Each experiment was associated with negative (without DNA template) controls. PCR products were analyzed on a 1.2% agarose gel. Sequence analysis of 16S rRNA products samples were performed using 16S universal primers by SenteBiolab (Ankara/TURKEY). Using the NCBI GenBank database, determined sequences were used to perform BLAST searches (Altschul et al. 1990). Comparison of approximately 1.400 bp fragments of 16S rRNA gene sequences of each isolate with other 16S rRNA sequences in the NCBI GenBank database (Altschul et al. 1990) were performed.

Optimum pH Range of All Bacteria

In order to determine the pH range in which the bacteria grows optimally, media at different pH values (pH3, pH4, pH5, pH6, pH7, pH8, pH9 and pH10) were prepared and incubated at 30°C for 24 hours.

Determination of *cry* Genes of Bacteria

PCR amplification of *cry* genes was performed of the toxin genes using *cry1*, *cry2*, *cry3* and *cry4* primers (Table 1). PCR was constructed according to the following conditions: pre-amplification 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 52-59°C for 60 s, and elongation at 72°C for 3 min. Reactions were totally in 50 µl; 1 µl of template DNA was mixed with 5 µl reaction buffer, 0.2 mM of each deoxynucleoside triphosphate (dNTP), 0.5 µM (each) with primer and 0.5 U with Taq DNA polymerase. PCR products were analyzed on a 1% agarose gel.

Table 1. *Cry* gene primers

Primer Name	Primer sequence	Sequence lenght (PCR amplification)	Tm (°C)
cry1Fw	CATGATTCATGCGGCAGATAAC		
cry1Rv	TTGTGACACTTCTGCTTCCCATT	277bp	55
cry2Fw	GTTATTCTTAATGCAGATGAATGGG		
cry2Rv	CGGATAAAATAATCTGGGAAATAGT	701bp	52
cry3Fw	CGTTATCGCAGAGAGATGACATTAAC		
cry3Rv	CATCTGTTGTTTCTGGAGGCAAT	604bp	54
cry4Fw	GCATATGATGTAGCGAAACAAGCC		
cry4Rv	GCGTGACATACCCATTTCCAGGTCC	439bp	59

RESULTS

In this study, 16 bacterial isolates; *Bacillus cereus* (GAP1), *Bacillus subtilis* (GAP2), *Bacillus wiedmannii* (GAP3), *Bacillus megaterium* (GAP4), *Pantoea rodasii* (GAP5), *Bacillus nakamurai* (GAP6), *Bacillus mobilis* (GAP7), *Bacillus pacificus* (GAP8), *Bacillus thuringiensis* (GAP9), *Lysinibacillus fusiformis* (GAP10), *Bacillus vallismontis* (GAP11), *Paenibacillus odorifer* (GAP12), *Bacillus velezensis* (GAP13), *Bacillus flexus* (GAP14), *Bacillus paramycoides* (GAP15) and *Lysinibacillus sphaericus* (GAP16) from *Apis mellifera* were isolated and characterized. The optimum pH range in which bacteria can grow was also determined (Table 2). When the pH results of the bacteria obtained in the study in Genbank are examined, it is seen that they confirm each other (https://www.ncbi.nlm.nih.gov/genbank/).These results are used to confirm the accuracy of the bacteria.

Table 2. Optimum pH range of bacteria.

pH	GAP 1	GAP 2	GAP 3	GAP 4	GAP 5	GAP 6	GAP 7	GAP 8	GAP 9	GAP 10	GAP 11	GAP 12	GAP 13	GAP 14	GAP 15	GAP 16
pH3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pH4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pH5	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
pH6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
pH7	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+
pH8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
pH9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
pH10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Some biochemical characteristics (API20E) of bacterial isolates are summarized in Table 3, including which growth medium is suitable for their growth.

Table 3: Biochemical characteristics of bacteria (API20E).

API20E Tests	GAP 1	GAP 2	GAP 3	GAP 4	GAP 5	GAP 6	GAP 7	GAP 8	GAP 9	GAP 10	GAP 11	GAP 12	GAP 13	GAP 14	GAP 15	GAP 16
GEL	-	-	+	+	-	+	-	-	+	+	-	+	-	-	+	-
GLU	+	-	+	-	-	-	+	-	+	-	-	-	+	-	-	-
MAN	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
INO	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
SOR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RHA	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
SAC	+	-	+	-	-	-	+	-	+	-	-	-	+	-	-	-
MEL	+	-	-	-	+	-	+	-	-	-	+	-	+	-	-	+
AMY	+	-	+	-	-	-	+	-	+	-	-	-	+	-	-	-
ARA	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
ONPG	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
ADH	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	+
LDC	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+
ODC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CIT	+	-	-	-	+	-	+	-	-	-	+	-	+	-	-	+
H2S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
URE	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+
TDA	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+
IND	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VP	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-

When the biochemical test results of the bacteria obtained in the study in Genbank are examined, it is seen that they confirm each other (https://www.ncbi.nlm.nih.gov/genbank/).These results are used to confirm the accuracy of the bacteria.

16S rRNA gene sequence analysis results of isolates are given in Table 4. The 16S rRNA partial gene sequences generated in this study have accession numbers MZ097346, MZ097347, MZ097348, MZ097349, MZ097350, MZ097351, MZ097352, MZ097353, MZ097354, MZ097355, MZ097356, MZ097357, MZ097358, MZ097359, MZ097360 and MZ097361, respectively. In the Table 4, the isolate name is the code that we gave in this study, the bacterium name is from the 16S identifiation, accession numbers are provided from GenBank and the similarity score is presented from GenBank for each strain identification.

As seen in Figure 1, cry gene analyzes were made and *cry 1* gene was only found in 8 bacteria, and *cry 3* gene in 3 bacteria. *cry 2* and *cry 4* genes were not observed in any bacteria. *cry 1* gene has 277 bp and *cry 3* gene has 604 bp. In the first lane (M) there is marker (1kb) and the bands are 250 bp, 500 bp, 750 bp, 1000bp, 1500 bp, 2000 bp and 2500 bp, respectively (from the bottom to top). The bands under the marker bands, which are seen in all four gel images (A, B, C and D), are the bands belonging to the primers, and their appearance indicates the quality of the gel. According to these results and looking at the marker, the bands corresponding to around 250 bp in A shows the presence of *cry1* gene. According to the results, bands seen around 500 bp in the B also indicates the presence of *cry3* gene. In addition, one sample of each of these bands was sent to the sequence and were confirmed. On the other hand, the bands seen in the gel images of *cry 2* and *cry 4* genes belong to the primers. As can be seen, these bands are below the marker bands.

Table 4. 16S results of bacteria.

Isolate name	Bacterium name	Accession number	Similarity
GAP1	*Bacillus cereus*	MZ097346	99%
GAP2	*Bacillus subtilis*	MZ097347	99%
GAP3	*Bacillus wiedmannii*	MZ097348	99%
GAP4	*Bacillus megaterium*	MZ097349	99%
GAP5	*Pantoea rodasii*	MZ097350	99%
GAP6	*Bacillus nakamurai*	MZ097351	99%
GAP7	*Bacillus mobilis*	MZ097352	99%
GAP8	*Bacillus pacificus*	MZ097353	99%
GAP9	*Bacillus thuringiensis*	MZ097354	99%
GAP10	*Lysinibacillus fusiformis*	MZ097355	99%
GAP11	*Bacillus vallismontis*	MZ097356	99%
GAP12	*Paenibacillus odorifer*	MZ097357	99%
GAP13	*Bacillus velezensis*	MZ097358	99%
GAP14	*Bacillus flexus*	MZ097359	99%
GAP15	*Bacillus paramycoides*	MZ097360	99%
GAP16	*Lysinibacillus sphaericus*	MZ097361	99%

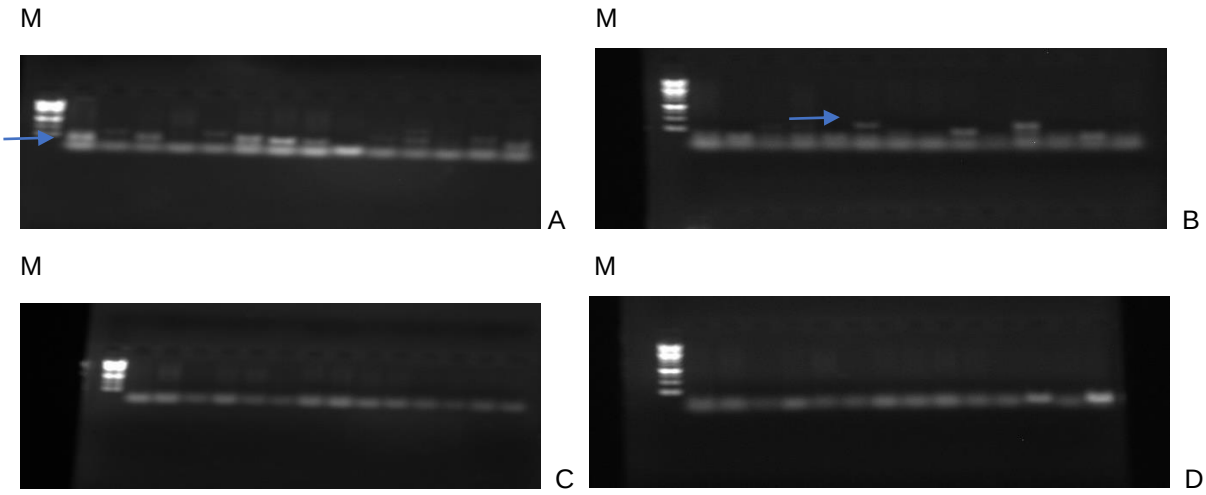

Figure 1: *cry* gene analysis of bacteria. A: *cry 1* gene PCR results, B: *cry 3* gene PCR results, C: *cry 2* gene PCR results, D: *cry 4* gene PCR results.

DISCUSSION

As a result of the study, 12 bacteria belonging to the genus *Bacillus*, two bacteria belonging to the genus *Lysinibacillus*, one bacterium belonging to the genus *Paenibacillus* and one bacterium belonging to the genus *Pantoea* were obtained. *Pantoea* species, which are common in plants and soil, belong to the Enterobacteriaceae family. *Pantoea* species are members of the normal flora of the human gastrointestinal tract and can be found in water, wastewater, soil, plants and foods such as fruit/vegetables. Many *Pantoea* species are known as plant disease agents and are used as biopesticides in the agricultural industry (Cruz et al. 2007). *Pantoea* species, known as plant pathogens, are microorganisms that develop following injuries with plant spines, and the most frequently isolated species is *Pantoea rodasii*, which was also identified from the GAP5 isolate (Kurşun et al. 2012).

The *Paenibacillus* genus includes some strains previously found in the *Bacillus* and *Clostridium* genera. *Paenibacillus odorifer* bacteria identified in GAP12 isolate are found in soil, water and various foods (Beno et al. 2020).

Members of the Bacillaceae family, which are important in terms of biological control, are Gram-positive, motile or non-motile rod-shaped bacteria that produce endospores. *Bacillus* and *Clostridium* genera in this family contain important insect pathogen species and are mostly separated from each other according to their oxygen needs. Species belonging to the genus *Bacillus* are aerobic or facultative anaerobic, while species belonging to the genus *Clostridium* are anaerobic. Both genera have rod-shaped cells that form chains (Tanada and Kaya 1993). Insect pathogens known in the genus *Clostridium* reproduce only in the insect gut and cause disease and never pass into insect hemocele. The genus *Bacillus* contains important insect pathogenic species. The most important of these is *Bacillus thuringiensis* bacteria, which is in the *Bacillus cereus* group. *B. thuringiensis* is a spore-forming soil bacterium that produces toxin in crystalline structure and has an insecticidal effect mostly against insects in Lepidoptera, Diptera and Coleoptera groups (Beegle and Yamamoto 1992). According to studies conducted in recent years, it has been found to be lethal among the insect groups of Hymenoptera, Homoptera, Orthoptera and Mallophaga, as well as on nematodes, ticks and protozoa (Feitelson et al. 1992, Feitelson 1993). The fact that insecticidal products obtained from *B. thuringiensis* bacteria do not cause infection on humans, non-target organisms and beneficial insects has increased the effective use of these products in the control harmful insects (Lacey et al. 2001, Seigel 2001). *B. thuringiensis*-derived products constitute 95% of the world biopesticide market. Many commercial companies have introduced products from *B. thuringiensis*. According to 1998 figures, more than 200 products of *B. thuringiensis* origin are used against pests only in the

USA (Schnepf et al. 1998). In addition, many *B. thuringiensis*-derived products are susceptible to synthetic chemical pesticides, obtained at a lower cost. Some other species belonging to the genus Bacillus are also used in the control of harmful insects. *Bacillus popilliae* (Dutky) is used in the control of some species belonging to the Scarabaeidae family, while *Bacillus sphaericus* Neide is used in the control of mosquito larvae (Klein and Jackson 1992). *B. popilliae* needs to be produced *in vivo*, and lower than expected levels of infection in many field applications reduces the potential of this bacterium to be used in large areas (Klein and Kaya 1995). Compared to *B. thuringiensis*, *B. sphaericus* is resistant to polluted habitats and environmentally friendly. Although it is more resistant to various factors, its biggest disadvantage is that the host spectrum is very narrow (Lacey and Undeen 1986, Charles et al. 1996, Nicolas et al. 1994). However, some fly species are resistant to this bacterium. It has been reported (Rao et al. 1995, Nielsen-Leroux et al. 1997).

The most important factor in *Bacillus* species are crystal (*cry* genes) genes. In the study, 16 *Bacillus* genus bacteria were obtained and *cry* gene analyzes were performed. In this context, the bacteria obtained are of great importance. According to the results of *cry* gene analysis 8 bacteria have *cry1* genes and 3 bacteria have *cry3* genes were obtained in bacteria. *cry2* and *cry4* genes could not be obtained in these bacteria. The most common *cry* gene types in nature are *cry1* and *cry3*. According to these results, the potential of using these bacteria against bee pests seems high.

For the honeybees to be healthy, under the chemical treatment practices, antibiotics are frequently used against bacterial diseases (Mutinelli, 2003). Antibiotic use weakens the immune system of the bees and leads to antibiotic-resistant bacterial pathogens (Doğaroğlu and Samancı, 2006; Barganska et al. 2011). Unfortunately, the fight against these bacteria is self-defeating. In previous studies, positive effects have been shown by the resistance of the bacterial flora in the bodies of bees against disease (Gilliam, 1997).Thus, the idea arises that if the naturally occurring microbial flora in the bodies of the bees are supported, the bees may be more resistant to disease (Tajabadi et al. 2013) In particular, the bacteria with probiotic properties found in the honey stomachs or intestines of honeybees have been observed to provide resistance against other bee pathogens (Forsgren et al. 2010).

In this study, it was aimed to determine the general bacterial flora of honey bees and the optimal growth conditions. We found that bacteria can be partially detected by using selective growth media.

Both similar and different bacteria were obtained previously from the honey bee intestinal flora, so we suspect that there could be regional differences of honey bee gut bacteria based on their floral diet selection (Yarılgaç, 2016). For future research, we aim to obtain and characterize local isolates to determine their biological significance in terms of improving honey bee health.

Conclusion

As a result, considering the definitions of the bacteria obtained in the study, 8 bacteria with the *cry 1* gene and 3 bacteria with the *cry 3* gene have the potential to be used in future biopesticide development studies. GAP6 (*Bacillus nakamurai*) bacteria, in which both *cry* genes (*cry 1* and *cry 3*) are common, should be studied in more detail. It is thought that it may have the potential to be a more potent biopesticide with the activity of both *cry* genes.

According to isolated bacteria and gene analyses, these bacteria should be tested primarily for use in maintaining honey bee health. Previously, some of these bacteria have been effective for manageing *Varroa* mites and especially *Galleria mellonella* pests. In addition to honey bee pests, future research can be carried out to develop bacterial biopesticides for different agricultural and forest pests in general.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

PHYSICOCHEMICAL ANALYSIS OF SUNFLOWER HONEY FROM BULGARIA

Bulgaristan Ayçiçek Ballarının Fiziko-Kimyasal Analizi

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ABSTRACT

Physicochemical properties of 27 sunflower honey samples from Bulgaria were investigated. The botanical origin of the samples was ascertained by pollen analysis. The honey samples displayed relative frequencies of *Helianthus annuus* L. pollen up to 41%. The ranges for water content (15.60–19.30%), reducing sugars (72.51–80.80%), sucrose (0.50–3.70%), diastase (9.00–20.80 Gothe units), hydroxymethylfurfural (HMF), (0.69–12.40 mg/kg), total acidity (17.70–36.00 meq/kg), electrical conductivity (0.23–0.48 mS/cm), proline (218.50 – 679.50 mg/kg), specific rotation (-20.20–(-12.30)) $[\alpha]_D^{20}$ were obtained. The results obtained also suggest that these honey samples are of good quality. The results are in agreement with standards of quality established by national and international regulations. Significant moderate correlation between electrical conductivity and specific rotation was found (r=0.582, p<0.05).

Keywords: Honey, sunflower honey, physicochemical properties, quality parameters

ÖZ

Bulgaristan'dan alınan 27 ayçiçeği balı örneğinin fiziko-kimyasal özellikleri araştırıldı. Örneklerin botanik kökeni polen analizi ile belirlendi. Bal numuneleri, %41'e varan oranlarda *Helianthus annuus* L. polen sıklığı göstermiştir. Su içeriği (%15.60 – 19.30), indirgeyici şekerler (%72.51 – 80.80), sakaroz (%0.50 – 3.70), diastaz (9.00 – 20.80 Gothe birimleri), hidroksimetilfurfural (HMF), (0.69 – 12.40mg/kg) için aralıklar), toplam asitlik (17.70 – 36.00meq/kg), elektriksel iletkenlik (0.23 – 0.48 mS/cm), prolin (218.50 – 679.50 mg/kg), spesifik rotasyon (-20.20 – (-12.30)) $[\alpha]_D^{20}$ elde edildi. Elde edilen sonuçlar da bu bal örneklerinin kaliteli olduğunu göstermektedir. Sonuçlar, ulusal ve uluslararası düzenlemeler tarafından belirlenen kalite standartları ile uyumludur. Elektriksel iletkenlik ile özgül rotasyon arasında orta düzeyde anlamlı korelasyon bulundu (r=0,582, p<0,05).

Anahtar Kelimeler: Bal, ayçiçeği balı, fiziko-kimyasal özellikler, kalite parametreleri

GENİŞLETİLMİŞ ÖZET

Giriş: Çiçek balı, bal arıları (*Apis mellifera* L.) tarafından bitkilerin nektarından üretilen doğal bir maddedir. Bal, tek çiçekli bitki veya çok bitki kaynağı olarak sınıflandırılır. Tek çiçekli bitki balı, ağırlıklı olarak nektar ve polen içeren bir bitki türü tarafından üretilir. Farklı çiçekli bitki balları, tipik melissopalinolojik ve fizikokimyasal özellikler gösterir. Çok kaynaklı bitki balı, hiçbiri baskın olmayan çeşitli bitkilerden nektar ve polenlerden üretilir. Bu çalışma, Bulgaristan'da üretilen ayçiçeği balının fizikokimyasal parametrelerini değerlendirmeyi amaçlamaktadır. Bulgaristan'dan alınan 27 ayçiçeği balı örneğinin fizikokimyasal özellikleri araştırıldı.

Gereç ve Yöntem: Bal örnekleri oda sıcaklığında cam kaplarda muhafaza edilmiştir. Çalışmada 2017 ve 2018 arıcılık sezonlarında hasat edilen arı balının temsili örnekleri kullanılmıştır. Örneklerin botanik kökeni polen analizi ile tespit edilmiştir. Bal numuneleri, %41'e varan oranlarda *Helianthus annuus* L. polen sıklığı göstermiştir. Bulgaristan Arı Balı Devlet Standardı 2673-80'e göre *H. annuus*'tan %40'a kadar polen içeren bal örnekleri ayçiçeği balı olarak sınıflandırılabilir. Analizler, numunelerin laboratuvara teslim edildiği tarihten itibaren bir ay içinde yapılmıştır. Polen analizi ve fizikokimyasal analizler Bulgaristan, Sofya'daki Veteriner Kontrol ve Ekoloji Merkez Laboratuvarında yapıldı.

Bulgular: Ayçiçeği balının şu parametreleri Avrupa Bal Komisyonu tarafından önerilen yöntemlere göre belirlendi: su içeriği, indirgeyici şekerler, sakaroz, diastaz, HMF, toplam asitlik, elektriksel iletkenlik, prolin ve spesifik rotasyon. Su içeriği (%15.60 – 19.30), indirgeyici şekerler (%72.51 – 80.80), sakaroz (%0.50 – 3.70), diastaz (9.00 – 20.80 Gothe birimleri), hidroksimetilfurfural (HMF), (0.69 – 12.40mg/kg) için aralıklar), toplam asitlik (17.70 – 36.00meq/kg), elektriksel iletkenlik (0.23 – 0.48 mS/cm), prolin (218.50 – 679.50 mg/kg), spesifik rotasyon (-20.20 – (-12.30)) elde edildi. Spesifik optik rotasyon, bal örneklerinin çiçek balından geldiğini, dönen negatif olduğunu (laevorotatory) gösterir. Numunelerin çoğu 350 mg/kg'a kadar prolin değerlerine sahiptir. HMF ortalama 2.82 mg/kg olarak belirlendi. Elde edilen sonuçlar da bu bal örneklerinin kaliteli olduğunu göstermektedir. Sonuçlar, ulusal ve uluslararası düzenlemeler tarafından belirlenen kalite standartları ile uyumludur. Elektriksel iletkenlik ile özgül rotasyon arasında orta düzeyde anlamlı korelasyon bulundu (r=0,582, p<0,05).

Sonuç: Sonuç olarak, tüm bal örnekleri %40'tan fazla *H. annuus* polen içerir ve ayçiçeği balı olarak sınıflandırılabilir. Ayçiçeği balının su içeriği, indirgeyici şekerler, sakaroz, diastaz, HMF, toplam asitlik, elektriksel iletkenlik, prolin ve özgül rotasyon parametreleri uluslararası yönetmeliklerle belirlenen kalite parametrelerini karşılamaktadır. Bu çalışma, Bulgaristan'dan ayçiçeği balının karakterizasyonu için faydalı bilgiler sağlamaktadır.

INTRODUCTION

Blossom honey is a natural substance produced by honey bees (*Apis mellifera* L.) from the nectar of plants. Honey is classified as unifloral or polyfloral. The unifloral honey is produced by one plant species containing predominantly its nectar and pollen. The different unifloral honeys show typical melissopalynological and physicochemical properties. The polyfloral honey is produced from nectar and pollen from various plants, none of which is predominant. In general, unifloral honeys are regarded as more valuable products. They have good quality and specific sensorial characteristics. Furthermore, market prices of honey are determined by its botanical origin. It can be said that monofloral honeys are more expensive than mixed polyfloral honeys. This is due to their pharmacological and organoleptic properties (Silva et al. 2009, Bilandžić et al. 2017). Thus, great attention has been paid for identification of unifloral honey by researchers (Wen et al. 2017). Honey is becoming an effective therapeutic agent by practitioners of conventional medicine due to its chemical composition. In this respect the composition of honey depends on its floral origin (De-Melo et al. 2018, Valdés-Silverio et al. 2018). According to Ahmed et al. (2016) and Saxena et al. (2010) honey includes a lot of substances. The main constituents of honey are carbohydrates and water. Phenolic compounds, amino acids, vitamins, minerals and enzymes are also presented in honey.

In Bulgaria there are low levels of local consumption of bee honey – under 200 g on average per capita compared to some countries in Western Europe (about 1 kg on average per year). Bulgarian beekeeping has a traditionally export character. According to Agro-statistics of the Ministry of agriculture and food the export of Bulgarian honey

for the period 2015 – 2017 increased from 9784 to 13302 tons per year (Aleksiev 2019). In Bulgaria very often the sunflower honey is mixed with polyfloral honey and it is sold as polyfloral honey.

Different unifloral honey types are produced in Europe. In Bulgaria, one of the most important honey types are black locust honey (*Robinia pseudoacacia*), lime honey (*Tilia spp.*), rape honey (*Brassica spp.*), sunflower honey (*H. annuus*). Some honey types such as black locust, lime, rape coriander, fennel honey were studied and described by Atanassova et al. (2012), Dinkov (2014) while data for others such as sunflower honey is insufficient. Sunflower is largely cultivated in many European countries because it represents to bees nectar and pollen (Persano Oddo and Piro 2004). To date, several studies have attempted to elucidate the sunflower honey produced in Bulgaria (Nikolova et al. 2014, Atanassova et al. 2012). The present study aims to evaluate physicochemical parameters of sunflower honey produced in Bulgaria. The obtained results were compared with national (Bulgarian State Standard for Bee Honey 3050-80 and Bulgarian State Standard 2673-80) and international regulations (Council Directive 2001/110 relating to honey (2002) and Codex Alimentarius (2001)).

MATERIALS AND METHODS

In this study, 27 sunflower honey samples were obtained mainly from Northwest, North and West Bulgaria. The honey samples (about 500 g) were kept in glass containers at room temperature. The study used representative samples of bee honey harvested in beekeeping seasons 2017 and 2018. The analyses were performed within one month from the date of receiving the samples at the laboratory. The pollen analysis was carried out by Bulgarian State Standard for Bee Honey 3050-80 and Bulgarian State Standard 2673-80. According to Bulgarian State Standard for Bee Honey 2673-80 honey samples with more than 40% *H. annuus* pollen is sunflower honey. The pollen analysis and physicochemical analyzes were done at the Central Laboratory of Veterinary Control and Ecology, Sofia, Bulgaria.

The physicochemical parameters water content, diastase, reducing sugars and sucrose, hydroxymethylfurfural, total acidity and electrical conductivity, proline content and specific rotation were determined according to the European Honey Commission recommended methods (Bogdanov et al. 1997):

-Water content is determined by the refractometric method (Abbe refractometer);

-Determination of diastase activity is after Schade method;

-For reducing sugars and sucrose is used Fehling's reagent;

-Hydroxymethylfurfural was determined after White (in mg/kg);

-Total acidity (meq/kg) by titration with 0.1 N sodium hydroxide with phenolphthalein indicator;

-Electrical conductivity was measured in 20% weight volume in water (the results are expressed in mS/cm);

-Proline content was determined spectrophotometrically. Proline stock solution with 0.8 mg/mL concentration was prepared.

Statistical analysis of the results was performed using SPSS 20.0 for Windows. Correlations between results were made using the Pearson's correlation coefficient (r), ($p<0.05$). All results are presented as minimal and maximal value, means ± standard deviation.

RESULTS

Melissoplaynological analysis is based on the identification of pollen by microscopic examination, and it needs highly specialized personnel. Another limitation of the method is that sometimes the pollen grains have similar morphologies and it is difficult to be recognized (Bogdanov et al. 2004). In many cases the melissoplaynological analysis presents the dominant pollen type in the honey. In general, all honey samples could be considered as sunflower honey according to their pollen content (up to 41% *H. annuus* pollen). According to Bulgarian State Standard for Bee Honey 2673-80 honey samples with up to 40% pollen samples from *H. annuus* can be classified as sunflower honey. The main species are presented in Table 1. The *H. annuus* pollen varies in the ranges 41 – 80%.

Table 1. Pollen analysis of studied honey samples (n=27)

Honey samples	Main species or family, %±SD
1	*Asteraceae* 80.1±4.2 from them *Helianthus annuus* – 72.1±3.4
2	*Asteraceae* 40.4±2.1 from them *Helianthus annuus* – 43.3±1.7
3	*Asteraceae* 84.5±4.4 from them *Helianthus annuus* – 71.8±4.3
4	*Asteraceae* 52.8±1.2 from them *Helianthus annuus* – 41.7±0.5
5	*Asteraceae* 63.8±3.3 from them-*Helianthus annuus* – 60.7±3.2
6	*Asteraceae* 70.3±3.7 from them-*Helianthus annuus* – 69.0±3.6
7	*Asteraceae* 52.9±2.8 from them *Helianthus annuus* – 48.7±2.5
8	*Asteraceae* 57.1±3.0 from them *Helianthus annuus* – 49.3±2.6
9	*Asteraceae* 74.9±3.9 from them *Helianthus annuus* – 73.9±3.8
10	*Asteraceae* 49.5±2.6 from them *Helianthus annuus* – 48.4±2.5
11	*Asteraceae (Helianthus annuus* – 69.3±4.6); *Apiaceae* (*Coryandrum sativum* – 3.8±0.2); *Brassicaceae* –2.5±0.2
12	*Asteraceae (Helianthus annuus* – 52.1±4.3); *Asteraceae* (*Cardus nutans* – 8.0±0.4)
13	*Asteraceae (Helianthus annuus* – 64.7±3.4); *Brassicaceae* – 9.0±0.5; *Apiaceae* (*Coryandrum sativum* –7.7±0.4)
14	*Asteraceae (Helianthus annuus* – 62.1±1.3); *Apiaceae* (*Coryandrum sativum* – 4.5±0.2); *Asteraceae* (*Cardus nutans* – 4.2±0.2)
15	*Asteraceae (Helianthus annuus* – 62.1±3.3)
16	*Asteraceae (Helianthus annuus* – 49.5±2.5)
17	*Asteraceae (Helianthus annuus* – 63.9±1.3)
18	*Asteraceae (Helianthus annuus* – 50.0±2.5)
19	*Asteraceae (Helianthus annuus* – 74.1±1.7)
20	*Asteraceae (Helianthus annuus* – 52.5±1.8)
21	*Asteraceae (Helianthus annuus* – 57.1±2.3)
22	*Asteraceae (Helianthus annuus* – 70.2±4.1)
23	*Asteraceae (Helianthus annuus* – 66.2±2.0)
24	*Asteraceae (Helianthus annuus* – 71.5±3.6)
25	*Asteraceae (Helianthus annuus* – 55.4±1.7)
26	*Asteraceae (Helianthus annuus* – 57.9±2.0)
27	*Asteraceae (Helianthus annuus* – 72.7±1.4)

Table 2 presents the results of the analysis of the honey samples: the average values and standard deviation of the physicochemical parameters (water content, reducing sugars, sucrose, diastase, HMF, total acidity, electrical conductivity).

Table 2. Physicochemical parameters of sunflower honey, n=27

Parameters	Min	Max	Mean±SD
Water content, %	15.60	19.30	17.76±0.96
Reducing sugars, %	72.50	80.80	76.15±2.60
Sucrose, %	0.50	3.70	1.72±1.01
Diastase, Gothe units	9.00	20.80	14.50±3.94
Hydroxymethylfurfural (HMF), mg/kg	0.69	12.40	2.82±2.65
Total acidity, meq/kg	17.70	36.00	23.73±7.23
Electrical conductivity, mS/cm	0.23	0.48	0.32±0.07

Taking into consideration that the honey water content has to be lower than 20% in general (Council Directive 2001/110 relating to honey, 2002 and Codex Alimentarius, 2001), the values obtained in this study were satisfactory. They ranged from 15.60 to 19.30%.

The main reducing sugars in honey are glucose and fructose. The results for reducing sugars were considered to be sufficient for identification of honey quality. The mean value and standard deviation for the reducing sugars and sucrose are determined as 76.15±2.60% and 1.72±1.01%, respectively. The results for the minimal and maximal values are presented in Table 2.

According to international regulation (Council Directive 2001/110 relating to honey (2002)) diastase activity must not be less than 8 Gothe units. All results are up to this value. In the present study the diastase activity varied by a large range (Table 2).

Hydroxymethylfurfural (HMF) was determined as 2.82 mg/kg on average. The highest value is under 13 mg/kg.

The mean total acidity value was below 50 meq/kg of honey and satisfied the European regulation for this parameter (Council Directive 2001/110 relating to honey (2002). The total acidity ranged from 17.70 to 36.00 meq/kg.

As expected, sunflower honey had the lower values of electrical conductivity (average 0.32 mS/cm). Significant moderate correlation between electrical conductivity and specific rotation was found ($r=0.582$, $p<0.05$).

The measured proline content of sunflower honey is shown on Figure 1. In the present study the proline content varies in very large ranges 218.50 – 679.50 mg/kg. The average values and standard deviation are 404.94±144.78 mg/kg. As can be seen from Figure 1 most of the samples have proline values up to 350 mg/kg.

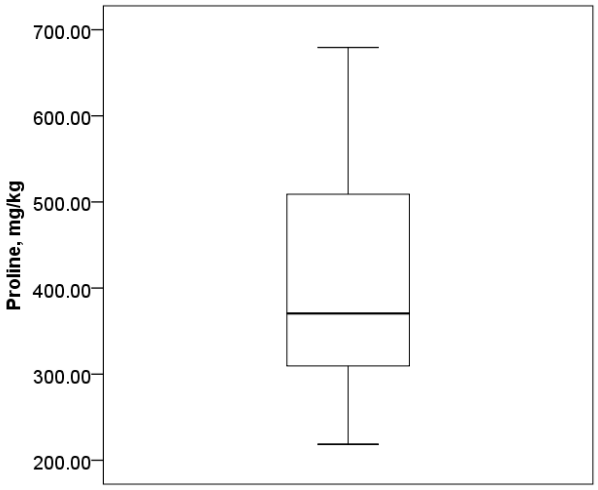

Figure 1. Box plot diagram of proline content in sunflower honey samples. Minimal, maximal and median values are presented.

Specific rotation of sunflower honey samples ranged from -20.20 to -12.30 $[\alpha]_D^{20}$ and mean value ± standard deviation (-17.23±2.43 $[\alpha]_D^{20}$). Sample 14 (value 22.20) is an outlier. The results are shown on Figure 2.

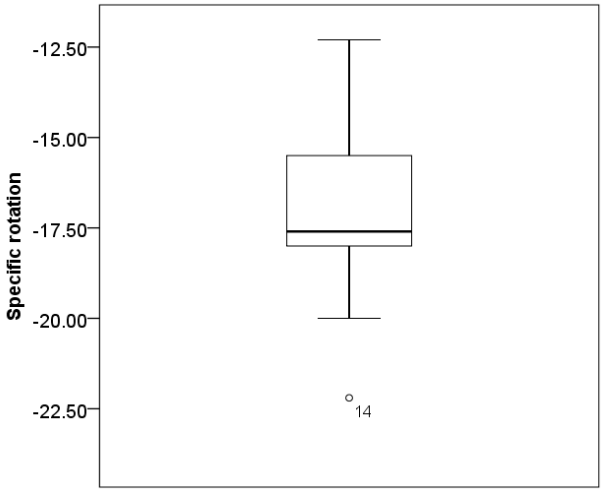

Figure 2. Box plot diagram of specific rotation on sunflower honey samples. Minimal, maximal and median values are presented

DISCUSSION

Melissopalinological analysis is very important tool in the analysis of honey. Pollen analysis is generally used to determine and confirm the botanical origin of the honey samples. The *H. annuus* pollen was

predominant in all honey samples, accounting for more than 40% pollen grains. Oroian and Ropciuc (2017) reported that their samples had around 60% of *H. annuus* pollen. In a recent study, Pauliuc and Oroian (2020) presented more than 45% pollen grains of *H. annuus* (ranges 50 – 92% pollen grains).

Water is quantitatively one of the most important components of honey. This parameter describes honey quality. Honeys with high levels of water (more than 20% in general) tend to ferment more easily. Its content can affect the storage of honey. Furthermore, the water content in honey depends on beekeeping practices and environmental conditions. Also, it can vary from year to year. The average value (17.76±0.96%) is in accordance with the results of Isopescu et al. (2014) for sunflower honey samples.

The sugar content mainly depends on nectar of the flowers used by the bees. Therefore, it varies in the different honey type. In this respect, the content of some sugars and the ratios between them are used to determine honey authenticity (Borrás et al. 2014). Some geographical and climatic conditions can also affect sugar composition of honey (Mărghitaș et al. 2009, Kaskonienė et al. 2010). Reducing sugars are the most abundant sugars in floral honey samples. The sucrose content is under the limits presented by Council Directive 2001/110 relating to honey (2002) and Codex Alimentarius (2001). Gropoşilă-Constantinescu et al. (2020) presented 74.8% reducing sugars for commercial sunflower honey samples. This result is very similar to the average value in the present study. Sahinler et al. (2009) reported higher values for sucrose content in sunflower honey (6.46±0.78%) which is higher than the maximal value for the Bulgarian sunflower honey samples (3.70%).

Enzyme diastase is derived from the glandular secretions of the honey bees. Diastase activity is one of the most important quality parameter. Very often it is used to determine if honey has been heated during storage. Diastase activity loss occurs as temperature increases. The average diastase activity in this study is comparable to those presented by Gropoşilă-Constantinescu et al. (2020) for sunflower honey (about 12 Gothe units). Juan-Borrás et al. (2014) evaluated the influence of the country origin on some physicochemical parameters including diastase activity. Their results for diastase activity in sunflower honey samples from Spain, Romania and Czech Republic are very similar to the results in the present study. The diastase activity varies not only according to its botanical origin and country origin but also due to high temperature and storage. Eremia et al. (2019) reported large ranges for diastase activity (15.02 – 30.69 Gothe units).

Hydroxymethylfurfural (HMF) can be found in low concentrations in honey. It is produced from fructose in the presence of free acids. The production of HMF depends on the temperature (Da Silva et al. 2016). Thermal treatment of honey can generate toxic HMF, thereby resulting in quality reduction. For the sunflower honey is typical quick crystallization (Persano Oddo and Piro 2004). This is due to high glucose content in this honey type. Glucose may crystallize at room temperature. For this reason, sometimes the sunflower honey is liquefied at high temperature. This over heating produces HMF. Based on the Codex Alimentarius (2001) and Council Directive 2001/110 relating to honey (2002), HMF level should not exceed 40 mg/kg. The obtained results for HMF are close to those previously reported by Sakača et al. (2019) and Pauliuc and Oroian (2020). The average HMF value and standard deviation (1.19±1.12 Gothe units) reported by Sakača et al. (2019) are consistent with our results. Juan-Borrás et al. (2015) found higher HMF values (37.4 mg/kg, 37.9 mg/kg, and 39.8 mg/kg) in sunflower honey. The authors noted that these values are unacceptable for raw honey. These outlying values are not frequent.

Acidity of honey depends on the presence of organic acids and inorganic ions. Acid measurement in honey evaluates honey fermentation (Belay et al. 2013). Lower value of acid indicates absence of fermentations. The average value of total acidity is comparable to this presented by Kivrak et al. (2017) for sunflower honey.

Values for electrical conductivity higher than 0.80 mS/cm are not typical for nectar honeys according to Codex Alimentarius (2001). The obtained minimal and maximal values are in agreement with the result presented by Persano Oddo and Piro (2004). The correlation between electrical conductivity and specific rotation was also supported by Belay et al. (2013) and Pridal and Vorlova (2002).

In the present study most of the samples have proline values up to 350 mg/kg. These results are confirmed by Wen et al. (2017). They received very similar data. For example, the range of proline content in sunflower honeys is from 214.06 to 601.11 mg/kg. Czipa et al. (2012) found higher values for

proline in sunflower honey. Large proline variation for sunflower honeys is typical. Although proline content has been considered as a useful parameter of honey quality, other parameters can also be used for honey quality identification. International regulations of honey quality do not present information for the proline content in honey. The proline content varied in the different honey types (Cotte et al. 2004, Keckes et al. 2013, Wen et al. 2017).

Specific optical rotation is a parameter which may distinguish blossom honeys (negative values) and honeydew honeys (positive values), (Persano Oddo and Piro 2004). The concentration of various sugars are responsible for specific rotation of honey. The results obtained indicate that honey samples were from blossom honey, rotating negative (laevorotatory). Significant moderate correlation between electrical conductivity and specific rotation was found ($r=0.582$, $p<0.05$).

Conclusion

All honey samples have more than 40% *H. annuus* pollen and can be classified as sunflower honey. The parameters water content, reducing sugars, sucrose, diastase, HMF, total acidity, electrical conductivity, proline and specific rotation of sunflower honey satisfied the quality parameters established by international regulations. This study provides useful information for characterization of sunflower honey from Bulgaria.

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Author contribution: Vanya Manolova, Ivayla Parvina, Todorka Yankovska – Stefanova, Ralitsa Balkanska collected the honey samples. Vanya Manolova and Ralitsa Balkanska designed the study and carried out the experiments. Ralitsa Balkanska wrote the manuscript.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

ANTIBACTERIAL EFFECTS OF ANATOLIAN PROPOLIS ON *PAENIBACILLUS LARVAE*

Anadolu Propolisinin *Paenibacillus larvae* Üzerine Antibakteriyel Etkisi

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ABSTRACT

***Paenibacillus larvae (P. larvae)* is a pathogenic bacterium causing American Foulbrood Disease (AFB) in honeybee larvae. It is necessary to develop alternative strategies for the control of this disease due to the serious honeybee colonies loses and the use of antibiotics. Recent studies are aimed at the investigating natural fighting agents against *P. larvae*. In our study, it was aimed to demonstrate potential antibacterial efficacy of ethanol extract of Anatolian Propolis (EAP) against *P. larvae* strains PB35 and SV35 which were isolated in Turkey. The total phenolic content (TPC) and flavonoid content (TFC) of EEAP were determined as 181.73±5.20 mg Gallic Acid Equivalents (GAE)/g, and 42.33±1.40 mg Quercetin Equivalents (QE)/g, respectively. It was found that EAP contains different amounts of ferulic, caffeic, coumaric acids, rutin, and caffeic acid phenethyl ester (CAPE). The antibacterial activity of the EAP was determined by using agar-well diffusion, microdilution, and Bioscreen C techniques. The Minimal Inhibition Concentration (MIC) values of the EAP were determined as 74.87 µg/ml against strain PB35 and SV35 using both microdilution and Bioscreen C teqnique. In both techniques, Minimal Bactericidal Concentration (MBC) values of the EAP were evaluated as 149 and 598.4 µg/ml against strain PB35 and SV35, respectively. The fact that EAP shows low concentrations of bacteriostatic (MIC) and bactericide (MBC) activity values against *P. larvae* strains, spore-forming bacilli, which are agents of AFB disease, suggests that it may be a potential source in AFB disease control.**

Keywords: American Foulbrood, Anatolian Propolis, MBC, MIC, *Paenibacillus larvae*

ÖZ

***Paenibacillus larvae (P. larvae)*, bal arısı larvalarında Amerikan Yavru Çürüklüğü hastalığına (AYÇ) neden olan patojenik bir bakteridir. *P. Larvae* tedavisinde kullanılan antibiyotikler bal arısı kolonilerinin ciddi kaybına neden olmakta ve bu nedenle antibiyotik kullanımına bağlı olarak bu hastalığın kontrolü için alternatif stratejiler geliştirmek gerekmektedir. Son çalışmalar, *P. larvae*'ya karşı doğal mücadele ajanlarını araştırmaya yöneliktir. Çalışmamızda Anadolu propolisi etanolik ekstresinin (EAP)**

Türkiye'de izole edilen *P. larvae* suşları PB35 ve SV35'e karşı potansiyel antimikrobiyal etkinliğinin ortaya konması amaçlanmıştır. EAP, toplam fenolik madde (TPC), flavonoid madde miktarları (TFC) ve bazı fenolik bileşikler açısından karakterize edilmiştir. Analizlere göre, EAP'nin total fenolik madde miktarı ve flavonoid madde miktarları sırasıyla 181,73±5,20 mg Gallik Asit Eşdeğeri (GAE)/g TFC olarak 42,33±1,40 mg Kuersetin Eşdeğeri (QE)/g ve farklı miktarlarda ferulik, kafeik, kumarik asitler, rutin ve kafeik asit fenetil ester (CAPE) içerdiği bulundu. EAP'nin antimikrobiyal aktivitesi, agar kuyu difüzyon, mikrodilüsyon ve Bioscreen C teknikleri kullanılarak belirlendi. EAP'nin Minimal İnhibisyon Konsantrasyon (MIC) değerleri, hem mikrodilüsyon hem de Bioscreen C tekniği kullanılarak PB35 ve SV35 suşuna karşı 74,87 µg/ml olarak belirlendi. Her iki teknikte de PB35 ve SV35 suşlarına karşı EAP'nin minimal bakterisidal konsantrasyon (MBC) değerleri sırasıyla 149 ve 598,4 µg/ml olarak belirlendi.

Anahtar kelimeler: Amerikan Yavru Çürüklüğü, Anadolu propolisi, MBC, MIC, *Paenibacillus larvae*

GENIŞLETILMIŞ ÖZET

Amaç: Dünyanın birçok yerinde koloni kaybına, toplu arı ölümlerine ve bal veriminin düşmesine neden olan çeşitli arı hastalıkları bulunmaktadır. Hastalıklar arasında en dikkat çekici olanlardan bir tanesi de Amerikan Yavru Çürüklüğü Hastalığıdır (AYÇ). Hastalık larva aşamasında arının orta bağırsak lümenini etkiler ve yetişkin arı hastalığına neden olmaz. *Paenibacillus* sp., gram pozitif, fakültatif anaerobik, katalaz negatif. endospor oluşturan bakterilerdir. *Paenibacillus larvae* kolonileri küçük, düzenli, çoğunlukla kaba, düz veya kabarık ve beyazımsı ile bej renklidir. Etkilenen larvada bir milyonun üzerinde spor üretebilir ve AYÇ oluşumuna neden olur. Oksitetrasiklin hidroklorür (OTC) gibi antibiyotiklerin kullanılması, etkilenen kolonilerin kaybının önlenmesi ve tedavisi için yaygın bir stratejidir. Ancak, antibiyotiklerin uzun süreli kullanımında çeşitli sorunlar oluşabilmektedir. Son zamanlarda bazı ülkelerde oksitetrasikline dirençli *P. larvae* izolatlarının olduğu birçok çalışmada gösterilmiştir. Bu nedenle, kovanlarda patojenik mikroorganizmaları kontrol etmek için propolis gibi doğal ürünlerin kullanımına artan bir ilgi vardır. Bu çalışmanın amacı, doğal bir kovan ürünü olan etanolik Anadolu propolis ekstraktının (EAP) *P. larvae* suşları (PB35 ve SV35) üzerindeki etkisini in vitro belirlemek ve AFB'nin kontrolü ve önlenmesi için alternatif bir ürün olarak etanolik Anadolu propolis ekstraktının kullanılabilirliğini araştırmaktır.

Gereç ve Yöntem: Yapılan bu çalışmada ham propolis Düzce Üniversitesi Arıcılık Uygulama ve Araştırma Merkezi'nden (DAGEM) 2016 yılında temin edildi. Ham propolis dondurularak öğütüldü ve %70'lik etanol ile ekstrakte edildi. Hazırlanan ekstraktın toplam fenolik madde miktarı Folin-Ciocalteu metoduna göre belirlendi. Ayrıca etanolik propolis ekstraktının toplam flavonoid madde miktarı, antioksidan kapasitesi ve fenolik profili belirlendi. *P. larvae* PB35 ve SV35 suşları morfolojik, biyokimyasal ve moleküler özelliklerine göre tanımlandı. Daha sonra etanolik Anadolu propolis ekstraktının antibakteriyel aktiviteleri, agar-well difüzyon yöntemi kullanılarak *P. larvae* PB35 ve SV35 suşlarına karşı test edildi. Minimal inhibisyon derişimi ve minimal bakteriyosidal derişimi tespit edildi. Etanolik Anadolu propolis ekstraktının *P. larvae* PB35 ve SV35 popülasyonları, gecikme fazları ve üstel fazlar üzerindeki etkileri Bioscreen kullanılarak incelendi.

Bulgular: Yapılan analizler neticesinde etanolik Anadolu propolis ekstraktının total fenolik madde miktarının 181,73±5,20 mg GAE/g, total flavonoid madde miktarının 42,33±1,40 mg QE/g olduğu ve propolis ekstraktının *p*-kumarik asit, ferulik asit, krisin ve pinocembrin bileşenleri açısından zengin olduğu tespit edildi. Etanolik Anadolu propolisinin *P. larvae*'ye ait iki farklı suş üzerine de etkili olduğu belirlendi

Sonuç: Bu çalışmanın sonuçları, etanolik Anadolu propolis ekstraktının AYÇ'yi ortadan kaldırmak için iyi bir potansiyele sahip olduğunu ve *P. larvae* tehdidine karşı doğal bir ilaç olabileceğini göstermektedir. Ayrıca spor oluşturan bir bakteri olan *P. larvae*'nin MBC değerlerinin elde edilmesi, AYÇ hastalık kontrolünde kullanım potansiyeline sahip olduğunu gösteren önemli bir sonuç olarak değerlendirilmektedir.

INTRODUCTION

In many parts of the world, there are various bee diseases which cause loss of colonies, collective bee deaths, and reduced honey yield. One of the

most remarkable of among the diseases is the American Foulbrood Disease (AFB). This disease in many countries "forced notifiable" disease is on the list (Genersch 2010). The disease affects the middle gut lumen at the larval stage and does not cause disease of adult bee. *Paenibacillus* sp. in the Firmicutes Phylum and Bacillales Ordo are gram-positive, facultative anaerobic, catalase negative endospore-forming bacteria. *Paenibacillus larvae* colonies were small, regular, mostly rough, flat or raised, and whitish to beige (Ash et al. 1993). It is a bacterium that can produce over one million spores in effected larva thus AFB occurs. A complete solution for the control and protection of the disease is not offered because bacterial spores can survive for a long time against physical conditions (Hrabák and Martínek 2007, Genersch 2010). Using antibiotics like oxytetracycline hydrochloride (OTC) is a common strategy for the prevention and treatment of affected colonies (Hansen and Brødsgaard 1999). However, there may be various problems with the extended use of these antibiotics. Recently many studies showed to be oxytetracycline resistant *P. larvae* isolates in some countries (USA, Canada, and Argentina) (Alippi 2000, Evans 2003).

Propolis is a natural bee product containing essential oils, waxes, phenolic, and flavonoids. Propolis is collected by honeybees from tree buds, leaf, and other botanical resinous sources for protection of hives from a number of threats. Propolis composition depends on mainly the botanical origin (Kuropatnicki et al. 2013, Baltas et al. 2016). There has been an increasing interest in the usage of natural products like propolis for controlling the pathogenic microorganisms in hives.

The aim of the study is to determine the effect of Ethanol Extract of Anatolian Propolis (EAP) on *P. larvae* strains and is to investigate the availability of EAP as an alternative product for the control and prevention of AFB. Until now, no one has tested Anatolian propolis extract against *P. larvae* using Bioscreen C techniques. All microorganisms (bacteria, mold, yeast, etc.) increase the turbidity of the liquid growth medium when growing and multiplying in it. Bioscreen C monitors this growth by measuring the turbidity of the medium in the wells of a microplate. These measurements are done kinetically and recorded as optical density (OD) measurements (Anonymous 2016). In our study, the values of propolis extract stopping the development of *P. larvae* were determined by monitoring them with Bioscreen C at different concentrations.

MATERIAL AND METHODS

Bacteria Strains and Propolis Sample

Paenibacillus larvae (*P. larvae*) PB35 and SV35 strains were obtained from Bee Diseases Laboratory, Samsun Veterinary Control Institute. The tested Anatolian Propolis sample was obtained from Düzce University Beekeeping Research, Development and Application Center (DAGEM) in Yığılca areas in Turkey, 2016. Raw propolis sample was obtained from directly plastic traps put under hive cover. The propolis sample was kept in a deep freezer (-20°C).

Identification of *P. larvae* strains

P. larvae PB35 and SV35 strains were identified according to their morphological, biochemical, and molecular characteristics. Properties of colony morphologies, Gram staining, endospore staining were used for morphological characterization of bacterial strains (CLSI 2015). For molecular analyses, 16S rRNA gene sequence was performed previously described by Sevim et al. (2017). The 16S rRNA sequences for *P. larvae* isolates were deposited in GenBank (NCBI, Bethesda, MD, USA) under accession numbers MW227606 (PB35) and MW227607 (SV35).

Extraction of Anatolian Propolis

Frozen raw propolis sample was grinded, and 5 g of powdered raw sample was dissolved in 50 mL 70% ethanol in a glass flask (500 mL), stirred on a shaker (Heidolph Promax 2020, Schwabach, Germany) at room temperature for 48 hours and after filtration, the extract was evaporated with a Rotary evaporator at 40^0C (HEIDOLPH Hei-VAP Value Digital G3) and stored at -20°C (Aliyazıcıoglu et al., 2013, Keskin et al. 2020).

Determination of Total Phenolic and Flavonoid Content

Total phenolic content of the Anatolian propolis was carried out according to Folin-Ciocalteu method (Singleton et al. 1999). The results were expressed as mg of gallic acid equivalents per g sample. Total flavonoid was measured by spectrometric assay (Fukumoto and Mazza 2000). The result was expressed as mg of quercetin equivalents (QE) per g sample.

Determination of Ferric Reducing Power Antioxidant Activity

The FRAP method is the most commonly used method for determining the antioxidant capacity of natural products, and it is a method based on the reduction of iron(III) ion in the Fe(III)-TPTZ complex by antioxidants (Benzie and Strain, 1996). Fe(III), which is reduced by the antioxidant substances in the solution, gives absorbtion at 593 nm. Results for FRAP activity were expressed as µmoL $Fe_2SO_4.7H_2O$/g.

Analyses of Phenolic Compounds in HPLC-UV

Phenolic compounds of the propolis were analyzed according to method described by Can et al. (2015). C_{18} analytical column C (150 mm x 4.6 mm, 5 µm; Fortis) was used by gradient elution (Can et al. 2015). 2 % acetic acid in water (A) and acetonitrile: water (70:30) (B) were utilized as mobile phase for elution. 25 µL samples were injected at 30°C and flow rate at 0.75 ml/min. Caffeic acid phenethyl ester (CAPE) compound was carried out the same colon with different analyses conditions (Can et al. 2015). A 0.1% formic acid in water and B 0.1% formic acid in acetonitrile and was monitored at 270 nm at 30°C.

Determination of Antibacterial Activity

Antibacterial activities of EAP were tested against *P. larvae* PB35 and SV35 strains using the agar-well diffusion method. PB35 and SV35 strains were suspended in 3 mL of MYPG broth. These bacterial suspensions were diluted to 10^6 cfu/ ml and then were plated on the surface of MYPG agars. Wells of 5 mm in diameter were cut from the dried agar using a sterile cork-borer. Each well was filled with 50 µL of EAP and the plates were incubated at 37°C for 48 h. Antibacterial activity was evaluated by calculating the clear zone of inhibition. Ampicillin (10 µg) was used as a control antibacterial agent. As a solvent control, 1/2 dilution of analytical grade ethanol was used (Sevim et al. 2017).

Determination of Minimal Inhibition Concentrations Value (MICs)

MICs values were determined by microdilution technique (CLSI, 2015). The microdilution technique was performed in microtiter plates in MYPG broth. Ethanolic propolis extract was serially diluted in plate wells with MYPG broth. In fresh cultures of *Paenibacillus larvae* PB35 and SV35 strains, 0.5 McFarland turbidity suspensions were prepared. Approximately 10 microliters of culture were added to each well containing serially diluted propolis extracts and incubated at 37°C for 48 h in order to determined MIC values. The MIC values were defined as the lowest concentration that showed no growth. Ampicillin (10 µg/ mL) was used as a standard antibacterial agent. MYGB medium was used as negative control.

Determination of Minimal Bacterisidal Concentration (MBC)

The minimum bactericidal concentration (MBC) is the least concentration of antimicrobial agent required to kill microorganisms (Andrews 2001). The MBC was determined for ethanol extracts of Anatolian propolis. After MIC and Bioscreen C determination of the EAP tested, an aliquot of 10 µl from all wells in which showed no bacterial growth was plated onto MYPG Agar plates without EAP. The plates were then incubated for 2 days at 37°C. The MBC value was determined as the lowest EAP concentration without growth on MYPG Agar plates (Andrews 2001).

Bioscreen C techniques

The effects of EAP on the *P. larvae* PB35 and SV35 populations, lag phases and exponential phases were studied by using Bioscreen C (Labsystems, Helsinki, Finland) incubator. Triplicates of 360 µL MYPG broth including serial dilution (1/2) concentration of EAP and 40 µL 10^6 cfu/ ml bacterial concentrations were added in the wells of the Bioscreen plate. Plates were then placed on Bioscreen C incubator and then incubated at 37°C. The optical density of the cell suspensions at 600 nm was monitored automatically in regular intervals of 30 min for 20 hours. Plates were shaken for 20 seconds before each measurement. The control wells contained the tested culture medium without EAP. The data were analyzed using the Excel software in Office 365 and by calculating the averages of three copies for each culture media type.

RESULTS

The PB35 and SV35 were isolated from the flower honey in the hives showing disease and honeycombs showing illness symptom, respectively. The strains were identified as *P. larvae* according to properties of morphological, biochemical, and

molecular. The PB35 strain has a cream appearance, rough colony morphology, and terminal spore. The PB35 strain was negative with respect to catalase production, Voges-Proskauer, citrate, and starch hydrolysis test. The PB35 strain was able to metabolize maltose. The SV35 strain has transparent appearance, rough colony morphology and terminal spore. All biochemical test of the SV35 strain that was only able to metabolize glucose was negative. Two strains PB35 and SV35 have penicillin and ampicillin resistance. While the strains PB35 was resistant to chloramphenicol, the strains SV35 was resistant to tetracycline and norfloxacin. The partial sequences of the 16S rRNA gene were used for further characterization of the bacterial isolates. The obtained sequences were used for a Blast search in the NCBI database and phylogenetic analysis. Using 16S rRNA gene sequences, dendrogram was constructed and 2 isolates were identified as *P. larvae* (Figure. 1). Results obtained for the amount of total phenolic content and phenolic composition of propolis sample were summarized in **Table 1.** Effect of EAP on vegetative growth of PB35 and SV35 were given in **Figure. 2** and **Figure. 3**.

Table 1. Characteristic properties of the Anatolian propolis.

Tablo 1. Anadolu propolisinin karakteristik özellikleri

Total phenolic content (mg GAE/ g)		181.73±5.20
Total flavanoid (mgQE/ g)		42.33±1.40
Total antioxidant capacity (FRAP) µmoL $Fe_2SO_4.7H_2O$/ g		680.70±6.80
Phenolic Composition (HPLC-UV analyses) (mg/ 100g)		
Phenolic acids	*Gallic acid*	-
	Protocatechuic acid	0.55
	p-OH benzoic acid	6.0
	Caffeic Acid	210
	Syringic Acid	-
	p-coumaric acid	124.0
	Ferulic acid	42.5
	t-cinnamic acid	1.68
Flavonoids	*Catechin*	-
	Rutin	58
	Epicatechin	6.40
	Resveratrol	-
	Daidzein	1.02
	Luteolin	7.40
	Myricetin	-
	Hesperetin	4.20
	Chrysin	46.80
	Pinocembrin	50.04
	Caffeic acid phenethyl ester (CAPE)	8.12

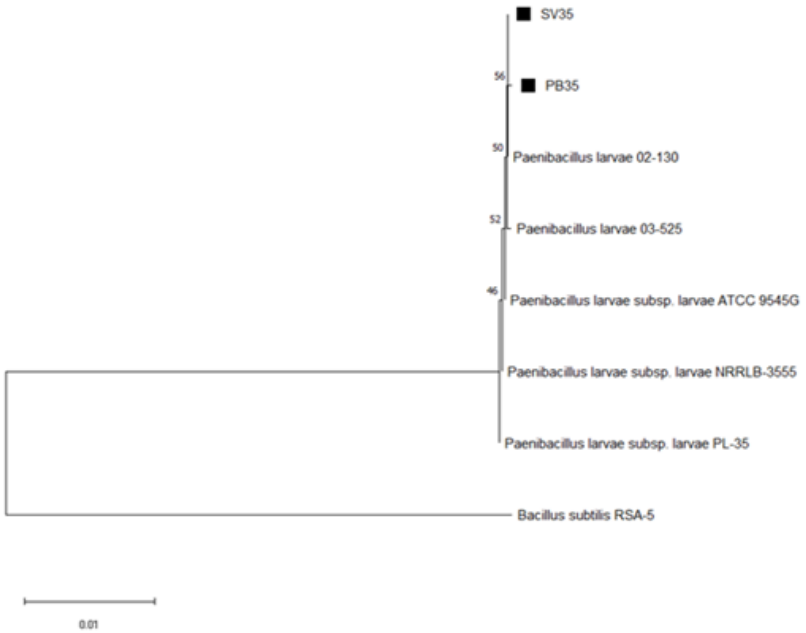

Figure 1. Phylogenetic analysis of the *P. larvae* PB35 and SV35 based on partial sequencing of the 16S rRNA gene. Neighborjoining analysis with p-distance method was used to construct the dendrogram. Bootstrap values shown next to nodes are based on 1000 replicates. *P. larvae* isolates are indicated with black squares. The scale on the bottom of the dendrogram shows the degree of dissimilarity. *Bacillus subtilis* RSA-5 was used as out group.

Şekil 1. 16S rRNA geninin kısmi dizilimine dayalı olarak P. larva PB35 ve SV35'in filogenetik analizi. Dendrogramı oluşturmak için p-mesafe yöntemiyle komşuluk analizi kullanıldı. Düğümlerin yanında gösterilen önyükleme değerleri, 1000 kopyayı temel alır. P. larva izolatları siyah karelerle gösterilmiştir. Dendrogramın altındaki ölçek, farklılığın derecesini gösterir. Dış grup olarak *Bacillus subtilis* RSA-5 kullanıldı.

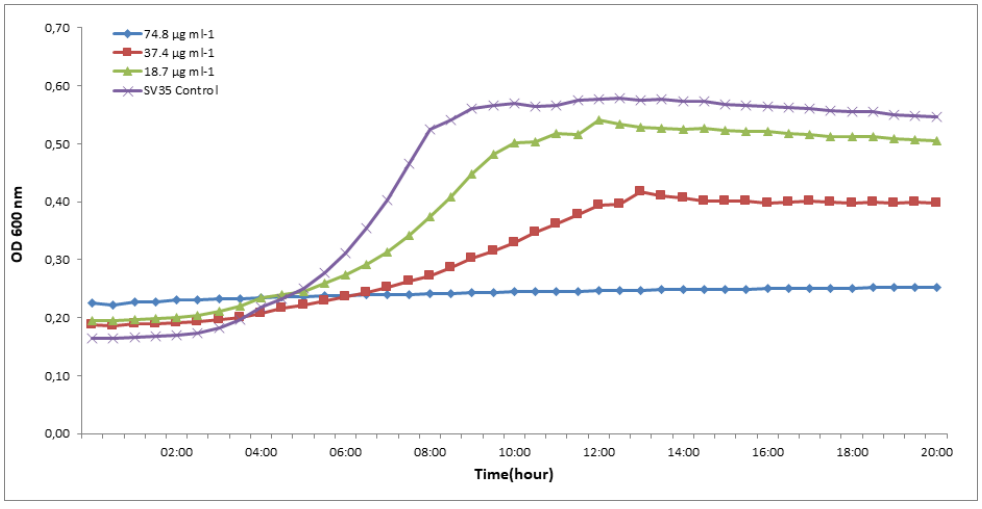

Figure 2. Effect of EAP on vegetative growth of SV35

Şekil 2. EAP'nin SV35'in vejetatif büyümesi üzerindeki etkisi

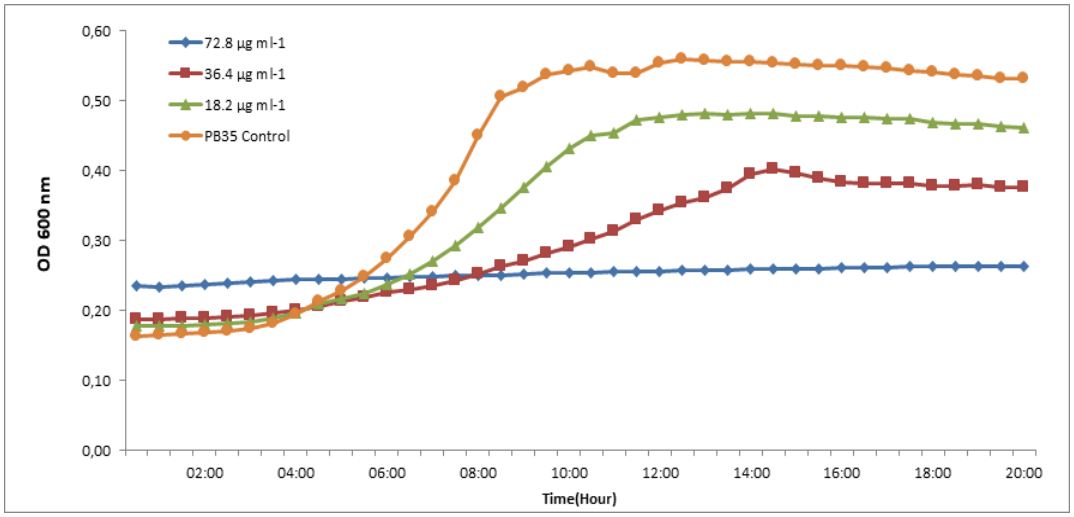

Figure 3. Effect of EAP on vegetative growth of PB35

Şekil 3. EAP'nin PB35'in vejetatif büyümesi üzerindeki etkisi

DISCUSSION

P. larvae are a major threat to hives. The infection of *P. larvae* cause a collapse in honeybee colonies. To protect the hives, it has been required effective antibacterial agents that do not leave residues in the bee products. For this reason, we tried to use Anatolian propolis as an antibacterial and prophylactic agent against P. larvae strains in this study.

P. larvae, caused AFB, is a serious problem in beekeeping worldwide and highly infectious. The spores of the pathogen are resistant to environmental conditions thus controlling the disease is more difficult. Adult worker bees, beekeeping equipment and products that are infected with *P. larvae* spores cause the spread of the disease within and among colonies. As a result, beekeepers often burn infected colonies to eliminate the source of infection (Bastos et al. 2008, Sevim et al. 2017). For beekeeping, the use of antibiotics is an alternative way to protect from AFB. Many antibiotics such as tetracycline derivatives (oxytetracycline and chlortetracycline), streptomycin, sulfonamides, tylosin, erythromycin, chloramphenicol, and lincomycin have been used to date. However, using them insensibly has led to both the accumulation of antibiotic residues in honey and in other hive products and to antibiotic resistant in strains of *P. larvae*. Therefore, the development of different methods such as the use of natural products to control AFB disease is crucial (Pellegrini et al. 2017, Sevim et al. 2017). It is known that propolis is a multifunctional honeybee product. Propolis extract has been identified as a natural alternative for controlling AFB (Antunez et al. 2008, Bastos et al. 2008). The inhibitory effect of propolis extract against *P. larvae* was attributed to the synergy between flavonoids and phenolic acids of propolis (Mihai et al. 2012). The higher phenolic contents have the higher biological active properties, as well as antimicrobial activity (Can et al. 2015, El Adaouia et al., 2020). When compared our result with different regions on propolis, Yığılca propolis contained a higher amount of total phenolic compounds than many samples (Bankova et al. 1995, Cabral et al. 2012, Rebiai 2015). Flavanoids have high pharmaceutical properties such as antioxidant, anti-inflammatory, and antimicrobial effects (Bankova et al. 1995, Salomão et al. 2004). In a study it has been reported there is a relationship between antimicrobial, bacteriostatic activities and phenolics of propolis (Baltas et al. 2016). The last studies have demonstrated that the inhibitory effect of propolis on bacteria depends on the synergism of many compounds in propolis. Mirzoeva et al. (1997) investigated the antibacterial activity of propolis ethanol extract. They concluded that this activity is highly related to the composition and concentration of propolis active compounds. Phenolic and flavonoid components of propolis were found to inhibit bacterial motility. It has been claimed that the

antibacterial activity of propolis extracts is related to the chemical composition of flavonoids (Sforcin 2007). It has been reported that the interaction between the two major groups (flavones/flavonols and flavanones/dihydroflavonols) was particularly important in inhibiting growth of honeybee pathogens such as *P. larvae* by Mihai et al. (2012). The antimicrobial effect of propolis extracts with different floral origins was tested against *P. larvae* strains isolated in Brazil. It has been reported that the antimicrobial activity of propolis has a minimum zone diameter of 20-12 mm and minimal inhibitory concentration of 1.7 and 0.12 mg/mL (Bastos et al. 2008). In another study performed by Isidorov et al. (2017), the antimicrobial activity of propolis samples (poplar, birch, and wire poplar) collected in the European climate zone was tested against the *P. larvae* and their antimicrobial activity was compared. The researchers declared that not only phenolic compounds but also some other compound classes like phenylpropanoids, hydroxycinnamyl sesquiterpenols, glycerides, and benzoates had an effect against the AFB.

Due to the evolution of resistance against antibiotics used in the treatment of AFB, this research is a significant first step in the identification of possible new active compounds within Anatolian propolis in the treatment of AFB in honey bee colonies. In case of success, this alternative application for the treatment of AFB does not leave any residue in pollen, honey, or wax (Bogdanov 2006, Lopez et al. 2007). The Anatolian propolis seems to be one of the best candidates for AFB treatment because of non residue, non toxic, and a natural bee product.

In our study, it is thought that it is important to determine the values of propolis extract that stop the development of *P. larvae* PB35 and SV35 strains by monitoring them with bioscreen C at different concentrations. It is thought that the spore-forming bacteria that cause this and similar diseases will make important contributions in determining the possible doses that can be used to prevent the transition from environmental sources to the hive and to the larvae. It is known that probably bees also need propolis production in order to protect them from *P. larvae* and similar pathogens. Honeybees collect resins with antimicrobial properties from various plant species, mix them with varying amounts of beeswax, and bring them back to their colony as propolis. The collection of antimicrobial compounds from the environment and their incorporation into social nest architecture as propolis is reported to be an effective but relatively undiscovered colony-level defense against pathogens (Banskota et al. 2001).

Conclusion

In conclusion, the results of this study showed that EAP is rich in ferulic, caffeic, coumaric acids, rutin, and caffeic acid phenethyl ester (CAPE). It has a good potential to eliminate AFB by its compounds and could be a natural drug against the threat of *P. larvae*. In addition, obtaining MBC values of *P. larvae*, a spore-forming bacterium, was evaluated as an important result showing that it has a potential for use for AFB disease control.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

PALYNOLOGICAL ANALYSES, CHEMICAL and MINERAL COMPOSITION OF SOME HONEYBEE POLLEN PELLETS

Bazı Bal Arısı Polenlerinin Palinolojik Analizleri, Kimyasal Ve Mineral Madde İçerikleri

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ABSTRACT

Bee products have gained popularity in recent years as food, dietary supplements and adjuvant products due to their extraordinary health properties. Globally, bee pollen is the second most consumed product after honey, has a special importance as a male reproductive unit of flowers, as well as a rich nutrient material, as it contains the oral secretions of the honeybee. In this study, palynological identification of bee pollen collected from apiaries from different regions was made. The bee pollen content was found to be consisting of dry matter between 71.47-81.38%, protein between 17.5-26.0%, fat 5.84-10.95%, and ash content 2.02-2.44%. Moreover, the most common mineral elements in pollen were calcium, potassium, magnesium, silicium, sodium and iron. Besides, heavy metals such as arsenic, cadmium and lead have been detected in bee pollen samples that is a result of the increased environmental pollution and have negative effects on health. Therefore, in bee pollen production, identification, determination of nutritional quality and standardization of pollen are very important for producers and consumers.

Keywords: Bee pollen, palynological analysis, protein and mineral content chemical composition

ÖZ

Arı ürünleri sağlık açısından olağanüstü özellikleri nedeniyle son yıllarda gıda, gıda takviyesi ve destekleyici ürünler olarak ilgi görmektedir. Dünyada baldan sonra tüketimi hızla artan arı poleni, çiçeklerin erkek üreme birimi olarak zengin bir besin maddesi yanında, bal arısının ağız salgıları içermesiyle ayrı bir öneme sahiptir. Bu çalışmada farklı bölgelerdeki arılıklardan toplanan arı polenlerinin olarak palinolojik tanımlaması yapılmıştır. Polen içeriğinin belirlenmesine yönelik yapılan analizlerde kuru madde %71,47-81,38 aralığında, protein %17,5-26,0 aralığında, yağ %5,84-10,95 ve kül miktarı ise %2,02-2,44 arasında bulunmuştur. Mineral madde kompozisyonuna bakıldığında en çok bulunanlar; kalsiyum, potasyum, magnezyum, silisyum, sodyum ve demir elementidir. Çevre kirliliğinin artmasının bir sonucu olan ve sağlık üzerine olumsuz etkileri bulunan ağır metallerden

arsenik, kadmiyum ve kurşun arı poleni örneklerinde tespit edilmiştir. Polen üretiminde özellikle ticari olarak satışı yapılan polenlerin tanımlanması, besinsel kalitesinin belirlenmesi ve standardizasyonu üretici ve tüketici açısından oldukça önemlidir.

Anahtar Kelimeler: Arı poleni, palinolojik analiz, protein, mineral madde

GENİŞLETİLMİŞ ÖZET

Amaç: Türkiye koloni varlığı ve ürettiği bal ile dünyanın önde gelen ülkelerinden biridir. Bal üretimi haricinde üretimi yaygınlaşan ikinci bir ürün de arı polenidir. Arı ürünlerinin doğal ürün olması ve tüketicilerin sağlıklı beslenme için bu ürünlere karşı olan talebi her gün artmaktadır. Bulunduğu topraklar ve sahip olduğu iklimler nedeniyle bitki çeşitliliği yönünden zengin olan Türkiye, arı poleni üretiminin çeşitlendirerek tüketiciye alternatifler sunması gerekmektedir. Bu çalışmada özellikle arıcılık üretimi açısından önemli bitkilerden elde edilen arı polenlerinin kimyasal ve mineral madde özelliklerinin ortaya konulması amaçlanmıştır.

Gereç-Yöntem: Örnekler İzmir, Balıkesir ve Afyon illerinden haftalık olarak Langstroth tip çekmeceli kovanlardan alınmış ve analizleri yapılıncaya kadar -20°C'de muhafaza edilmiştir. Örneklerin palinolojik inceleme ve tayinleri Bilisik v.d. (2008) metoduna göre ışık mikroskobu ile yapılmıştır. Polen örneklerinde (%) kuru madde, kül miktarı, protein ve yağ tayini yapılmıştır (AOAC 2000, Almeida-Muradian v.d., 2005, Commission 2009). Son olarak mineral madde analizi (NMKL-186, 2007) yapılarak arı poleni örneklerinin analiz sonuçları değerlendirilmiştir. İstatistiksel analizler JMP Pro 13 (SAS) programı kullanılarak tekrarlı ANOVA yöntemiyle yapılmıştır.

Bulgular: Polen örneklerinde kuru madde %71,47-81,38, protein %17,50-26,00, yağ %5,84-10,95 arasında ve kül miktarı %2,02-2,44 arasında tespit edilmiştir. Polen örneklerinde en çok bulunan mineraller potasyum, kalsiyum, magnezyum, silisyum, demir ve sodyumdur. *Scabiosa* L. ve *Salix* L. polenlerinin magnezyum yönünden, *Castanea sativa* Mill. polenlerinin ise demir, manganez ve sodyum minerali yönünden zengin olduğu belirlenmiştir. Ayrıca arı poleni örneklerinde ağır metallerden arsenik, kurşun ve kadmiyum elementleri tespit edilmiştir. En çok arsenik ve kurşun ağır metalleri *Scabiosa* L. poleninde tespit edilmiştir.

Sonuç: Polen örneklerinin kimyasal analiz sonuçları daha önceki yapılmış çalışmalar ile uyumlu gözükmektedir. Türkiye'de üretilen arı polenleri özellikle Ca, K, Mg, Fe, Si ve eser elementleri açısından iyi bir mineral kaynağı olarak değerlendirilebilir. En önemli konu artan çevre kirliliğine karşı polende oluşabilecek sağlık risklerinin (ağır metal gibi) tespit edilmesine yöneliktir. Arı poleninin riskli gruplar (yaşlılar, hamileler, hastalar) tarafından tüketilebileceği düşünüldüğünde, hem üreticilerin hem de tüketicilerin ortaya çıkabilecek bu soruna karşı dikkatli olmaları gerekmektedir. Arı poleni hakkında bilimsel çalışmalar yapıldıkça polen kullanımının sağlık için önemi artmaktadır.

Özellikle ticari bir ürün olan arı poleninin piyasada doğru tanıtılması önemlidir. Bu nedenle ticari firmaların büyük çaplı polen stoklarının palinolojik analizinin yapılması ve kalitelerinin ortaya konulması üretici ve tüketici için oldukça önemlidir.

INTRODUCTION

Proteins, sugars, lipids, amino acids, vitamins and mineral elements are the main components in bee pollen (BP) (Szczęsna 2007a, Hassan 2011, Liolios et al. 2016, Velásquez et al. 2017, Lilek et al. 2021). BP is important for human health and nutrition. The chemical components of BP differ based on plant species. There is a correlation between chemical composition and botanical origin of the pollen (Taha 2015). BP consists of 20.0-30.4% protein, 2.8-50.2 mg/kg carotenoids and 22.8-918.4 mg/kg phenolic components (Velásquez et al. 2017). BP has high sugar and protein content and relatively low lipid content. Furthermore, pollen is a rich source of vitamins and other bioactive compounds. It was reported that 8 important minerals (calcium, iron, copper, chromium, manganese, molybdenum, phosphorus, and zinc) were found and six of them were high enough to meet dietary requirements (Sattler et al. 2016, Mărgăoan et al. 2014). Most of the daily consumption requirements of these minerals can be met with the consumption of 20-30 g of pollen (Ialomiteanu 1978). According to a

systematic review of more than 100 studies, BP consists of an average of 54.22% (18.50–84.25%) carbohydrates, 21.30% (4.50–40.70%) protein, 5.31% (0.41–13.50%) lipids, 8.75% (0.15– 31.26%) fiber, and 2.91% (0.50–7.75%) ash. Besides, the presence of mineral content in BP was reported as: 4951.61 mg/kg (3.06–13366.60 mg/kg) potassium, 4157.86 mg/kg (234.40–9587.00 mg/kg) phosphorus, 1751.22 mg/kg (1.09 –5752.19 mg) /kg) calcium, 1246.99 mg/kg (44.00–4680.53 mg/kg) magnesium, 46.97 mg/kg (0.10–105.80 mg/kg) zinc, and 197.41 mg/kg (2.60–1180.00 mg/kg) iron (Thakur and Nanda 2020).

BP consumption has increased in recent years, particularly due to its nutritional value and therapeutic applications. Quantification of mineral components is of great importance to evaluate both toxicity and beneficial effects of essential elements (Costa et al. 2019). It shows that bee products contain essential macro elements (K, P and S) and micro elements (Zn and Fe) in concentrations depending on the bee product type. However, the presence of toxic heavy metals makes it necessary to investigate the quality of bee products before using them as nutritional supplements. Since bee products are quite heterogeneous, they also differ in element content depending on environmental factors. Therefore, it is necessary to develop standards that regulate acceptable inorganic pollutant levels. Moreover, since bees and their products are considered an effective biological monitoring tool, analysis results can reflect the state of the environment that affects the health and well-being of both humans and bees (Matuszewska et al. 2021). In the present study, mineral content, protein, fat, dry matter, and ash content in some monofloral BP and mixed BP produced in Turkey were investigated.

MATERIALS AND METHODS

The 7 different BP samples were collected between April and July 2020. Samples 3 and 4 were taken from Izmir, sample 2 was taken from Balikesir and the other samples (1,5,6,7) were taken from Afyon. All pollen samples were harvested weekly from Langstroth type drawer hives under similar conditions and stored at -20°C until analysis.

Palynological Identification of Pollen Samples

To determine the plant sources used by honeybees in the region, fresh pollen granules were prepared as specimens by separating them according to their colors (Wodehouse 1935). Palynological identifications were made using a light microscope (Olympus BX41) and the percentages of pollen belonging to each taxon in each sample were determined (Almeida-Muradian et al. 2005, Bilisik et al. 2008, Mărgăoan et al. 2013).

Chemical Analysis of Pollens

Moisture Analysis: Moisture content of pollen samples was determined by Radwag 50/NH moisture analyzer (103˚C, 3-4 hour) by drying the samples until they reached a constant weight (Commission 2009).

Ash Analysis: Pollen samples were weighed into porcelain crucibles (2 g) and burned in a muffle furnace at 600ºC until no black spots remained in the sample. Then, after cooling in the desiccator, weighing was made and the results were calculated as % (AOAC 2000).

Protein Assay: The amount of protein was determined according to the Dumas method. Leco FP-528 nitrogen/protein analyzer is used to determine the protein amount of the samples (AOAC 2000). Pollen samples were burned in the protein analyzer. The amount of nitrogen obtained as a result of combustion was multiplied by 5.60 and the amount of protein was calculated (Rabie et al. 1983, Sorkun et al. 2010; Çakıcı et al. 2018).

Fat Assay: The amount of fat was determined using diethyl ether with the Soxhlet extraction method in a semi-continuous solvent extraction system (Almeida-Muradian et al. 2005). The results were calculated as % of weight loss (Yetim and Keskin 2008).

Mineral analysis: Pollen samples were burned in a microwave system at 200 ºC with nitric acid and hydrogen peroxide. Then, the minerals were determined with the ICP-MS device (NMKL-186 2007).

Statistical Analysis

Statistical analyzes were performed in JMP Pro 13 (SAS) software using the repeated ANOVA method. Chemical analysis of the samples was done in duplicate, and mineral analyzes were performed in triplicate. Results were expressed as mean ± standard deviation. The heatmap and principal components plots were prepared using the Clustvis webtool (https://biit.cs.ut.ee/clustvis/).

RESULTS

From the total samples used in the study, one of the pollen types was found to be more than 90%. Thus, sample 1 was found to be predominat in *Salix* (94.17%), sample 2 in *Castanea sativa* (99.50%) and sample 3 in *Scabiosa* pollen (99.40%). In addition to these samples, which can be considered monotypic, no dominant pollen was observed in other samples, and they were accepted as polytypic. Majority of the pollen was represented by *Brassicaceae* (38.10%) and *Echium* sp. (34.01%) in the 4th sample, while *Brassicaceae* (32.69%) and *Apiaceae* (30.77%) families in the 5th sample *Salix* (51.33%) and *Rosaceae* (30.51%) in the 6th sample, and *Papaver* (44.50%) in the 7th sample (Table 1).

Table 1. Percentage of pollen types represented in studied pollen pellets.

Çizelge 1. Polen örneklerini temsil eden polen türleri (%)

Taxa / Sample	1	2	3	4	5	6	7
Acer						10,90	
Apiaceae					30,77		9,09
Asteraceae							1,21
Brassicaceae	0,82			38,10	32,69		5,45
Castanea sativa		99,5					
Centaurea		0		3,40			
Cistus		0,5		2,72	6,73		9,09
Echium				34,01			1,82
Eucalyptus				9,52			7,88
Fabaceae					0,96		
Juglans						3,63	
Lamiaceae				1,36	2,88		
Onobrychis					0,96		1,82
Papaver				2,04	16,35		44,85
Plantago					0,96		
Poaceae					4,81		
Portulaca			0,60				
Ranunculaceae						2,18	
Roemeria					1,92		3,64
Rosaceae	5,01			4,76		30,51	15,15
Salix	94,17					51,33	
Sarco/Poterium					0,96		
Scabiosa			99,40				
Symphytum						1,45	
Trifolium				4,08			

In this study, dry matter was between 71.47-81.38%, protein 17.5-26.0%, fat 5.8-10.95% and ash content between 2-2.44% (Table 2). Chestnut pollen was the monofloral pollen with the highest protein content (23.5%). It can be said that the fat composition of mixed pollen is higher than monofloral pollen.

Table 2. Chemical analysis pollen samples (%)

Çizelge 2. Çalışılan örneklerin kimyasal analiz ortalama ve standart sapma sonuçları (%)

Sample	Dry matter	Protein	Fat	Ash
1	71.470±0.028	19.000±1.414	5.847±0.953	2.163±0.185
2	78.480±0.212	23.500±0.707	8.596±1.063	2.209±0.004
3	79.480±0.000	18.500±0.707	6.420±0.364	2.287±0.022
4	76.595±0.162	20.500±0.707	10.148±0.062	2.448±0.112
5	72.685±0.063	17.500±0.707	7.486±0.487	2.025±0.134
6	81.380±0.430	17.500±0.707	10.951±1.330	2.287±0.358
7	73.065±0.374	26.000±0.000	7.937±0.929	2.129±0.038

mean ± standard deviation

The most abundant minerals in the pollen samples in our study are potassium, calcium, magnesium, silicium, iron and sodium (Table 3). It was determined that *Scabiosa* and *Salix* pollen were rich in magnesium, while *Castanea sativa* pollen was rich in iron, manganese and sodium elements (Table 3).

Table 3. Mineral content profile of honeybee pollen pellets (mg/kg)

Çizelge 3. Bal Arısı Polenlerinin Mineral Madde Profili (mg/kg)

Mineral composition	Pollen samples		
	1	2	3
Calcium (Ca)	413.871 ± 3.725	363.390 ± 7.926	358.020 ± 10.382
Potassium (K)	4470.637 ± 40.075	4644.076 ± 0.0	4751.461 ± 86.225
Magnesium(Mg)	910.500 ± 21.642	440.501 ± 16.346	990.769 ± 8.459
Sodium (Na)	17.435 ± 0.571	41.017 ± 1.179	23.432 ± 0.396
Boron (B)	9.141 ± 0.176	8.359 ± 0.176	18.909 ± 0.418
Iron (Fe)	50.877 ± 17.234	74.818 ± 1.918	55.843 ± 2.484
Aluminium(Al)	32.490 ± 1.395	48.425 ± 2.111	79.690 ± 0.709
Silicium (Si)	288.192 ± 11.987	388.754 ± 13.186	501.398 ± 23.981
Vanadium (V)	0.136 ± 0.054	0.329 ± 0.024	0.664 ± 0.093
Chrome (Cr)	1.049 ± 0.048	1.553 ± 0.070	2.360 ± 0.103
Manganese (Mn)	19.568 ± 0.812	79.122 ± 5.693	8.883 ± 0.029
Cobalt (Co)	0.160 ± 0.077	0.279 ± 0.074	0.300 ± 0.074
Nickel (Ni)	0.184 ± 0.059	4.277 ± 0.115	1.560 ± 0.060
Copper (Cu)	5.698 ± 0.070	8.943 ± 0.409	10.416 ± 0.139
Zinc (Zn)	23.882 ± 0.603	31.260 ± 1.098	20.236 ± 1.041
Selenium (Se)	0.019 ± 0.030	0.021 ± 0.029	0.033 ± 0.025
Strontium (Sr)	2.670 ± 0.054	0.475 ± 0.014	0.646 ± 0.271
Molybdenum (Mo)	0.085 ± 0.034	0.077 ± 0.082	0.125 ± 0.030
Silver (Ag)	0.038 ± 0.007	0.019 ± 0.002	0.043 ± 0.003
Thallium (Tl)	0.003 ± 0.000	0.016 ± 0.000	0.014 ± 0.000
Antimony (Sb)	0.016 ± 0.001	0.051 ± 0.002	0.017 ± 0.010
Barium (Ba)	1.247 ± 0.040	2.222 ± 0.873	1.095 ± 0.048
Beryllium (Be)	0.012 ± 0.003	0.019 ± 0.005	0.027 ± 0.007
Arsenic (As)	0.020 ± 0.016	0.029 ± 0.024	0.108 ± 0.031
Lead (Pb)	0.034 ± 0.011	0.050 ± 0.009	0.455 ± 0.019
Cadmium (Cd)	0.050 ± 0.017	0.017 ± 0.003	0.012 ± 0.009

*mean ± standard deviation (n:3)

Table 3- Continuation Mineral content profile of honeybee pollen pellets (mg/kg)

Çizelge 3-Devamı. Bal Arısı Polenlerinin Mineral Madde Profili (mg/kg)

Mineral composition	Pollen samples			
	4	5	6	7
Calcium (Ca)	540.314 ± 11.359	241.489 ± 6.053	343.457 ± 8.752	431.849 ± 9.089
Potassium (K)	4690.504 ± 0.890	4633.516 ± 27.419	4769.771 ± 22.986	4599.277 ± 0.0
Magnesium(Mg)	906.868 ± 14.109	498.734 ± 10.497	566.264 ± 21.951	849.189 ± 8.752
Sodium (Na)	64.653± 1.212	22.443 ± 0.200	16.925 ± 0.563	15.830 ± 0.433
Boron (B)	32.269 ± 1.117	14.431 ± 0.345	12.148 ± 0.247	10.967 ± 0.193
Iron (Fe)	39.263 ± 1.105	47.036 ± 0.634	42.773 ± 1.353	50.877 ± 1.859
Aluminium(Al)	33.281 ± 0.957	21.254 ± 1.381	56.729 ± 7.101	8.993 ± 0.215
Silicium (Si)	447.230 ± 23.279	360.060 ± 7.221	546.316 ± 33.621	355.795 ± 19.982
Vanadium (V)	0.287 ± 0.010	0.241 ± 0.005	0.268 ± 0.018	0.045 ± 0.001
Chrome (Cr)	1.681 ± 0.064	1.314 ± 0.069	1.761 ± 0.078	1.605 ± 0.043
Manganese (Mn)	14.802 ± 0.228	12.201 ± 0.288	70.357 ± 0.715	16.984 ± 0.274
Cobalt (Co)	0.111 ± 0.010	0.343 ± 0.035	0.168 ± 0.018	0.165 ± 0.054
Nickel (Ni)	0.556 ± 0.225	1.350 ± 0.027	0.409 ± 0.018	0.515 ± 0.013
Copper (Cu)	7.665 ± 0.141	7.032 ± 3.858	6.460 ± 0.095	10.577 ± 0.818
Zinc (Zn)	20.875 ± 1.156	16.850 ± 0.020	20.013 ± 1,415	23.132 ± 0.610
Selenium (Se)	0.024 ± 0.008	0.016 ± 0.035	0.014 ± 0.009	0.020 ± 0.060
Strontium (Sr)	0.965 ± 0.031	0.602 ± 0.025	0.486 ± 0.020	0.526 ± 0.279
Molybdenum (Mo)	0.460 ± 0.015	0.261 ± 0.128	0.166 ± 0.053	0.218 ± 0.077
Silver (Ag)	0.030 ± 0.006	0.025 ± 0.006	0.051 ± 0.004	0.028 ± 0.005
Thallium (Tl)	0.062 ± 0.002	0.009 ± 0.000	0.010 ± 0.000	0.006 ± 0.000
Antimony (Sb)	0.084 ± 0.003	0.030 ± 0.002	0.0243 ± 0.001	0.007 ± 0.001
Barium (Ba)	1.173 ± 0.160	0.677 ± 0.220	1.509 ± 0.051	0.416 ± 0.010
Beryllium (Be)	0.018 ±0.004	0.016 ± 0.001	0.023 ± 0.007	0.010 ± 0.003
Arsenic (As)	0.087 ± 0.059	0.039 ± 0.023	0.025 ± 0.003	0.015 ± 0.016
Lead (Pb)	0.119 ± 0.034	0.046 ± 0.013	0.050 ± 0.012	0.015 ± 0.003
Cadmium (Cd)	0.010 ± 0.005	0.027 ± 0.011	0.017 ± 0.003	0.042 ± 0.002

*mean±standard deviation (n:3)

In the pollen samples of our study, the highest amount of heavy metals arsenic (0.108 mg/kg) and lead (0.455 mg/kg) were detected in the 3rd sample which consists of 99.40% of *Scabiosa* pollens, and the highest amount of cadmium (0.050 mg/kg) was detected in the 1st sample which consists of 94.17% *Salix* pollens.

DISCUSSION

In our study, moisture content varied between 19-27%, protein 17.5-26.0%, fat between 5.8-10.95% and ash content between 2-2.44% (Table 2). Similarly, moisture content was found to be 16.9-31%, ash 1.34-2.81%, protein 13.16-24.14% and fat 1.33%-5.47% in BP samples from Romania (Spulber

et al. 2018). In another study conducted in Brazil, it was determined that the moisture content was between 3.45-4.85%, protein was between 18.55-22.6%, and fat was between 4.80-5.07% (Carpes et al. 2009). In the pollen samples collected throughout the year in eastern Saudi Arabia, the composition was as follows; 20.16% spring pollen, 19.27% summer pollen, 18.02% autumn pollen, and 20.14% winter pollen (Al Kahtani et al., 2020). In Brazil physico-chemical analyzes of two pollen samples were done. For both pollen, protein content (24.8 ± 2.4 g 100 g-1), total sugar content (36.2 ± 1.1 g 100 g-1), lipid content (4.0 ± 0.3 g 100 g-1), and ash (2.6 ± 0.05 g 100 g -1) were determined within the limits, however, it was determined that moisture levels (6.6 ± 2.2 g 100 g-1) of both samples were not in accordance with the values recommended by the laws (<4 g 100 g-1) (Lorini et al., 2020). In another study, eight monofloral BP samples were collected from different apiaries in Morocco. Botanical origins of BP samples were determined using scanning electron microscopy (SEM), and physicochemical parameters (pH, moisture, ash and mineral contents) were investigated. It was shown that pH was between 4.19 ± 0.17 and 4.82 ± 0.36, humidity was between 10.7 ± 0.04 and 26.8 ± 0.01, and ash content was between 1.81% ± 0.10 and 4.22 ± 0.08 for the samples. The protein content has been reported to be between 19.86 ± 0.36 mg/100 g BP, and 30.32 ± 0.12 mg/100 g BP (Asmae et al., 2021).

It was demonstrated that BP samples were rich in mineral elements. Potassium, calcium, magnesium, silicon, iron, and sodium were the most abundant mineral elements. Pollen sources in the present study consist of a significant amount of silicium mineral distinct from those reported in other studies (Szczesna 2007b; Carpes et al. 2009). The samples with monotypic *Salix* and *Scabiosa* pollens were found to be magnesium rich, whereas the ones with monotypic *Castanea sativa* pollen were rich in iron, manganese, sodium, and zinc composition. It can be foreseen that all pollen samples have characteristic mineral composition which could be seen in heatmap (Figure 1) and PCA plots (Figure 2).

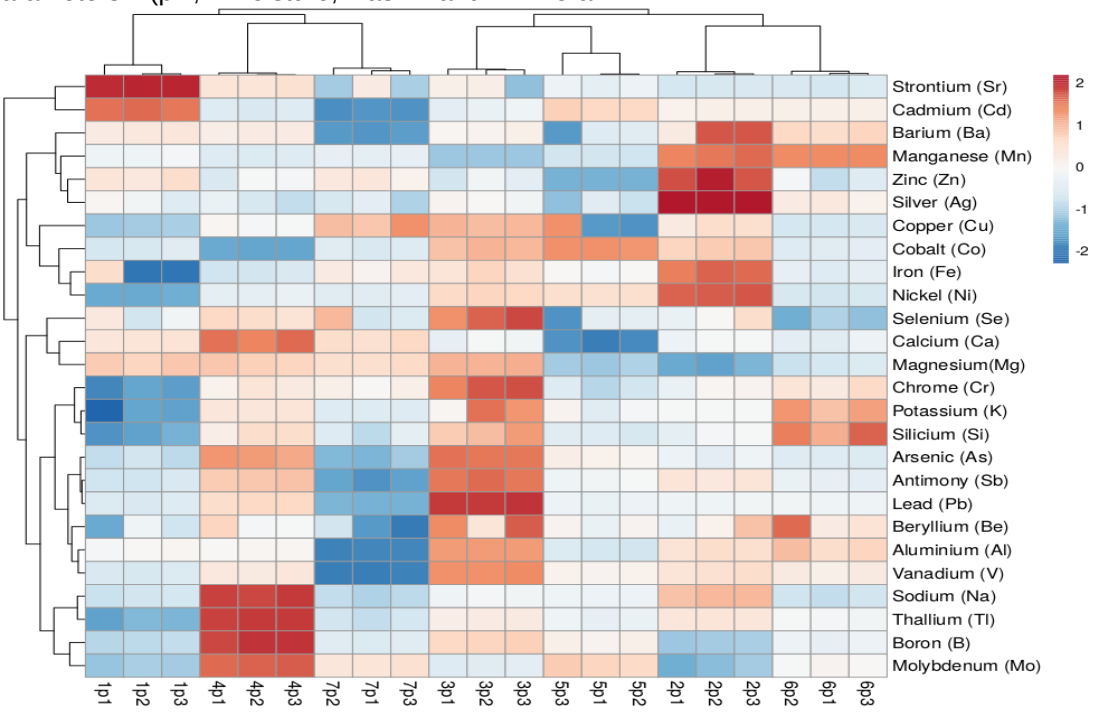

Figure 1. Heatmap plot of mineral content in pollen samples

Şekil 1. Polen örneklerindeki mineral maddelerinin Heatmap haritası

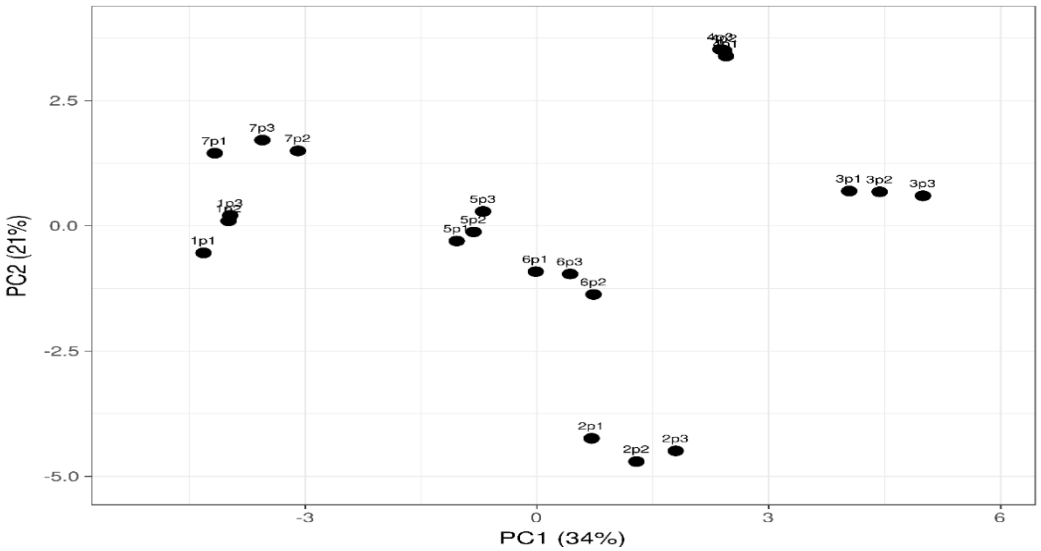

Figure 2. PCA plot according to the mineral content in pollen samples

Şekil 2. Polen örneklerindeki mineral maddelerinin temel bileşenler analizi (PCA)

In BP samples collected from the Northeast Anatolia Region the chemical composition was determined as 8.60±0.43 mg/kg of copper, 35.30±2.13 mg/kg of zinc, 1113.33±61.00 mg/kg of magnesium, and 1034.00±53.86 of calcium. These amounts were stated to be at a level that would contribute to the daily mineral need (Uçar et al. 2018). It has been reported that the most abundant minerals in BP samples from Morocco were potassium and magnesium, and heavy metals were not detected (Asmae et al., 2021). In the study carried out with the pollen samples of Turkey and Russia, it was determined that there was a statistical difference in the composition of the minerals between the regions where the pollen was collected. It has been reported that Russian pollen samples are richer in potassium, while Turkey's pollen samples are rich in potassium, phosphorus, magnesium, and iron elements (Özcan et al. 2019). In a study conducted in Romania, *Prunus spp*. pollen contained the highest Fe (150.9±1.11 mg/kg), while the highest Mg level (1505±1.43 mg/kg) was detected in *Brassica* pollen. The other pollen samples in the study were found to be consisting of 1980-4284 mg/kg of potassium, 474.3-1505 mg/kg of magnesium, 1155-4335 mg/kg of calcium, 21.7-58 mg/kg of iron, and 20.2-59.5 mg/kg of zinc (Spulber et al. 2018). In another study with pollen samples from China, South Korea, and Poland, it is reported that there are differences in mineral compositions between countries. For example, 762 mg/kg of calcium, 26.3 mg/kg of manganese, 1305 mg/kg of magnesium, 36.8 mg/kg of zinc, 9.3 mg/kg of copper, 65.4 mg/kg of iron, 3903. mg/kg of potassium, and 739 mg/kg of sodium was reported in Polish samples (Szczesna 2007b). In BP collected in Brazil, phosphorus, potassium, calcium and magnesium minerals were found to be at the highest levels, respectively (Carpes et al. 2009).

In the research carried out on pollen samples from Turkey and Russia, Russian samples had 0.11-0.19 mg/kg of cadmium, and 0.18-0.64 mg/kg of lead, whereas 0.04-0.14 mg/kg of cadmium and 0.25-0.60 mg/kg of lead was found in Turkish samples (Özcan et al. 2019). In fresh BP from Bulgaria, lead was determined as 0.467-0.483 mg/kg and cadmium as 0.022-0.029 mg/kg (Dinko and Stratev 2016). In Serbia, cadmium was detected as 0.004-0.125 mg/kg in pollen samples (Kostić et al. 2015). According to Campos et al. (2008), the highest amount of Pb and Cd in fresh BP should not exceed 0.03 mg/kg and 0.5 mg/kg, respectively. In addition, the composition of other minerals should be as follows; 4000-20000 mg/kg of potassium, 200-3000 mg/kg of magnesium, 200-3000 mg/kg of calcium,

11-170 mg/kg of iron, 30-250 mg/kg of zinc, 2-16 mg/kg of copper, and 20-110 mg/kg of manganese.

It is known that the chemical composition of pollen sources varies according to the type of plant from which the pollen is produced. Similar to our results, it is reported that BPs of different origins collected throughout Serbia contain potassium, calcium and magnesium as major elements, and are also extremely rich in iron and zinc, which are very important nutrients. It has been reported that the mineral composition of BP is more dependent on the type of pollen producing plant than its geographical origin (Kostić et al. 2015). Moreover, in the study conducted in Brazil, it was reported that the mineral composition changed according to the place and year of pollen production (Morgano et al. 2012).

Conclusion

The biochemical content of BP varies depending on the plant diversity, the period and place it was produced (Taha and Al-Kahtani 2020), and the effect of the season/period in which the pollen was taken on pollen diversity and yield should also be considered. It is important for the producer to define the contents of BP, which is sold as a commercial product, and to create a demand according to its quality.

BP produced in Turkey can be considered as a good mineral source, especially in terms of Ca, K, Mg, Fe, Si and trace elements. Recently, BP has become a prominent product due to the increasing interest and consumer demand for natural products. Both its components and its use in apitherapy make pollen an important product. However, due to the increase in environmental pollution in recent years, BP taken from apiaries close to residential areas, industrial facilities and highways should be controlled in terms of heavy metals. Considering that BP can be consumed by risky groups (the elderly, pregnant women, patients), both producers and consumers should be careful about health problems that may arise.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

A MICROSCOPY AND MOLECULAR STUDIES OF NOSEMA CERANAE INFECTION IN MAZANDARAN PROVINCE OF IRAN

Nosema ceranae Enfeksiyonunun İran, Mazandaran İlinde Mikroskopik ve Moleküler Çalışması

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ABSTRACT

***Nosema ceranae* as a fungal parasite has been reported from the *Apis mellifera* in all continents. It causes reduced longevity, depopulation, decreased production, and colony losses in honeybee colonies. This study aimed to determine the prevalence of *N.ceranae* in the apiaries of Mazandaran province. In this study, we randomly selected 320 hives from thirty-two apiaries and collected twenty old honeybees from the apiaries. The collected samples were examined by microscopy and molecular methods. The results of the microscopic examinations showed that 78.12% of apiaries were infected with *Nosema* spp. In addition, *N. ceranae* was identified 84.37% of apiaries by PCR, while no samples were infected by *N. apis*. Blast analysis of the sequenced samples confirmed the presence of *N. ceranae* infection in the apiaries. Based on the obtained results, a high frequency of *N.ceranae* was detected in apiaries in Mazandaran province.**

Keywords: *Nosema ceranae*, Honeybee, PCR, Iran

ÖZ

***Nosema ceranae*, tüm kıtalarda *Apis mellifera*'dan mantar paraziti olarak rapor edilmiştir. Bal arısı kolonilerinde yaşam süresinin azalmasına, nüfus azalmasına, üretimin azalmasına ve koloni kayıplarına neden olur. Bu çalışmada Mazandaran ili arılıklarında N.ceranae yaygınlığının belirlenmesi amaçlanmıştır. Bu çalışmada, otuz iki arılıktan rastgele 320 kovan seçtik ve arılıklardan yirmi yaşlı bal arısı topladık. Toplanan örnekler mikroskop ve moleküler yöntemlerle incelendi. Mikroskobik incelemelerde arılıkların %78,12'sinin Nosema spp belirlendi. Ayrıca *N. ceranae*, PCR ile %84.37'sinde tespit edilirken, hiçbir numune *N. apis* ile enfekte olmamıştır. Sıralı örneklerin gen hizalama analizi sonucunda arılıklarda *N. ceranae* enfeksiyonunun varlığı doğrulandı. Elde edilen sonuçlara göre Mazandaran ilindeki arılıklarda yüksek oranda *N.ceranae* tespit edilmiştir.**

Anahtar Kelimeler: *Nosema ceranae*, Bal arısı, PCR, İran

GENİŞLETILMIŞ ÖZET

Amaç: Çalışmanın amacı, Mazandaran ili arılıklarında *N.ceranae* yaygınlığının mikroskopik ve moleküler yöntemlerle belirlenmesidir.

Gereç ve yöntem: Bu çalışmada, otuz iki arılıktan rastgele 320 kovan seçilmiştir. Her bir kovanda, her bir kovanın çevre çerçevelerinden 20 yaşlı işçi arı toplanmıştır. Bal arılarının karınları normal tuzlu su

çözeltisinde öğütüldü. Süspansiyon süzüldü ve santrifüjlendi. Süpernatantlar çıkarıldı ve Pelletler doymuş salin solüsyonu ile karıştırıldı. Birkaç mililitre süpernatan alındı ve solüsyonun geri kalanı atıldı. Süpernatanlar, tekrarlanan santrifüjleme yoluyla damıtılmış su ile yıkandı. Nihai pelet, Nosema sporunu tanımlamak için mikroskobik olarak incelendi. Bal arılarının geri kalan karın içeriği DNA ekstraksiyonu için kullanıldı. Her numunede Nosema türlerinin saptanması için bir multipleks PCR tahlili kullanıldı. En güçlü banda sahip beş pozitif amplikon da gen dizilimi için seçildi.

Bulgular: Mikroskobik incelemelerin sonucunda arılıkların %78.12'sinin Nosema spp. belirlendi. Ek olarak, *N. ceranae*, PCR ile arılıkların %84.37'sinde tespit edilirken, hiçbir örneğe *N. apis* bulaşmamıştır. Mikroskobik ve moleküler yöntemler arasında adil bir sonuç elde edildi. Ayrıca, fumagillin reçete edilmesinin arılık boyutunda, arılıklarda Nosema spp enfeksiyon oranı üzerinde anlamlı bir etkisi olmamıştır. Sıralı örneklerin gen sıralama analizi, arılıklarda *N. ceranae* enfeksiyonunun varlığını doğruladı.

Tartışma ve sonuç: Birçok çalışmanın sonuçları, *N. ceranae* enfeksiyonunun dünya çapında bir dağılıma sahip olduğunu göstermiştir (Klee ve ark. 2007). *N. ceranae* yaygınlığı Türkiye'de %15-100 (Ivgin Tunca ve ark. 2016), İtalya'da %63 (Papini ve ark. 2017), Polonya'da %80,6 (Michalczyk ve ark. 2011), %95-97 Macaristan'da (Csáki ve diğerleri 2015), Bulgaristan'da %77 (Shumkova ve diğerleri 2018), Kanada'da %41-91 (Emsen ve diğerleri 2016) ve Suudi Arabistan'da %56 (Ansari ve diğerleri 2017).

Bu çalışmada, mikroskopik ve PCR sonuçları arasında adil bir uyum gözlemlenirken, diğer çalışmalar iki yöntem arasında önemli bir uyum olduğunu bildirmiştir (Khezri ve diğerleri 2018, Papini ve diğerleri 2017). Bal arılarında Nosema enfeksiyonunun tanımlanması ve ayrımı için PCR yönteminin duyarlılığı ve özgüllüğünün ışık mikroskobundan daha yüksek olduğu açıktır (Michalczyk ve ark. 2011). Bununla birlikte, sporlar gözlemlenmesine rağmen, iki örnekte PCR sonuçları negatifti. Sonuçlar, eksik DNA ekstraksiyonu veya esnek duvarların DNA ekstraksiyonu üzerindeki önleyici etkisi ile ilgili olabilir (Webster ve ark. 2004). Bu çalışmada izole edilen *N. ceranae* dizileri, Çin'de toplanan ve GenBank veri tabanında depolanan *N. ceranae* dizileriyle yüksek düzeyde homolojiye sahiptir.

Moleküler inceleme, bu çalışmada arılıklarının %87,37'sinin yalnızca *N. ceranae* ile enfekte olduğunu göstermiştir. Sonuçlarımız, İran arı kovanlarında *N. ceranae*'nin tek nosemosis etkeni olduğunu belirleyen diğer moleküler çalışmalarla uyumludur (Nabian ve ark. 2011, Khezri ve ark. 2018, Mohhamadian ve ark. 2018). Bu çalışmada *N. ceranae*'nin yüksek oranda yaygınlığı, bölgelerdeki subtropikal iklim ile ilgili olabilir. *N. ceranae* enfeksiyonlarının oranı, diğer bölgelere kıyasla ılıman iklimlerde daha baskın görünmektedir, oysa *N. apis* şu anda daha soğuk iklimlerde daha yaygın olabilir (Fries 2010).

Elde edilen sonuçlara göre İran'ın Mazandaran eyaletindeki arılıklarda yüksek oranda *N.ceranae* tespit edilmiştir. Ayrıca, fumagillin kullanımı, enfekte olmuş kolonilerde nosemayı kontrol etmek için yeterli değildir.

INTRODUCTION

Nosemosis is a significant disease in honey bees around the world (Bailey and Ball 1981). Nosemosis is caused by unicellular fungi belonging to class Microsporidia (OIE 2019). Recent molecular research of the SSU rRNA gene was shown a new definition of the *Nosema* –Vairimorpha clade. Although *Nosema* species are genetically close to *Vairimorpha*, but their morphological and developmental features of two groups are very similar. However the taxonomy of *Nosema* species is not yet well established (Tokarev et al. 2020). *Nosema* spores are found in feces and are ingested, directly or indirectly, by adult bees. A higher rate of *Nosema* infection is observed in worker bees compared to drones and queens, probably due to the cleaning activities of worker bees in the hive (Bailey and Ball 1981). The spores then develop in the epithelial cells in the bees' midgut and affect their digestive functions. The spores are expelled in the feces and are able to maintain their infectivity for a long time in cold and heat conditions for several years (Fenoy et al. 2009). The causative agents of nosemosis are *N. apis* and *N. ceranae* that infect *Apis mellifera*, with different frequency depending on the area (Fries 2010). *Nosema apis* is distributed especially in cold and temperate regions. It more common during spring and winter. *Nosema ceranae* is a new species of microsporidium isolated for the first time from *Apis cerana*, a bee species widespread in Southeast Asia (Fries et al. 1996). The natural infection of *Apis mellifera* with *N.*

ceranae was reported in Spanish apiaries (Higes et al. 2006). The clinical signs of *N. ceranae* infection in adult honeybees are different from *N. apis.* The most prominent symptom in *N. apis* infection is dysentery, while intestinal disorders are not observed in *N. ceranae* infection. The affected honeybees tend to die away from the hive, resulting in progressive depopulation of the colonies (OIE 2019, Fries 2010).

The microscopic spores of *N. apis* are barely morphologically distinguishable from those of *N. ceranae*. It is only possible to make an accurate diagnosis through PCR (OIE 2019). Epidemiological studies have indicated a high prevalence of *Nosema* spp. in honeybee colonies in the northern half of Iran (Lotfi et al. 2009, Tavassoli et al. 2009, Razmaraii and Karimi 2010, Moshverinia et al. 2012). However, molecular studies have shown only *N. ceranae* infection in the apiaries in different provinces of Iran (Nabian et al. 2011, Khezri et al. 2018, Mohhamadian et al. 2018).

Mazandaran province is located in the Caspian climate, and with abundant flowering plants, it is one of the essential centers of beekeeping in Iran. *Nosema ceranae* infection was reported the first time from Iranian apiaries in this province (Nabian et al. 2011). This study aimed to determine the prevalence of *Nosema ceranae* in the apiaries of Mazandaran province by microscopy and molecular assays.

MATERIALS AND METHODS

Study area

Mazandaran province is located between the Caspian Sea and Alborz Mountain, extending from latitude 35°45’ to 37°10' and longitude 50°15' to 54° (Fig.1). The Alborz Mountains separate the Mazandaran province from the plateau and prevent Caspian's humidity from extending over the country, and also cause high annual precipitation consisting of snow in the highlands and rain in the lowlands. The abundant precipitation provides suitable conditions for natural vegetative growth in the province (Kazembeyki 2003).

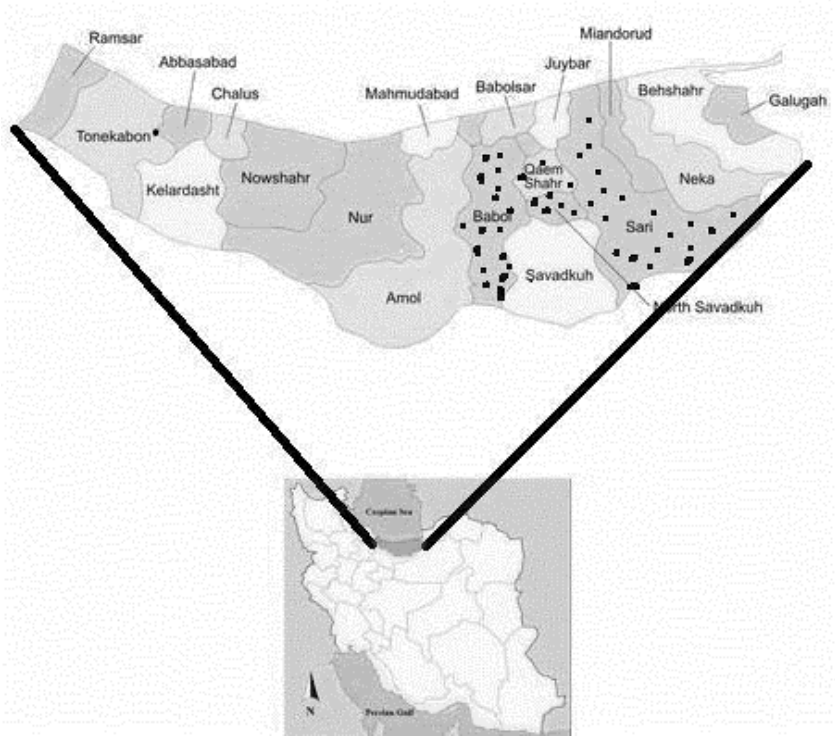

Figure 1. Map of sample collection in the Mazandran Province, Iran.

Samples collection

We collected samples from 320 hives. The sample size was calculated using a 95% confidence level with 5% desired absolute precision (Thursfield 1986), based on the prevalence of *Nosema* spp. infection (59%) that was previously reported in Azerbaijan province (Razmaraii and Karimi 2010). Thirty-two apiaries were randomly selected and sampled from April to March 2017 in Mazandaran province. The apiaries were located in the Sari, Ghaemshar, Babol and Savadkooh areas (Fig. 1).

After visiting the determined apiaries, the data for each apiary, we obtained from the beekeeper the apiary address, the name of the owner, bee population. Then, the samples were collected from 10 seemingly healthy hives in each apiary, consisting of 20 old worker bees from peripheral frames of each hive (200 honeybees in each apiary) (OIE 2019). The collected bees were put in storage containers and transported immediately to the laboratory under cold conditions.

Samples preparation Twenty honeybees' abdomens from each hive were ground up in 5 ml of normal saline solution. The suspensions were filtered through two layers of muslin to remove coarse bee parts and then centrifuged at 2500 g for 5 min. and the supernatants removed. Pellets of isolated spores were mixed with saturated saline solution and again centrifuged at 2500 g for 5 min. Some milliliters of supernatants were taken and the rest of the solution was discarded. The supernatants were washed three times with distilled water and each time they were centrifuged at 2500 g for 3 min and the upper parts were discarded. The finel pellets were resuspended in 1.5 ml of distilled water. The final pellets were One drop of the sample was put on a slide and covered with a slip and examined by a light microscope at ×400 magnification. The rest of the homogenate was transferred to an Eppendorf tube at kept at -20°C until use.

DNA extraction and Duplex- PCR

Total genomic DNA of homogenate samples was extracted according to the protocol of a DNA isolation kit (Molecular Biological System Transfer (MBST), Tehran, Iran). A Multiplex PCR method was used to simultaneous detection of two *Nosema* species in isolate DNA. (Martín-Hernández et al. 2007). Briefly, in amplification of Duplex-PCR four oligonucleotide PCR primers, 5'-GGCGACGATGTGATATGAAAATATTAA-3' as *N. ceranae* forward, 5'-CCCGGTCATTCTCAAACAAAAAACCG-3' as *N. ceranae* reverse, and 5'-GGGGGCATGTCTTTGACGTACTATGTA-3' as *N. apis* forward and GGGGGGCGTTTAAAATGTGAAACAACTATG -3' as *N. apis* reverse were used Amplification was conducted in 25 µl reaction volumes (Accupower PCR premix kit, Bioneer®, South Korea) with a final concentration of each dNTP of 250 µM in 10 mM Tris-HCl pH 9.0, 30 mM KCl and 1.5 mM $MgCl_2$, 1U Taq DNA polymerase and 10 pmol of each PCR primer (Takapouzist Co. Iran), Then 1 µl of DNA template was added to each reaction . The remaining 25 µl reaction volume was filled with nuclease-free distilled water. The thermocycler program consisted of 94°C for 2 min, followed by 10 cycles of 15 s at 94°C, 30 s at 61.8°C, and 45 s at 72°C, 20 cycles of 15 s at 94°C, 30 s at 61.8°C, and 50 s at 72°C plus an additional 5 s of elongation for each successive cycle, and a final extension step at 72°C for 7 min. The PCR products were electrophoresed in a 2% agarose gel with TBE buffer and visualized using ethidium bromide and UV-eliminator. A visible band at 321 bp for *N. apis* and 218bp for *N. ceranae* was produced in the PCR. The positive controls were prepared from the infected honeybees in the last study (Moshverinia et al. 2012) and the nuclease free distilled water as a negative control for each PCR amplification.

Gene sequencing

Five positive amplicons with the strongest band were selected, purified and sent to gene sequencing (Bioneer Inc, Seoul, Korea). The primers which were previously used for the PCR product of *N. ceranae* were applied for the sequencing reactions. Assembling and editing of sequenced nucleotides was performed using CLC software (CLC Main Workbench, Version5.5).

Statistics analysis

The relationship between *Nosema* infection rate and different variables such as the size of apiary and use and non-use of fumagillin was analyzed by the Chi-square test. A significant association was identified when a p-value of less than 0.05 was observed. The agreement between the molecular and microscopic tests was showed as a Kappa- coefficient. The agreement as poor if Kappa- coefficient between 0.2 and 0.4, moderate if between 0.4 and 0.6, substantial if 0.6 and 0.8 and good if it exceeds 0.8 and 1, (Petrie and Watson 2006).

RESULTS

In this study, *Nosema* spp. infection was detected in 78.12% of apiaries (25/32) by microscopy method (Fig. 2) and 84.37% of apiaries (27/32) by PCR. *N. ceranae* was the only species of *Nosema* identified. (Fig. 3). A poor agreement was observed between the microscopy and PCR methods (Table.1). (Kappa= 0.389). No significant statistical differences were identified between the prevalence of *N. ceranae* infection in apiaries by population and the use of fumagillin (Table. 2) ($p>0.05$). A blast search against GenBank revealed the highest similarity (100%) with *N. ceranae* 16SrRNA partial sequence from China (Sequence ID: MF099642.1).

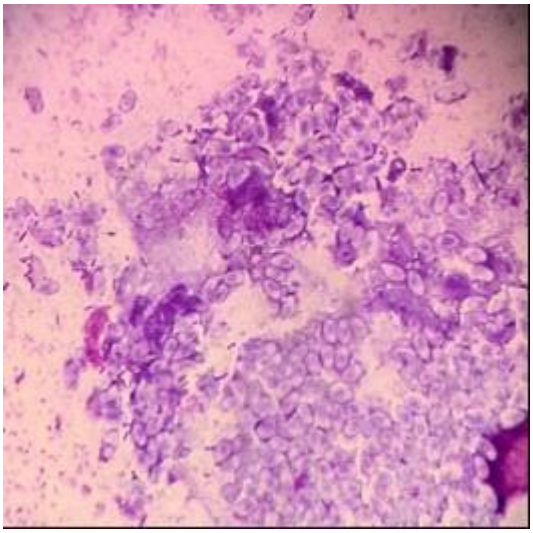

Figure2. *Nosema* spores stained by Giemsa under a light microscope (1000×).

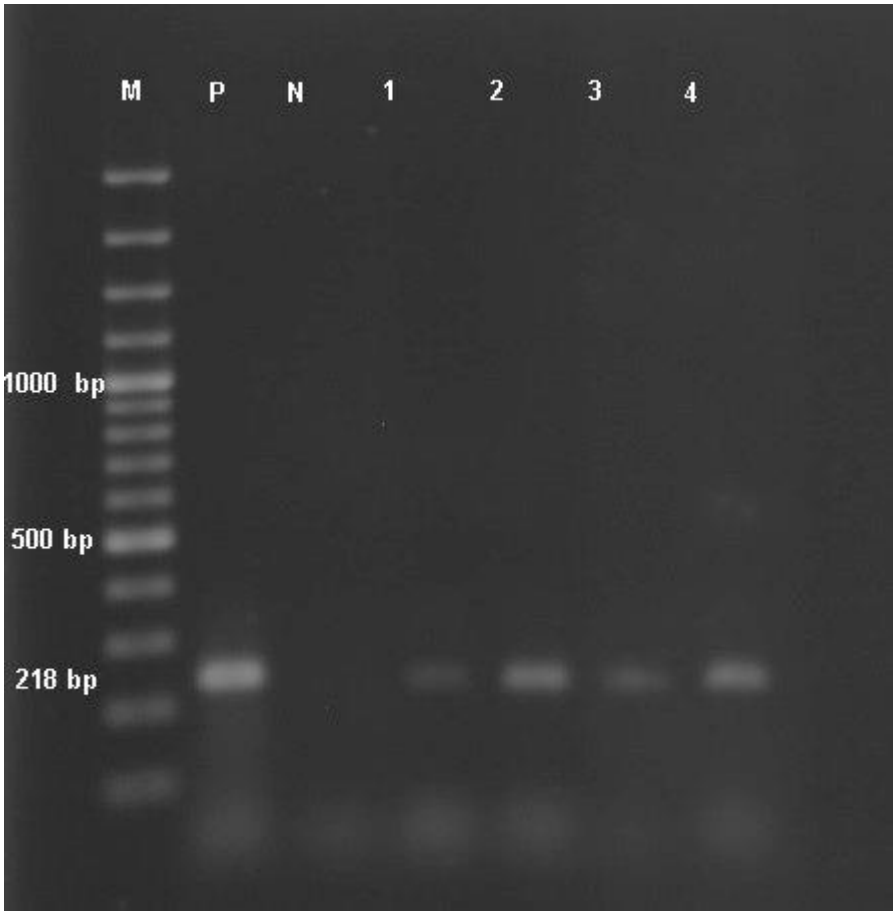

Figure 3. Electrophoresis results of SSUrRNA gene with special primers, M: Marker, P: Positive control, N: Negative Control, 1, 2, 3 and 4: *Nosema* Positive samples (218bp)

Table 1. Comparison of detection *Nosema* spp. infection in apiaries by Microscopy and PCR

Variable	PCR		Total
The size of apiary	Negative N	Positive N	
10-100 hives	4	18(81.8)	22
>100 hives	1	9(90)	10
Prophylaxis drug			
Fumagillin use	1	10 (90.9)	11
Fumagillin no use	4	17(80.9)	21
Total	5	27 (4.5)*	32

Table 2. Frequency of *N.ceranae* infection by size of apiaries

Results	Number	(%)
Both Microscopy and PCR (+)	23	71.8
Both Microscopy and PCR (-)	3	9
Microscopy (+), PCR (-)	2	6.2
Microscopy (-), PCR (+)	4	12.5

DISCUSSION

The frequency of *Nosema* spp. infection was 78.12% in apiaries in the present study based on microscopic examination. The rate of *Nosema* spp. infection was reported to be 50%-90% in Iranian apiaries in different regions by a microscopy method (Razmaraii and Karimi 2010, Moshverinia et al. 2012, Khezri et al. 2018). The frequency of *Nosema* spp. infection was reported to be 22.4%-35.4% in Germany (Gisder et al. 2010), 78.6%-94.6% in Balkan countries (Stevanovic et al. 2011), and 20.59% in Saudi Arabia (Ansari et al. 2017) by microscopic examination. The differences in the reported prevalence of *Nosema* infection may depend on the climate of each country, health management practices in apiaries, and sampling and diagnostic methods. Two studies were reported *N. apis* infection in Iranian apiaries by microscopic method (Razmaraii and Karimi 2010, Mashverinia et al. 2012). The results of these studies are questionable, because, the spores of two Nosema species are very similar and there is no morphological index for two species differentiation.

Molecular examination showed that 87.37% of the apiaries of this study were infected with *N. ceranae* only. Our results are consistent with other molecular studies that determined that *N. ceranae* was the only causative agent of nosemosis in Iranian apiaries (Nabian et al. 2011, Khezri et al. 2018, Mohhamadian et al. 2018). The high prevalence of *N. ceranae* in this study may be related to subtropical climate in the areas. The proportion of *N. ceranae* infections appears to dominate in warmer climates compared to more temperate regions, whereas *N. apis* presently may be more prevalent in colder climates (Fries 2010).

The results of many studies have shown that *N. ceranae* infection has a worldwide distribution (Klee et al. 2007). The prevalence of *N. ceranae* was15%-100% in Turkey (Ivgin Tunca et al. 2016), 63% in Italy (Papini et al. 2017), 80.6% in Poland (Michalczyk et al. 2011), 95%-97% in Hungary (Csáki et al. 2015), 77% in Bulgaria (Shumkova et al. 2018), 41%-91% in Canada (Emsen et al. 2016), and 56% in Saudi Arabia (Ansari et al. 2017).

In the present study, a fair agreement was observed between microscopy and PCR results, while other studies have reported substantial to good agreement between the two methods (Khezri et al. 2018, Papini et al. 2017). It is clear that the sensitivity and specificity of PCR method is higher than light microscopy for identification and differentiation of *Nosema* infection in honeybees (Michalczyk et al. 2011). However, the PCR results were negative in two samples, even though the spores were observed. The results may be related to incomplete DNA extraction or the prevention effect of the resilient walls on DNA extraction (Webster et al. 2004). The isolated *N. ceranae* sequences in this study had high-level homology with *N. ceranae* sequences of *N. ceranae* collected in China that were deposited in the GenBank database. The Fumagillin as an antibiotic extracted from *Aspergillus fumagitus* has been used for treatment of nosemosis in apiaries for several years. Recent studies have been shown that fumagillin is a carcinogenic substance and its residue in honey is dangerous for human health. (Van den Heever et al. 2014). For this reason, European countries have banned its use in apiary. Nevertheless, it is still used as a drug for nosomiasis treatment in Iran (Moradi, 2019) and other countries (McCallum et al. 2020; Glavinic et al. 2021). We also investigated the effectiveness of preventive fumagillin treatment in this study. The results showed that the level of *Nosema* spp. infection did not differ between treated and untreated colonies. An experimental study showed that *N. ceranae* is not very sensitive to low doses of fumagillin, and it can actually cause hyperproliferation of *Nosema* spp. in infected honeybees. (Williams et al. 2010). Our findings showed that *N. ceranae* at a high frequency are the only causative agent of nosemosis in Mazandaran

province. Furthermore, the use of fumagillin was not adequate for controlling nosemosis in infected colonies.

Ethics statement: Study protocols and methodologies were revised and approved by the Ethical Committee at Ferdowsi University of Mashhad, Khorasan Razvi Province, Iran

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Conflict of interest: The authors declare that they have no conflict of interest.

Author contributions: A.S collected samples and performed all experiments. G.R.R was the supervisor of project and analyzed the data and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

EFFECTS OF THE ISOLATION CONDITIONS ON MORPHOLOGY AND PERFORMANCE OF HONEY BEES

İzolasyon Koşullarının Bal Arılarının Morfolojisi ve Performansına Etkileri

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ABSTRACT

Keeping honey bee colonies in isolated areas can cause inbreeding. The inbreeding over a long period is good for the purity of bee subspecies but also has some negative effects including the low performance of colonies. This study was performed on hybrid honey bee colonies placed in an isolated location for about five years to check the effects of inbreeding on them. The bees were able to mate with each other during this period of time without introducing new bee queens or bee packages. Some morphological characteristics and parameters were studied to test the purity of the bees and the presence of any negative effects due to inbreeding. The results showed the presence of variations between studied colonies without any negative effects on bee morphology, hygienic behavior, bee larvae development, brood rearing activity, and food storing activity. The study highlighted the absence of deleterious effects on honey bees due to inbreeding under isolation conditions.

Key Words: Inbreeding, *Apis mellifera*, hygienic, endogamy, purity

ÖZ

Bal arısı kolonilerinin izole alanlarda tutulması akrabalı üremeye neden olabilir. Akrabalı yetiştirmenin uzun bir süre boyunca yapılması, arı alt türlerinin saflığı için iyidir ancak aynı zamanda kolonilerin düşük performansı da dahil olmak üzere bazı olumsuz etkileri de vardır. Bu çalışma, akrabalı yetiştirmenin etkilerini kontrol etmek için yaklaşık beş yıl boyunca izole bir yere yerleştirilen hibrit bal arısı kolonileri üzerinde yapıldı. Arılar, bu süre zarfında yeni ana arılar veya arı paketleri sunmadan birbirleriyle çiftleşebildiler. Arıların saflığını ve akrabalı yetiştirmeden kaynaklanan olumsuz etkilerin varlığını test etmek için bazı morfolojik özellikler ve parametreler incelenmiştir. Sonuçlar, arı morfolojisi, hijyenik davranış, arı larva gelişimi, yavru yetiştirme aktivitesi ve gıda depolama aktivitesi üzerinde herhangi bir olumsuz etki olmaksızın çalışılan koloniler arasında varyasyonların varlığını göstermiştir. Çalışma, izolasyon koşulları altında akrabalı yetiştirme nedeniyle bal arıları üzerinde zararlı etkilerin olmadığını vurguladı.

Anahtar Kelimeler: Akrabalı yetiştirme, Apis mellifera, hijyenik, endogami, saflık

GENİŞLETİLMİŞ ÖZET

Amaç: İzole bir çöl bölgesinde akrabalı yetiştirmenin arı kolonileri üzerindeki etkisini bilmek.

Gereç ve Yöntem: Çalışma güneyde (Abu Simbel bölgesi) izole bir alanda bulunan bir arı kovanı üzerinde gerçekleştirilmiştir. Konum (22° 24' 21.6" K, 31° 32' 41.64" E), arıların akrabalı yetiştirmeye maruz kalmasını sağlamak için bu arılığa yeni kraliçeler veya arı paketleri eklenmedi.

Bu çalışmayı yapmak için 2019 yazında arılıktan rastgele seçilen aynı güçte altı kovandan bal arısı örnekleri toplandı. Elli işçi arı alkollü kavanozlara alınarak morfometrik analize hazırlandı. Kanatlar ve bacaklar arılardan ayrıldı, cam slaytlara monte edildi ve net görüntüler için 1200 dpi'de bir Hp Deskjet 2050A ile tarandı. Görüntüler daha sonra El-Aw ve arkadaşlarına göre tarama teknolojisi (SPT) kullanılarak ön uzunluk ve genişlik ile arka bacakların tibial ve baziler uzunluklarını ölçmek için kullanıldı (2012). Ölçülen özellikler daha sonra kovanlar arasındaki farkları ortaya çıkarmak için istatistiksel analize tabi tutuldu.

Abu Shaara ve Ahmed (2015) tarafından yürütülen bir çalışmada, Carniolan melezlerinden alınan işçi arı örneklerinin morfolojik özellikleri ölçülmüş ve yayınlanmıştır. Yayınlanan karakteristik aralıklar, melez arılar için normatif değerler olarak alınmıştır. İzole edilmiş arılıktaki arıların ölçülen özellikleri daha sonra, izole edilmiş arılarda akrabalı yetiştirme nedeniyle meydana gelen değişiklikleri belirlemek için standart değerlerle karşılaştırılmıştır.

-Hijyenik davranış: Pupaları öldürmek için kuluçka tarağının ortasındaki 50 kapalı hücre küçük bir iğne ile delindi. Kolonilerin hijyenik davranışını değerlendirmek için 24 saat sonra temizlenen hücreler sayıldı. Bu test bir ay arayla üç kez tekrarlandı ve ardından her kovan için üç testin ortalaması hesaplandı. Ortalamalar, kolonilerdeki hijyenik davranışın tutarlılığını kontrol etmek için istatistiksel olarak karşılaştırıldı.

-Yumurtadan ergine gelişim:

Yumurtaların normal olarak larvalara, larvalardan pupalara ve pupalardan yetişkinlere yumurtadan çıkma yeteneği değerlendirildi. Her kovanda yaklaşık 100 yumurta içeren bir alan işaretlendi ve ardından yumurtadan çıkana kadar olan gelişim kaydedildi. Bu deney: Eylül ve Kasım aylarında iki kez tekrarlandı.

-Damızlık yetiştirme ve yiyecek depolama faaliyetleri.

Eylül, Ekim ve Kasım aylarında kuluçka ve yiyecek depolama faaliyetleri kaydedildi. Bu aktiviteleri ölçmek için inç2'ye bölünmüş bir çerçeve (Jeffree 1958) kullanıldı. Kaydedilen araçlar daha sonra kovanlar arasındaki tutarlılığı bulmak için karşılaştırıldı.

Sonuç:

1. Arı morfolojisi:

Ölçülen morfolojik özellikler, işçi arılar için kaval kemiği uzunluğu ve arı erkek arılar için ön kanat uzunluğu dışında, ölçülen özelliklerin çoğunda önemli farklılıklar kaydedildiğinden çok yüksek özdeş değerlerin olmadığını göstermiştir. Bu, beş yıllık izolasyondan sonra arıların hala melez bir yapıya sahip olduklarını yansıtmaktadır. Bununla birlikte, minimum, medyan ve maksimum değerler açısından tanımlayıcı veriler, kolonilerin saflık ve özelliklerin tutarlılığına yöneldiğini desteklemektedir. Bu nedenle, işçi arılar tarafından paylaşılan özdeş değerler %28 ile %96 arasında değişmektedir ve bu benzerlikler izolasyon koşullarında yaklaşık beş yıllık akrabalı yetiştirmenin etkisiyle açıklanabilir.

2. Hijyenik davranış:

Toplam ortalamalara göre %80'den fazla uzaklaştırma yüzdesi ile sadece beş kovanın hijyenik trendle kabul edilebileceği açıktır. Elde edilen sonuçlar ışığında, incelenen kolonilerde 5 yılı aşkın akrabalı yetiştirmenin hijyen eğilimini olumsuz etkilemediği görülmüştür.

3. Yumurtadan ergine gelişim:

İzole lokasyonda bulunan kovanlarda arıların gelişimi normaldi ve zamanla akrabalı yetiştirmeden olumsuz etkilenmedi. Çalışma yerinin çöl doğasına rağmen arıların gelişimi üzerinde herhangi bir zararlı etkisinin olmadığı açıktır. Ayrıca, izolasyon koşullarında akrabalı yetiştirme nedeniyle arıların gelişimi herhangi bir genetik sürüklenmeden etkilenmemiştir.

4. Kuluçka yetiştirme ve yiyecek depolama faaliyetleri:

Mısır'ın melez arılarının zorlu çevre koşulları altında normal olarak kuluçkaya yatma yeteneği, Abou-Shaara ve arkadaşları (2013b) tarafından önceki çalışmalarda, bir çöl bölgesinde gerçekleştirilen mevcut çalışma ile uyumludur. Tüm koloniler aynı güce sahipti, ancak kuluçka yetiştirme ve yiyecek

depolama faaliyetleri, araçlara bağlı olarak kovandan kovana değişiyordu. Ancak, kovanlar arasında önemli farklılıklar sadece kuluçka faaliyetinde tespit edilmiştir. Genel olarak, bu çalışma, hibrit arılardan thelytokous olmadan saf arı elde etmek için yaklaşık beş yıllık bir sürenin yeterli olmadığını vurgulamaktadır.

INTRODUCTION

Honey bees, *Apis mellifera* L., are considered as typical social insects. They live in colonies consist of one reproductive female (queen), thousands of infertile females (workers), and hundreds of males (drones). The presence of one reproductive female increases the possibility of inbreeding in bee colonies through endogamy. However, honey bees naturally avoid inbreeding as queen mates in the air with a lot of bee drones (Taber and Wendel 1985, Moritz et al. 1996, Cobey 2007) at specific sites called drone congregation areas (Zmarlicki and Morse 1963). Such high number of drones ensures the genetic variations inside the colonies. The number of sperm inside the spermatheca of a mated queen can reach to 4.54 million (Kaftanoglu and Peng 1982). Sperm can stay alive for a long period inside the spermatheca, and secretions from spermathecal glands have a key role in this (den Boer et al. 2009). Studies have shown that mating with high number of drones is better than mating with a single drone, and sufficiently with seven mates as the minimum (Tarpy et al. 2012, Delaplane et al. 2015).

The queens under normal conditions can lay high number of eggs per day (Moore et al. 2015). Two types of eggs are laid by the queens: fertilized eggs and unfertilized ones (Ratnieks and Keller 1998). The fertilized eggs hatch to new workers or queens. The fertilization of eggs ensures the genetic variations as a result of the combination of genetic materials from the egg cells and sperm cells, especially the complementary sex determiner gene should be heterozygous in females (Beye et al. 2003). Drones are mainly result from the unfertilized eggs. The characteristics of bee drone are typical to its mother without huge variations between them. The inbred queens can lay fertilized eggs in worker cells which develop into weak drone larvae, but such larvae are rapidly eaten by workers (Woyke 1963, Woyke 1964). Basically, many apiaries are placed close to each other, and this ensures the presence of high number of drones at the drone congregation areas and from various genetic sources. Under certain circumstances, apiaries can be established in isolated areas including islands, newly reclaimed desert areas or mountainous areas.

The main problem of the isolated areas is the possibility of exposing bees to inbreeding and the low performance of the colonies due to endogamy. The main benefit of inbreeding is increasing the purity of bee stocks and can enhance some traits of bee colonies including calmness (Bienefeld et al. 1989). Most of honey bee populations are characterized by the average level of inbreeding due to the features of their life and biology. Only hybrid populations of honey bees can be characterized with outbreeding (Ilyasov et al., 2015; 2016). The purity of honey bees as well as the negative effects of inbreeding can be screened by studying bee morphology and colony performance parameters. Morphological characteristics can be utilized to examine the purity of bee subspecies (Bienefeld et al. 1996, Radloff et al. 2003, Abou-Shaara et al. 2013a), and are impacted by inbreeding (Roberts 1961, Brückner 1979). Hygienic behavior depends on genetic characteristics of bees without great influence of environmental conditions or colonies status on it (Bigio et al. 2013, Xonis et al. 2015), and can be improved through selection and breeding (Palacio et al. 2000, Pernal et al. 2012, Gerula et al. 2015). Behaviors and performance of bees can be impacted by inbreeding (Brückner 1980; Oldroyd and Goodman 1988, Bienefeld et al.1989, Cermak 1996). The small morphological characteristics, low hygienic ability, abnormal development of bees, and low bee activities are expected to bee colonies under isolation conditions because of inbreeding.

Therefore, this study was performed on honey bee colonies placed at an isolated location for a period of about five years. These colonies were able to mate with each other only and no new queens or bee packages were introduced to this location. Some morphological characteristics of worker and drone bee samples were measured. Also, hygienic behavior, development of bee larvae, and activities of the colonies were studied. These parameters were employed to check the purity of the studied colonies as well as the negative effects of inbreeding on them.

MATERIALS AND METHODS

1. Study location and honey bee colonies

An apiary was established in February 2015 in an isolated location in the south of Egypt (Abu Simbel region) (Abou-Shaara and Kelany, 2021). The location (22° 24' 21.6" N, 31° 32' 41.64" E) of this apiary is shown in Fig. 1. Twenty hives with hybrids of Carniolan honey bees were placed in this apiary. The queens were replaced frequently from the same colonies and mated with drones from the same colonies over a period of about five years. Some bee colonies were lost during this period. However, no new queens or bee packages were introduced to this apiary to ensure the exposure of bees to inbreeding.

Fig.1: The location of the apiary (black arrow).

2. Morphology of worker bees

Six hives with similar strength of seven frames covered with bees were randomly selected from the apiary to perform this study during summer 2019. From each hive 50 worker bees were collected from brood combs in jars contain alcohol and were then prepared for the morphometric analysis. The wings and legs were separated from the bees and were mounted on glass slides and scanned using Hp Deskjet 2050A at 1200 dpi to obtain clear images. The images were then used to measure forewing length and width, and lengths of tibia and basitarsus of the hind legs using the Scan Photo Technique (SPT) according to El-Aw et al. (2012). The measured characteristics were then subjected to the statistical analysis to detect the variations between the hives.

The morphological characteristics of bee worker samples from hybrids of Carniolan bees were measured and published in a study by Abou-Shaara and Ahmed (2015). The ranges of the published characteristics were considered as the standard values for the hybrid bees. Then, the measured characteristics of the bees from the isolated apiary were compared with the standard values to identify the changes in the isolated bees due to inbreeding.

3. Morphology of honey bee drones

From each hive ten drones were collected during late summer, and then were dissected and some morphological characteristics were measured as mentioned with worker bees. The measured characteristics were compared to check the consistency between hives. Low numbers of drones were collected from each hive because there were few drones in them.

4. Hygienic behavior

In each hive, 50 sealed cells at the middle of the brood comb were pierced with a tiny needle to kill the pupae. The number of cleaned cells was then

counted after 24h to assess the hygienic behavior of the colonies. This test was repeated three times with one month interval and then the mean of the three tests was calculated for each hive. The means were statistically compared to check the consistency of the hygienic behavior in the colonies.

5. The development from eggs to adults

The ability of eggs to hatch normally into larvae and from larvae to pupae and from pupae to adults was assessed. In each hive, an area containing about 100 eggs was marked and then the development until hatching was recorded. This experiment was repeated twice during September and November.

6. Brood rearing and food storing activities

The activities of brood rearing and food storing were recorded during September, October, and November. To measure these activities, a frame divided into inch^2 (Jeffree 1958) was used. The recorded means were then compared to find out the consistency between the hives.

7. Statistical analysis

Normality of the data was checked using Kolmogorov-Smirnov and Shapiro-Wilk tests. For morphological characteristics, descriptive statistics were firstly calculated while means were compared using the non-parametric test (Kruskal-Wallis test). For the hygienic behavior data, Kruskal-Wallis test was used to compare means. The analysis of variance (ANOA) was utilized to identify the significant differences between means of brood rearing and food storing activities. The analysis at a significance level of 0.05 was conducted using the SPSS v. 16.

RESULTS

1. Worker Morphology

The descriptive statistics showed the presence of variations between hives based on mean and median values but with somewhat similar minimum and maximum values for the measured characteristics (Table 1). These variations were confirmed by the Kruskal-Wallis test as significant differences were found in forewing length (Chi-Square=24.312, df=5, p= 0.000<0.05), forewing width (Chi-Square=45.819, df=5, p= 0.000<0.05), and basitarsus length (Chi-Square=33.289, df=5, p= 0.000<0.05) while no significant differences were found in tibia length (Chi-Square=6.748, df=5, p= 0.240>0.05). The percentages of worker bees with the identical minimum, median and maximum values from the six hives were 44 to 62% to forewing length, 28 to 72% to forewing width, 48 to 72% to the tibia length, and 54 to 96% to the basitarsus length.

The comparison between the measured characteristics and the standard values measured by Abou-Shaara and Ahmed (2015) are presented in Table 2. It is evident that the hives shared from 48 to 68%, from 26 to 54%, from 10 to 60%, from 6 to 44%, from 26 to 56%, and from 22 to 54% with the standard values for hive 1 to 6, respectively.

Table 1: Means (mm) ± S.E. of measured body characteristics of worker bees from the six hives. Also, descriptive statistics of measured characteristics for the six hives are presented.

Hive	Forewing length	Forewing width	Tibia length	Basitarsus length
1	8.57±0.01	2.97±0.01	2.86±0.01	2.02±0.01
2	8.54±0.01	2.95±0.01	2.86±0.01	2.04±0.01
3	8.49±0.01	2.85±0.01	2.84±0.01	2.06±0.01
4	8.46±0.01	2.90±0.01	2.87±0.02	2.01±0.01
5	8.48±0.01	2.88±0.01	2.86±0.01	1.95±0.01
6	8.51±0.02	2.91±0.01	2.81±0.01	2.05±0.01
Minimum	8.2-8.3	2.6-2.8	2.6-2.7	1.7-1.9
Maximum	8.7	3-3.2	3-3.1	2.1-2.3
Median	8.4-8.6	2.9-3	2.8-2.9	2-2.1

Table 2: Number of bees with identical values with the standard values measured by Abou-Shaara and Ahmed (2015) from the six hives.

Characteristics Standard value range (mm)	**Hive number**	1	2	3	4	5	6
Forewing length Range (8.64-8.74)	Number of bees **Total (%)**	34 **68**	25 **50**	18 **36**	12 **24**	16 **32**	21 **42**
Forewing width Range (3.02-3.06)	Number of bees **Total (%)**	24 **48**	16 **32**	5 **10**	3 **6**	13 **26**	11 **22**
Tibia length Range (2.88-2.96)	Number of bees **Total (%)**	34 **68**	27 **54**	30 **60**	18 **36**	27 **54**	27 **54**
Basitarsus length Range (1.98-2.04)	Number of bees **Total (%)**	25 **50**	13 **26**	13 **26**	22 **44**	28 **56**	23 **46**

2. Drone morphology

The descriptive statistics showed the presence of variations between hives (Table 3). These variations were confirmed by the Kruskal-Wallis test as significant differences were found in forewing width (Chi-Square=14.97, df=5, p= 0.01<0.05), tibia length (Chi-Square=14.87, df=5, p= 0.01<0.05), and basitarsus length (Chi-Square=22.10, df=5, p= 0.00<0.05) while no significant differences were found in forewing length (Chi-Square=8.59, df=5, p= 0.12>0.05).

Table 3: Means (mm) ± S.E. of measured body characteristics of bee drones from the six hives. Also, descriptive statistics of measured characteristics for the six hives are presented.

Hive	Forewing length	Forewing width	Tibia length	Basitarsus length
1	11.21±0.04	3.95±0.07	3.58±0.03	2.49±0.03
2	11.31±0.05	4.12±0.03	3.62±0.04	2.69±0.03
3	11.19±0.04	4.02±0.05	3.62±0.01	2.61±0.03
4	11.22±0.04	3.88±0.05	3.49±0.03	2.50±0.03
5	11.18±0.05	4.01±0.04	3.71±0.04	2.65±0.03
6	11.13±0.05	3.86±0.05	3.61±0.04	2.62±0.02
Minimum	11-11.1	3.7-4	3.4-3.6	2.4-2.5
Maximum	11.4	4.1-4.3	3.7-3.9	2.8-2.9
Median	11.05-11.4	3.85-4.15	3.45-3.7	2.5-2.7

3. Hygienic behavior

The three tests showed consistency in the results except hive 2 and 6 (Table 4). The percentages of the cleaned cells in the first test ranged from 44 to 100% and from 74 to 100% in the second test, and 100% to all hives in the third test. The lowest value was recorded only to hive 6 during the first test. The overall means ranged from 79.33 to 100% without statistical variations between the six hives according to the Kruskal-Wallis test (Chi-Square= 5.168, df= 5, p=0.39>0.05).

Table 4: Hygienic behavior tests for the six hives. Percentages of cleaned cells after 24h are presented.

Test number	Hive number					
	1	**2**	**3**	**4**	**5**	**6**
1	100	94	100	100	100	44
2	100	74	80	70	100	94
3	100	100	100	100	100	100
Mean ± S.E.	**100±0.00**	**89.33±7.86**	**93.33±6.67**	**90±10**	**100±0.00**	**79.33±17.75**

4. The development from eggs to adults

In all the studied hives, eggs were able to hatch normally into larvae and from larvae to pupae, and from pupae to adults with percentages of 100%.

5. Brood rearing and food storing activities.

Only sealed brood area differed significantly (ANOVA, df= 5, F= 7.627, p=0.02<0.05) between the hives. Hive 4 and 5 had the lowest brood rearing area. Stored pollen area (ANOVA, df= 5, F= 0.84, p=0.54>0.05) and stored honey area (ANOVA, df= 5, F= 2.375, p=0.10>0.05) exhibited insignificant variations (Table 5).

Table 5: Brood rearing and food storing activities (Means ± S.E. in inch^2) for the six hives. Means marked with different letters denote to the presence of significant differences according to Tukey HSD test.

Hive	Sealed brood area	Stored pollen area	Stored honey area
1	349.67±64.23ab	31.33±10.26	227.33±21.94
2	397.67±42.88a	54.00±35.67	557.00±59.28
3	460.67±57.77a	73.33±33.54	427.33±162.74
4	117.67±15.34b	27.33±13.22	136.67±10.36
5	142.67±76.52b	33.00±9.00	387.67±157.98
6	352.00±9.86ab	20.67±6.96	271.67±53.16

DISCUSSION

Bee morphology

The measured morphological characteristics showed the absence of very high identical values as significant differences were recorded in most measured characteristics except tibia length for worker bees and forewing length for bee drones. This reflects that the bees after five years of isolation still had hybrid nature. However, the descriptive data in terms of minimum, median, and maximum values support that the colonies tend towards purity and consistency of characteristics. Therefore, the identical values shared by worker bees ranged from

28 to 96%, and these similarities can be explained by the impact of inbreeding over about five years under the isolation conditions. The similarities are not very high likely because the period of five years is not sufficient to turn bee colonies into pure bees instead of their hybrid status.

Hybrid bees are mostly larger than inbred bees (Roberts 1961). In the present study, the morphological characteristics of worker bees under isolation conditions (i.e. inbred bees) showed similarities (from 6 to 68%) with the standard values of hybrid bees measured by Abou-Shaara and Ahmed (2015). Therefore, the bees were impacted slightly by the inbreeding especially hive 4 because some of the measured characteristics were less than the standard ranges of the hybrid bees.

The means of forewing length, forewing width, and basitarsus length for the six hives ranged from 8.46 to 8.57mm, 2.88 to 2.97mm, and 1.95 to 2.06mm, respectively. The values of these characteristics in respect are 8.23, 2.78, and 1.96 mm for the Egyptian bees, *Apis mellifera lamarckii*, (Data Bank, Oberursel Frankfurt, Germany), and 9.17, 3.19, 2.42 mm for the Carniolan bees, *Apis mellifera carnica* (Yakoub 2002). Therefore, the obtained values for the studied hives were moderate between the values of the two subspecies. This confirms the hybrid status of these bees between the Egyptian and Carniolan bees.

Hygienic behavior

t is clear that only five hives can be considered with a hygienic trend with a removal percentage of more than 80% based on the overall means. Hive 6 was different than the other hives due to the low hygienic ability during the first test. In light of the obtained results, the inbreeding over 5 years did not impact the hygienic trend negatively in the examined colonies. The obtained results are in line with those found by Abou-Shaara et al. (2018) for hybrid bees of Egypt, as the percentages of the cleaned cells ranged from 82 to 89%. However, the present study is on the contrary with the study by Balhareth et al. (2012) on hybrid bees of Egypt under Saudi Arabia conditions, as the mean of the cleaned cells was 79.32% to the studied colonies. This can be attributed mainly to the arid conditions of Saudi Arabia, which negatively impacted bee populations and subsequently their hygienic trend.

The development from eggs to adults

The development of bees in the hives located at the isolated location was normal and has not been impacted negatively by inbreeding over time. It is known that egg hatching is negatively impacted by unsuitable levels of temperature and relative humidity (Al-Ghamdi et al. 2014), and harsh conditions can passively affect egg hatching and development of bees (Abou-Shaara et al., 2017). So, it is evident that the study location has no deleterious effects on the development of bees although it is a desert nature. Also, the development of bees was not impacted by any genetic drift due to inbreeding under isolation conditions.

Brood rearing and food storing activities

The ability of the hybrid bees of Egypt to rear brood normally under harsh environmental conditions was confirmed in previous studies by Abou-Shaara et al. (2013b) and Kelany (2018), and this is in line with the present study which was performed at a desert region. All colonies had the same strength but brood rearing and food storing activities varied from hive to another based on means. However, significant differences between hives were detected only in brood rearing activity. The low brood rearing activity in hive 4 and 5 than the other hives can be partially explained by inbreeding. The absence of significant variations between hives in the stored pollen and honey areas suggested the same ability of hives to store food. In fact, effects of inbreeding on honey production can be negative (Bienefeld et al. 1989) or positive (Cermak 1996). These indicate that effects of inbreeding on food storing activity are not constant and can be affected by other factors than inbreeding. In a similar way, Oldroyd and Goodman (1988) found that colonies with hybrid queens did not have higher honey production than colonies with inbred queens. Overall, the present study highlights that the period of about five years is not sufficient to obtain pure bees from the hybrid bees without *thelytoky*. For comparison, the thelytokous Cape honey bees, *Apis mellifera capensis*, showed high levels of heterozygosity after inbreeding for 10 years (Oldroyd et al. 2011) and 20 years (Smith et al. 2019).

Conclusion

The small morphological characteristics, low hygienic ability, abnormal development of bees, and low bee activities are expected to bee colonies after five years from isolation as a result of inbreeding. On the contrary, the study showed no deleterious effects

on the morphology or performance of honey bees after isolation for about five years. The bees had noticeable and significant variations in the measured parameters without low levels of hygienic behavior or abnormal development of bees, suggesting that the negative effects of inbreeding were not high.

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

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

FORAGING ACTIVITY OF MANAGED BEE POLLINATOR (*Apis cerana indica)* IN BITTER GOURD CROPPING SYSTEM IN INDIA

Hindistan'da Acı Kabak Yetiştirme Sisteminde Bakılmış Arı Tozlayıcısının (*Apis cerana indica*) Yayılma Faaliyetleri

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ABSTRACT

Honey bees play an important role in crop pollination of bitter gourd flowers. An experiment was conducted at Coimbatore District to observe the foraging activity and pollination efficiency of Indian bee (*A. cerana indica)* in Bitter gourd (*Momordica charantia* L.). The foraging activity and number of foragers/ flower/ min was recorded in male and female bitter gourd flower. Bitter gourd fruit set and yield was assessed under three conditions pollinator exclusion, bee pollination and open pollination. No. of foragers/ flower/ minute (abundance of pollinators) and floral handling time in seconds (foraging rate) of Indian bees recorded on male flower (0.88 bees) and (6.52 sec) was higher than in female flower (0.57 bees) and (3.61 sec) respectively. The peak foraging activity of *A. cerana indica* noticed between 08:00-10:00 hours with 1.2 foragers/ 5 mins. Pollination efficiency index of Indian bee was 747035.5. Foraging activity at the hive entrance was maximum at 08:00 to 10:00 hours. Colony growth parameter of *A. cerana indica* was increased 67.85% in respect of the sealed honey area and 15.07% in respect of the adult population. The results of pollination studies show, the number of fruits/plant (17.4 fruits) and fruit yield per hectare (41.13 t/ha) was higher in managed bee pollinated plot than to open pollination condition (16.2 fruits) and (37.25 t/ha) and no fruit set was recorded in pollination exclusion condition.

Key words: Bitter gourd, *Apis cerana indica*, foraging activity, pollination efficiency

ÖZ

Bal arıları, acı kabak çiçeklerinin mahsul tozlaşmasında önemli bir rol oynamaktadır. Acı kabakta (Momordica charantia L.) Hint arısının (A. cerana indica) yiyecek arama aktivitesini ve tozlaşma etkinliğini gözlemlemek için Coimbatore Bölgesinde bir deney yapılmıştır. Erkek ve dişi acı kabak çiçeğinde yiyecek arama aktivitesi ve toplayıcı/çiçek/dk sayısı kaydedilmiştir. Acı kabak meyve tutumu

ve verimi, tozlayıcı dışlama, arı tozlaşması ve açık tozlaşma olmak üzere üç koşul altında değerlendirilmiştir. Hint arılarının erkek çiçek (0.88 arı) ve (6.52 sn) dişi çiçekten (0.57 arı) daha yüksek olduğu kaydedilmiştir. (3.61 sn) sırasıyla. A. cerana indica'nın pik arama aktivitesi, 1.2 toplayıcı / 5 dakika ile 08:00-10:00 saatleri arasında fark edildi. Hint arısının tozlaşma verimlilik indeksi 747035,5 olmuştur. Kovan girişinde yiyecek arama faaliyeti en fazla 08:00-10:00 saatleri arasında olmuştur. A. cerana indica'nın koloni büyüme parametresi kapalı bal alanına göre %67.85, ergin popülasyona göre ise %15.07 artmıştır. Tozlaşma çalışmalarının sonuçları, idareli arı ile tozlanan parsellerde meyve/bitki sayısı (17,4 meyve) ve hektar başına meyve veriminin (41,13 t/ha), açık tozlaşma koşuluna (16,2 meyve) ve (37,25 t/ha) göre daha yüksek olduğunu göstermektedir ve tozlaşma dahil edilmedğinde hiçbir meyve tutumu kaydedilmemiştir.

Anahtar kelimeler: Acı kabak, Apis cerana indica, yiyecek arama etkinliği, tozlaşma etkinliği

GENİŞLETİLMİŞ ÖZET

Çalışmanın amacı: Çalışmanın amacı, Hindistan'daki acı kabak yetiştirme sisteminde bal arısı Apis cerana indica'nın yiyecek arama aktivitesini ve tozlaşma etkinliğini değerlendirmekti.

Malzemeler ve yöntemler: Çalışma Coimbatore ilçesinde yürütülmüş, tozlayıcıların yiyecek arama faaliyetlerini incelemek için on bitki rastgele etiketlenmiş ve bitki başına 3 çiçek haftalık aralıklarla gözlemlenmiştir.

Tozlayıcının pik aktivitesi sırasında yiyecek arama davranışı hayır olarak kaydedildi. toplayıcılar /çiçek/dakika (tozlayıcıların bolluğu) ve bireysel arı tozlayıcısı/çiçek/dakika (toplayıcılık oranı) tarafından haftalık aralıklarla bir kronometre kullanılarak harcanan süre ve no. 0600-1800h arasında iki haftada bir 5 dakikalık bir süre boyunca ziyaret eden/çiçek açan toplayıcıların oranı. Arı gövdelerindeki gevşek polen taneleri ölçülmüş ve no. vücuttaki gevşek polen taneleri × yiyecek arama oranı × acı kabak çiçekleri üzerindeki tozlayıcıların bolluğu.

Yönetilen arıların tozlaşma verimliliği, tozlayıcı dışlama (T1), arı tozlaşma (T2) ve açık tozlaşma koşulunda (T3) olduğu gibi farklı tozlaşma modlarında değerlendirildi, verim değerlendirmesi yapmak için çiçeklenme başladıktan sonra tedavi başına 350 dişi çiçek etiketlendi. Kovan girişinde yiyecek arama faaliyeti 08:00-10:00, 12:00-13:00 ve 16:00-18:00 saatleri arasında 5 dakika süre ile sayılmıştır. Koloni büyüme parametreleri, 1cm2'lik şeffaf OHP levha ızgara yöntemi kullanılarak numaralandırıldı.

Sonuçlar: Acı kabak, erkek ve dişi çiçek oranı 25:1 olan tek evcikli bir mahsuldür ve genellikle açık tozlaşma koşullarında (bal arılarının daha az aktivitesinden dolayı) zayıf tohumla sonuçlanır. Bu çalışma sonucunda A. cerana indica'nın çiçek üzerinde gözlemlenen aktivitesinin 06:00-10:00 saatleri arasında zirve yaptığı ve 17:00-18:00 saatleri arasında herhangi bir aktiviteye rastlanmadığı ortaya çıkmıştır. A. cerana indica, verimi ve meyve tutumunu arttırdığı için acı kabak ekosisteminde etkili tozlayıcı olarak kabul edilmiştir. Kovan girişindeki aktivite, toplayıcıların maksimum hareketinin 08:00-10:00 saatleri arasında gözlemlendiğini ve deney süresi boyunca koloni büyümesinin kapalı bal alanına göre %67.85 ve ergin popülasyona göre %15.07 olduğunu göstermiştir. Meyve tutumu (17,4/bitki), meyve ağırlığı (255,3 g/meyve) ve 41.3t/ha verim, açık tozlaşmaya kıyasla kontrollü arı tozlamalı parselde maksimum olmuştur.

Çözüm: Mevcut çalışmamız, A. cerana indica ile yönetilen arı tozlaşma koşulunda, açık tozlaşma koşulu ve tozlayıcı dışlama gibi diğer tozlaşma modlarıyla karşılaştırıldığında, daha yüksek meyve tutumu ve meyve ağırlığı açısından farklı tozlaşma modlarının verim değerlendirmesinin gözlemlendiğini ortaya koymuştur. tozlayıcı hariç tutma durumunda, meyve tutumu kaydedilmemiştir.

INTRODUCTION

Pollination deficit is commonly noticed in many cucurbits, due to monoecious nature of flowering, which warrants the role of pollinators for fruit set and quality seed development. Honey bees play a crucial role in 80% crop pollination and at the same time contribute to the production of 1.6 million tonnes of honey (FAO, 2015). Managed bee pollination with *A. cerana indica* colonies increase the seed yield in many agricultural and horticultural crops, as for example for sunflower 79%, for mustard 55%, for safflower 64%, for coconut 40%, for gourds and for

litchi 20%. The estimated losses due to insufficient pollination in India were about Rs. 10,000 to Rs. 55,000 per hectare in cross pollinated crops (Mohapatra *et al*. 2019).

Bitter gourd (*Momordica charantia* L.) is widely cultivated in Tropical and Sub Tropical countries and popularly is called ampalaya, balsam pear, karela or bitter cucumber. In bitter gourd the male flower (staminate) blooms first followed by female flowers (pistillate) in the ratio of 19:12 (Deyto and Cervancia 2009) or 25:1 (Deshpande *et al*. 1979). Anthesis starts between 3.30 am to 7.30 am (Pal and Maurya 1972) and stigma receptivity lasts for 24h after anthesis. The bright yellow color flower attracts many pollinators. The flat structure and the opening position of the flower favours them for easy access to pollen and nectar. In bitter gourd, honey bees were the most dominant pollinator which constitutes 74.98 % followed by other pollinators such as lepidopterans (4.92%), coleopterans (3.58%), dipterans (4.35%) and hemipterans (3.19%) (Jignesh and Pastagia 2021). Fruit development starts from second to fifth day after pollination and insufficient pollination leads to drying of fruit on fifth day or results in curling of fruit which affects the marketable quality of the fruit. Hence, an experiment was conducted, to study the foraging activity, to measure the pollen grains on bee bodies and the colony growth of managed bees and to evaluate the pollination potential of Asiatic hive bees in bitter gourd.

MATERIALS AND METHOD

A field experiment was conducted at Telugupalayam area of Coimbatore District in India during 2021, to study the foraging behaviour of *A. cerana indica;* for this, ten plants were randomly tagged and 3 flowers per plant was observed at weekly intervals

Foraging activity of managed bee pollinators on bitter gourd flowers

Abundance of pollinators was recorded as no. of foragers/ flower/ minute and foraging rate as time spent by individual bee pollinator/ flower/ minute on both male and female flowers; both were recorded using a stopwatch at weekly intervals during peak pollinator activity. Peak foraging activity was recorded at an hourly interval from 06:00-18:00 h no. of foragers visited/flower for a period of 5 minutes at fortnightly interval (Yogapriya *et al*. 2019).

Pollination efficiency index

Loose pollen grains were counted by collecting foraging bees in the field at peak hours of foraging between 08:00 h and 10:00 h using a sweep net and bees are transferred to a glass vial containing 70% alcohol, shaken vigorously to unload the pollen grains from their body. Volume made up to 5ml. An aliquot of 0.01ml was taken and observed under a microscope by using a haemocytometer. Repeated for 5 replications and the total number of pollen grains in 5ml of solution were calculated (Kumar *et al*. 2012).

Pollination Efficiency Index = No. of loose pollen grains on the body × foraging rate × abundance of pollinators on bitter gourd flowers.

Evaluation of pollination potential of managed bee pollinators

The yield of bitter gourd was assessed with different modes of pollination. The experiment was conducted in Randomized Block Design (RBD) with three treatments and seven replication, 5 plants per replication (10 female flower per plant totally 350 flowers) were tagged after initiation of flowering to carry out yield assessment. Treatments included a) pollinator exclusion (T1), where female flowers were covered with sleeve cages before it begins to bloom (Fig. 1); one week after flowering the cages were removed; b) bee pollination (T2), where three frame strength Indian bee colonies were shifted to bitter gourd field in Telugupalayam, at the time of initiation of flowering in 10% plants; c) while open pollinated condition (T3) which was considered as control.

Yield parameters

Number of fruits per plant, fruit weight and yield

The number of fruits in tagged plants were counted and the fruit weight recorded. Randomly harvested ten fruits from tagged plants were weighed using a weighing balance and fruit weight was recorded. Fruits in each tagged plant were harvested, weighed and the yield was calculated and converted into yield per hectare (Manchare *et al*. 2019).

Foraging activity of *A. cerana indica* at hive entrance

The number of returning foragers with nectar (corbicula without pollen), pollen (corbicula with pollen) and number of outgoing foragers at the hive entrance was counted for the day during 08:00-

10:00 h, 12:00-13:00 h and 16:00-18:00 h for a period of 5 minutes.

Colony growth of *A. cerana indica* placed in bitter gourd field

Colony growth parameters as for example sealed brood area, sealed honey area, pollen storage area and adult bee population were enumerated using transparent OHP sheet grid of 1cm^2 and observations recorded at 15 days interval, % increase in colony growth parameters was compared (Fig. 2).

Statistical Analysis

Data were analyzed using the ANOVA (Analysis of Variance) and least significant difference (LSD) was performed at P=0.05 levels of significance. All other calculations are performed using the MS Excel.

Figure 1: Sleeve cage

(a) (b)

Figure 2: Colony growth parameter by OHP sheet gird method

(a) (b)

Figure 3: Foraging activity of Indian bee on bitter gourd flowers

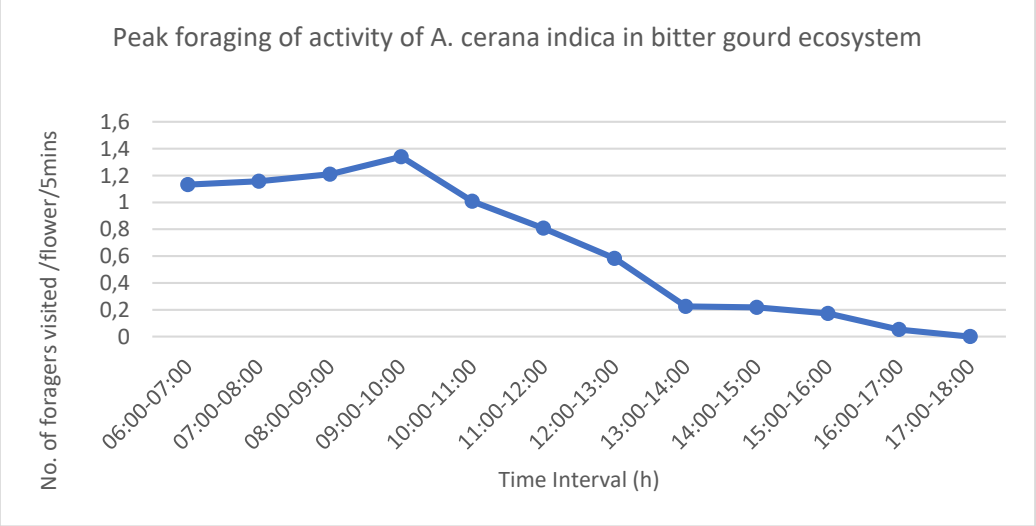


Figure 4: Peak foraging of Indian bees in bitter gourd

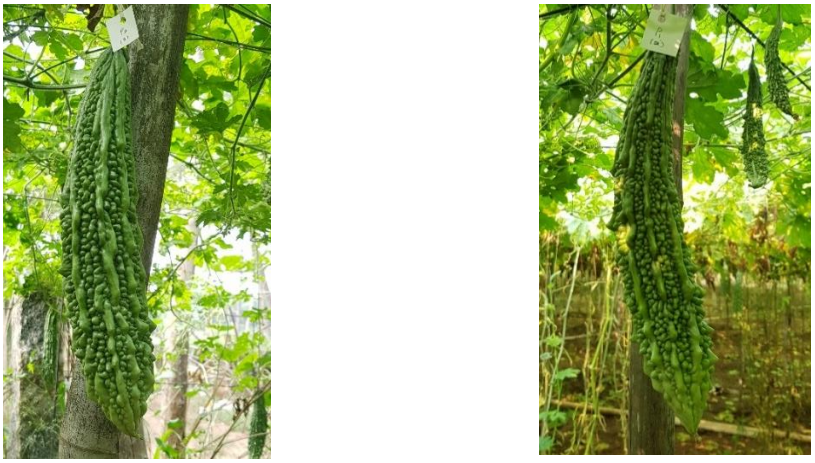
(a) Incoming nectar forager and outgoing bees

(b) Incoming forager with pollen load in corbicula

Figure 5: Foraging activity at the hive entrance

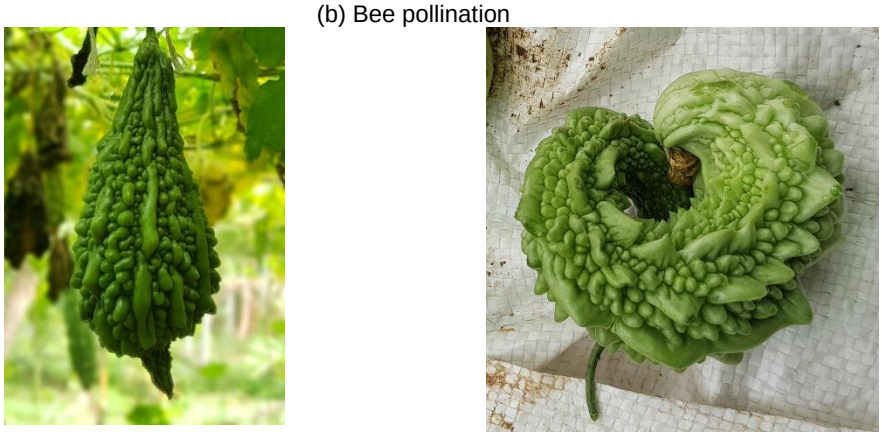
(a_1) (a_2)

(a) Pollinator exclusion



(b_1) (b_2)

(b) Bee pollination



(c_1) (c_2)

(c) Open pollinated condition

Figure 6: Fruit set in different mode of pollination

RESULTS

The result of the foraging activity of *A. cerana indica* showed that the abundance of forager bees was higher in male flower than female flowers (Table 1). The maximum number of bees in male flower were 1.15 bees/ flower/ minute and minimum were 0.56 bees/ flower/ minute, whereas female flower abundance of bees was maximum 0.90 bees/ flower/ minute and 0.26/ bees/ minute (Fig. 3).

Maximum time spent by a forager per flower in male flower was 8.83 seconds and minimum were 3.99 seconds, while in female flower its maximum and minimum time spent was 5.65 and 2.60 seconds respectively (Table 2).

The peak foraging activity of *A. cerana indica* (No. of foragers visited / flower / 5min) was maximum (Fig. 4) during 06:00-10:00 h (1.21-2.29). The foraging activity gradually declined after 10:00-11:00 h (1.01) and no activity was observed between 17:00-18:00 h. The declining of the activity between12:00 – 17:00 h was mainly due to the nature of bitter gourd flowers, which starts drying at about 12:30 h (Deyto and Cervancia 2009).

Pollination efficiency index

Pollination efficiency of *A. cerana indica* was calculated by counting the number of loose pollen grains adhering on the body (130200 pollen grains), multiplying by the abundance (0.88 no. of foragers/minute) and by foraging rate of *A. cerana indica* (6.52). Pollination efficiency index of *A. cerana indica* was found to be 747035.5 (Table 3).

Foraging activity of *A. cerana indica* at hive entrance

The foraging activity of *A. cerana indica* was recorded at the hive entrance in a day at a different time interval. The result revealed that the mean of incoming nectar foragers was higher (47.36) than incoming pollen foragers (16.5). Forager movement was maximum at 08:00 to 10:00 h (53.8) followed by 12:00 to 13:00 h (32.1) and minimum activity was at 16:00 to 18:00 h (33.53) (Table 4) (Fig. 5).

Colony growth parameter of *A. cerana indica*

Colony growth parameter was recorded periodically in *A. cerana indica* hive kept in bitter gourd field. The result shows that increase in sealed honey area, pollen storage area, sealed brood and colony population. The colonies recorded 67.85% increase of sealed honey area, 45.9% increase in pollen area and 34.41% increase of sealed brood. Adult population also increased from 2244 to 3924 bees per hive which accounts for and 15.07% increase (Table 5).

Effect of *A. cerana indica* on pollination and yield of bitter gourd

The maximum fruit set was found to be in bee pollination condition and it was 17.4 fruits/plant while 16.2 fruits/plant on average was recorded in open pollination condition (T3). The fruit weight was higher (255.3g/fruit) in bee pollination (T2) than open pollination condition (248.6g/fruit). The yield in bee pollination condition was 41.13t/ha followed by open pollination condition (37.25t/ha). In pollinator exclusion (T1), no fruit set was observed under sleeve caged condition (Fig. 6) (Table 6).

Table 1. Abundance of Indian bees on bitter gourd

	No. of foragers/flower/ min ± S.D								
	15th day	30th day	45th day	60th day	75th day	90th day	105th day	120th day	Mean
Male flower	0.56±0.04	0.67±0.071	0.9±0.03	1.05±0.15	1.15±0.03	0.97±0.014	0.96±0.004	0.81±0.091	0.88
Female flower	0.38±0.15	0.26±0.07	0.51±0.06	0.55±0.17	0.90±0.014	0.88±0.15	0.62±0.063	0.60±0.05	0.57
Mean	0.47	0.47	0.68	0.80	1.03	0.92	0.79	0.65	0.73

Note: *Mean of 10 plant observations; S.D: Standard Deviation

Table 2. Foraging activity of Indian bee in bitter gourd

Floral handling time (forager/flower/ min) in seconds ± S.D									
	15th day	30th day	45th day	60th day	75th day	90th day	105th day	120th day	Mean
Male flower	3.99±1.06	5.36±0.8	6.70±0.06	6.23±0.103	8.83±2.15	7.65±0.14	7.61±0.9	5.82±0.6	6.52
Female flower	2.49±1.36	3.61±0.74	2.74±1.19	2.60±0.702	5.65±0.4	4.70±0.13	3.89±0.30	3.20±0.84	3.61
Mean	3.24	4.48	4.72	4.42	7.24	6.18	5.75	4.51	5.07

Note: *Mean of 10 plant observations; S.D: Standard Deviation

Table 3. Pollination efficiency index of *A. cerana indica* on bitter gourd

Bee species	Abundance (No. of foragers/ min)	Foraging rate (Foraging activity in flower /seconds)	Number of loose pollen grains on the body*	Pollination index (Abundance × Foraging rate × Loose pollen grains)
A. cerana indica	0.88	6.52	130200	747035.5

Note: *Mean of five observations under stereo zoom microscope.

Table 4. Foraging activity of *A. cerana indica* at hive entrance during peak flowering period.

Foraging Time	08:00-10:00h	12:00-13:00h	16:00-18:00h	Mean
Incoming nectar forager	60.1 (7.74) [a]	32.4 (5.69) [c]	49.6 (7.04) [b]	47.36
Incoming Pollen forager	33.2 (5.758) [a]	14 (3.72) [b]	23 (1.492) [c]	16.5
Outgoing bees	68.1 (8.24) [a]	49.9 (7.04) [b]	28 (5.27) [c]	48.5
Mean	53.8	32.1	33.53	
C.D. (P=0.05)	-	-	-	0.346

Note: *Mean of five observations, figures in parentheses are √(x+0.5) (square root) transformed values. In rows means followed by different alphabets are significantly different at 5% level LSD

Table 5. Colony growth parameter of *A. cerana indica* in bitter gourd

Days (15 days interval)	Sealed honey area (cm^2)	% Increase in sealed honey area	Pollen storage area (cm^2)	% Increase in pollen storage area	Sealed brood area (cm^2)	% Increase in sealed brood area	Adult bee population	% Increase of bee population
15	37	-	46	-	165	-	2244	-
30	56	51.35	71	39.13	215	30.3	3410	51.96
45	94	67.85	89	45.9	289	34.41	3924	15.07

Note: *Mean of two observations.

Table 6. Effect of different mode of pollination on bitter gourd yield

Modes of pollination	No. of female flowers Observed	No. of picking /plant	No. of fruits /plants	Fruit weight (g)	% Increase in fruit weight	Yield of 10 plants (kg)	Yield (t/ha)	% Increase in yield (t/ha)
Pollinator Exclusion**	350	0	0	0	-	0	0	-
		(0.71)^{c}	(0.71)^{c}	(0.71)^{c}	-	(0.71)^{c}	-	-
Bee pollination (*A. cerana indica)*	350	13.57	17.4	255.3	2.6	44.42	41.13	10.28
		(3.89)^{a}	(4.34)^{a}	(16.03)^{a}	-	(66.58)^{a}	-	-
Open pollinated condition	350	10.86	16.2	248.6	-	40.27	37.25	-
		(3.52)^{b}	(4.23)^{b}	(15.82)^{b}	-	(63.91)^{b}	-	-
S.E (d)		0.011						
C.D. (P=0.05)		0.024						

Note: *Mean of five observations, **No fruit set was observed in pollinator e-xclusion. figures in parentheses are √(x+0.5) (square root) transformed values. In columns, means followed by alphabet are significantly different at 5% level LSD.

DISCUSSION

Bees are the most reliable and utilized pollinators in bitter gourd. Managed pollination with honey bees is the effective method in which the bee hives shifted to the field at the time of flowering in 10 % plants. The above-mentioned results of abundance of *A. cerana indica* was 0.73 bees/ flower/ minute (overall mean of male and female flower) and foraging activity was 5.07 seconds are in accordance with result of *Yogapriya et al*. (2019) who reported that the abundance of *A. cerana indica* in bitter gourd flowers was 0.90 individual/ 5mins/ m^2 and the average time spent by the individual bee in each flower was 3.91 second.

Peak foraging activity of *A. cerana indica* was maximum at 06:00-10:00 h (1.21-2.29) and this is in line with the result of Yogapriya *et al*. (2019) who also observed the maximum foraging activity at 06:00-10:00 h (2.88/ m^2/ 5min) and minimum activity at 14:00-16:00 h (0.345 seconds/ m^2/ 5min). Nidangundi *et al*. (2005) reported that the foraging activity of *A. cerana indica* started from 08:00 h and peak activity was recorded during 10:00 h (12.51 bees/ m^2/ 5min). The maximum activity of *A. cerana indica* was also observed at 10:00 h with 3.69 bees/ m^2/ 5min (Jignesh and Pastagia 2021). Kumar *et al*. 2012, recorded the foraging activity of *Halictus* sp. at 08:00 – 10:00 h (3.47 bees/m^2 /5 mins) followed by *Megachile* sp. at 08:00 – 10:00 h (2.07 bees/m^2/5 mins) and minimum activity of *A. dorsata* at 08:00 – 10:00 h (1.67 bees/m^2/5 mins).

Our results showed that pollination efficiency index *A. cerana indica* was 747035.5 are in line with Singh and Mall (2020) who observed that the activity of *A. cerana indica* in cucumber had a pollination efficiency index value of 320718.5. Kumar *et al*. (2019), also reported that in bitter gourd the maximum number of pollen grains in *A. dorsata* was 170000 (pollination index was 132600), followed by *Megachile* sp. 80000 pollen grains (84000 pollination index) *Halictus* sp. was 60000 (86400 pollination index).

Sowmiya *et al.* (2018) reported that sealed honey area was increased from 58.4 to 81.9 cm^2, pollen storage area from 40.2 to 65.9 cm^2, sealed brood area from 63.9 to 89.9 cm^2 and adult population from 2171 to 3305 bees per hive when they were placed in moringa (*Moringa oleifera)* orchard during the experimental period which supports our finding that significant increase in sealed honey area, sealed brood, pollen area and colony population were recorded in Indian bee hive kept in bitter gourd field during the experiment.

The results of present findings are in accordance with the result of Ngawang *et al*. (2017) who reported maximum fruit set of 87.14% under bee pollination condition and 65.21% under open pollination condition respectively. Deyto and Cervancia (2009) also recorded higher fruit set 78% in bitter gourd under natural pollination. The effect of bee pollination in bitter gourd with higher fruit weight of 129.20g followed by open pollination condition (72.09g) and (62.44g) pollinator exclusion. The yield in bee pollination condition was 118.87 quintals followed by open pollination 68.63 and caged plots 45.23 without bees. Rajasekhar (2001) reported higher fruits of (22.37) per plot under managed pollination in watermelon, with two colony and 20.75 fruits one colony per plot.

Conclusion

Pollination deficit under open pollination condition is results in lower yield of bitter gourd. *A. cerana indica* is the effective managed pollinator of bitter gourd and its cross-pollination activity significantly enhances the yield parameters. Colony growth parameter of *A. cerana indica* was also increased during the period of experiment. Hence, managed bee pollination with *A. cerana indica* considered as can be best approach for the farmers as well as bee keepers to enhance the bitter gourd yield and honey yield respectively.

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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

EVALUATION OF BOTANICAL EXTRACTS FOR THE MANAGEMENT OF GREATER WAX MOTH, *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) UNDER STORED CONDITIONS

Büyük Mum Güvesi, *Galleria Mellonella* Linnaeus'un (Lepidoptera: Pyralidae) Depolanmiş Durumda Yönetimi İçin Botanik Özütlerin Değerlendirilmesi

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ABSTRACT

In an experiment conducted to evaluate the efficacy of four botanical extracts (Solvent extraction) *viz*., Sweet flag (*Acorus calamus*), Turmeric (*Curcuma longa*), Sweet basil (*Ocimum basilicum*), Medicinal Coleus (*Coleus forskohlii*) in comparison with three Essential oils (Steam distillation) *viz*., Eucalyptus (*Eucalyptus globulus)*, Peppermint (*Mentha piperita*) and Lemongrass (*Cymbopogan citratus*) against the greater wax moth, *Galleria mellonella*, it was observed that Sweet basil, *O. basilicum* at 5% was found to be effective against *G. mellonella* with a maximum of 89.29% larval mortality followed by Sweet flag (*A. calamus*) with 65.71% mortality. Medicinal coleus, (*C. forskohlii*) with 56.66% mortality and Turmeric (*C. longa*) with 45.47% mortality in the descending order of efficacy. Among essential oils tried, Peppermint oil (*M. piperita*) has resulted in 80.24% larval mortality, followed by Eucalyptus oil (*E. globulus*) with 69.05% mortality and Lemongrass oil, *C. citratus* (50.48%). The botanical treatment with Sweet basil, *O. basilicum* (21.81%) resulted in minimum comb weight loss by *G. mellonella* followed by Sweet flag, *A. calamus* (29.04%). Among the essential oil, Peppermint oil, *M. piperita* (24.56%) afforded less comb weight loss, indicating the effectiveness of treatment in controlling the larval damage.

Key words: *Galleria mellonella* L., Botanicals, Essential oils, Larval mortality

ÖZ

Dört botanik ekstraktın (Çözücü ekstraksiyonu) etkinliğini değerlendirmek için yapılan bir deneyde, Tatlı bayrak (*Acorus hint kamışı*), Zerdeçal (*Curcuma longa*), Tatlı fesleğen (*Ocimum basilicum*), tıbbi Coleus (*Coleus forskohlii*) üç Essential ile karşılaştırıldığında yağları (Steam distilasyon) yani, Okaliptüs (*Eucalyptus globulus*), Nane (*Mentha piperita*) ve Limon otu (*Cymbopogan citratus*) büyük mum güvesi *Galleria mellonella*'ya mücadelesi için karşılaştırılmıştır. Tatlı fesleğen, *O. basilicum*'un %5 oranında *G. mellonella*'ya karşı maksimum %89.29 larva ölüm oranı ile etkili, bunu %65.71 ölüm oranı ile Tatlı bayrak (*A. hint kamışı*), azalan etkinlik sırasına göre %56.66 ölüm oranı ile tıbbi coleus (*C. forskohlii*) ve %45.47 ölüm oranı ile Zerdeçal (*C. longa*) takip etmektedir. Denenen uçucu yağlar

arasında Nane yağı (*M. piperita*) %80.24 larva ölümü ile sonuçlanmıştır. Bunu %69.05 ölüm oranı ile Okaliptüs yağı (*E. globulus*) ve Limon otu yağı, *C. citratus* (%50.48) izlemiştir. Tatlı fesleğen, *O. basilicum* (%21.81) ile yapılan botanik tedavi, *G. mellonella* tarafından minimum petek ağırlığı kaybı ve ardından Sweet flag, *A. kalamus* (%29.04) ile sonuçlandı. Uçucu yağlar arasında, Nane yağı, *M. piperita* (%24.56), daha az petek ağırlığı kaybı sağladı, ve bu da tedavinin larva hasarını kontrol etmedeki etkinliğini göstermektedir.

Anahtar kelimeler: *Galleria mellonella* L., Bitkiler, Uçucu yağlar, Larva ölümleri

GENİŞLETİLMİŞ ÖZET

Çalışmanın amacı: Balmumu güvesi, *Galleria mellonella* Linnaeus, bal arısı kolonilerine yönelik, kolonilerin tamamen yok olmasına ve bal verimi kaybına yol açan ciddi tehditlerden biridir. Balın kirlenmesine neden olan balmumu güvelerini kontrol etmek için sentetik pestisitlerin kullanılması, bal arısı kolonileri ve hedef olmayan organizmalar üzerinde zararlı etkileri olmaktadır. Bu sorunları üstesinden gelmek için çevre dostu botanik böcek öldürücüler kullanılabilir. Sonuç olarak, büyük mum güvesini ekonomik olduğu kadar verimli bir şekilde yönetebiliriz.

Malzemeler ve yöntemler: Botanik özler ve uçucu yağlar kullanılarak depolanan koşullar altında daha büyük mum güvesini kontrol etmek için bir deney yapıldı. Bu amaçla, kitle çoğaltma için etkilenen arı kolonilerinden daha büyük balmumu güvesi larvalarını topladık. Kafes koşullarında larvalar mumlu taraklarla beslendi. Erginlerin ortaya çıkmasından sonra çiftleşme kafesine bırakıldılar. Yumurtalar bu kafesten toplandı ve yumurtadan çıkmalarına izin verildi. Bu işleme biyoanaliz tamamlanana kadar devam edildi. Tıbbi ve aromatik bitkilerin yapraklarından botanik ekstraktların ve uçucu yağların hazırlanmasında Mikrodalga destekli distilasyon ünitesi ve buhar distilasyon ünitesi kullanılmıştır. Örnekler tarladan toplandı ve birkaç gün kurutuldu. Kurutulduktan sonra numuneler, çözücü olarak hekzan varlığında damıtma işlemi için ince toz haline getirildi. Seçilen bitkisel özütlerin etkinliğini belirlemek için balmumu peteklerine püskürtülen ekstraktlar ve larvaların beslenmesine izin verildi. Daha sonra ölüm yüzdesi, püskürtmeden 48 saat sonra hesaplandı. Aynı zamanda, *G. mellonella'*nın hasar potansiyelini bulmak için peteğin son ağırlığı da kaydedildi.

Sonuçlar: Denenen botanik ekstraktlardan Tatlı fesleğen (*O. basilicum*), *G. mellonella*'*nın* yönetiminde en yüksek ölüm oranı (%89.29) ve daha düşük hasar potansiyeli (%21.81) açısından etkili bulunmuş, bunu Sweet flag (A), Hint kamışı %65.71 ölüm oranı ve %29.04 hasar ile izlemiştir. Uçucu yağlar söz konusu olduğunda, Nane (*M. piperita*) %80.24 larva ölümü ve %24.56 ile minimum zarar potansiyeli bildirmiştir. Potansiyel böcek öldürücü özelliklere sahip olmaları nedeniyle, bu iki botanik *G. mellonella'nın* çevre dostu yönetimi için önerilebilir.

Çözüm: Mevcut çalışmamız, depolama koşullarında *G. mellonella'yı* kontrol etmek için botanik insektisitlerin en iyi alternatif olduğunu ortaya koymuştur. Hedef dışı organizmalar ve insanlar için tehlikeli bir etki oluşturmazlar. Botanik böcek öldürücülerin konsantrasyonunu artırarak, en yüksek ölüm yüzdesine sahip olabiliriz.

INTRODUCTION

Honey bees (*Apis* sp.) are the effective pollinators and potential resource-insects known for their abundant offerings of honey, wax, propolis, royal jelly, *etc*. and hence bee keeping is one of the economically viable ventures. Among many factors challenging bee keeping, two species of wax moth *viz*, Greater wax moth, *Galleria mellonella* L. and Lesser wax moth, *Achroia grisella* L. are considered to be harmful to bee combs both under field and storage conditions.

The larvae of wax moths are potential threat to bee keeping due to their damage by feeding on wax, pollen and larvae of honeybees (Milam 1970). The use of chemical pesticides such as Para Dichloro Benzene, Sulphur and Calcium Cyanide are harmful to honeybee colonies (Grout 1946; Whitcomb 1967). Due to the toxicological hazards of synthetic pesticides to honey bees and their hive products, contamination and persistence could be the global challenges (Pirali and Silva 2010). During severe infestation, the combs get destroyed which leads to the absconding of bee colonies. Infestation of wax moth was recorded about 90% in the combs of *Apis dorsata* (Mahindre 1983) and in case of *A. mellifera* colonies it was about 16-19% (Brar *et al*. 1985). To protect human health and environmental quality,

natural plant based products especially essentials oils are subjected to control the Insect pests (Currie and Gatien 2006). Plant based insecticides are having potential impact on the control of *Galleria mellonella* (Bolchi 1979; Eischen and Dietz 1987). The botanical pesticides are encouraged over chemical pesticides because of having less toxicity to non-target organisms and the capacity to degrade quickly (Isman 2006).

MATERIALS AND METHODS

Experimental location

The experiment was conducted at the Apiary of Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India during April 2021.

Mass culturing of Greater wax moth

The greater wax moth larvae were mass reared on aged honey combs in insect cages, under laboratory conditions, at an ambient temperature of 27±1°C. The plastic insect rearing boxes were used for mass culturing of *G. mellonella*. Based on the length and size of the larvae, different instars (Fig. 2d) were identified. The larval length and diameter ranges from 1-30mm and 0.12-7.0mm respectively (Paddock 1918). The later instar larvae (Fig. 2b&c) begin to spin the cocoon (Fig. 3a) and pupate inside that. Larval development lasts 6-7 weeks. The pupation was on the edges of the plastic lid. The pupal development of greater wax moth ranges from 6-55 days and it varies with season and temperature (Williams, 1997). Mostly, growth and size increase occur during the last 2 instars. There are 7 larval moults throughout its development (Ellis *et al*. 2013). After the emergence of adult (Fig. 4), moths were transferred into the separate plastic container for the purpose of mating process. The paper bits were added into that mating cage to provide the surface for egg laying (Fig. 1b). Eggs are laid in clusters (Fig. 1a). The newly hatched larvae (Fig. 2a) were allowed to grow continuously and the larvae thus cultured were utilized for assessing the efficacy of botanicals *viz*., *A. calamus* (Sweet flag), *C. longa* (Turmeric), *O. basilicum* (Sweet basil), *C. forskohlii* (Medicinal coleus), *E. globulus* (Eucalyptus), *M. piperita* (Peppermint) and *C. citratus* (Lemongrass) for the management of *G. mellonella*.

Preparation of botanical extracts and essential oils

The plant samples (Table 1) were obtained from the field at germplasm collections maintained by the department of medicinal plants and aromatic crops and botanical garden of TNAU.

Table 1. List of medicinal and aromatic plants used against the Greater wax moth, *G. mellonella*

Sl. No.	Common name	Botanical name	Family	Plant parts used
1.	Sweet flag	*Acorus calamus*	Acoraceae	Rhizome
2.	Turmeric	*Curcuma longa*	Zingiberaceae	Rhizome
3.	Sweet basil	*Ocimum basilicum*	Lamiaceae	Leaves
4.	Medicinal coleus	*Coleus forskohlii*	Lamiaceae	Leaves and tubers
5.	Eucalyptus	*Eucalyptus globulus*	Myrtaceae	Leaves
6.	Peppermint	*Mentha piperita*	Lamiaceae	Leaves
7.	Lemongrass	*Cymbopogan citratus*	Poaceae	Whole plant

The plant samples were collected, shade dried for 4-5 days, ground into powder and plant extracts were obtained by using microwave assisted extraction unit. A sample of 2gm each of the plant powder was weighed and put into the extraction cells along with 20ml Hexane as a solvent. The extraction cells were then fixed into the unit and the process was continued for one hour. Later, the plant extracts were collected from the unit and filtered with Whatman filter paper. Essential oils were extracted by steam distillation, which is the most common way to extract aromatic compounds from a plant. The combination of heated steam and gentle pressure allows the essential oil to be released from the microscopic

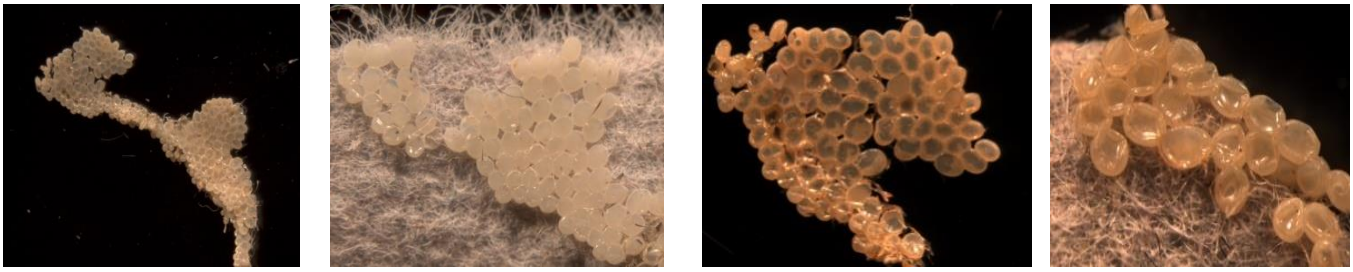
protective sacs of plant samples. As the vapour mixture flows through a condenser and cools, it yields a layer of oil and a layer of water. The essential oil rises to the top and was separated from the water.

Bioassay

Different concentrations of plant extracts (*A. calamus, C. longa, O. basilicum* and *C. forskohlii*) and essential oils (*E. globulus, M. piperita* and *C. citratus*)were prepared for fixing effective dose to evaluate the bio-efficacy of selected plant extracts against different instars of wax moth larvae. Accordingly, different concentration levels *viz*., 2%, 3% and 5% were prepared by suitable dilution with distilled water for plant extracts and a preliminary bioassay was conducted on a piece of old comb consisting of 100 larvae without segregating instars. Based on the observation of larval mortality, the 5% concentration was fixed to be tested against different instars. The aged or dark honey combs were cut into 5 gram weighed rectangular (35sq.cm) pieces and placed into the plastic containers. Each instar of wax moth larvae at 20 numbers. were released onto uniform size aged comb pieces and the treatments were imposed by giving a spray (1ml) by using an atomiser. After spraying, the combs were allowed for drying at room temperature. Each treatment was replicated thrice. Observations on larval mortality were taken 48 hours after spray and the larval mortality (%) was calculated (Taye and Mekonen, 2019).

Assessment of damage potential of Greater wax moth

The effectiveness of treatments was assessed by allowing 20 numbers of larvae on 5gm of aged treated combs without any hive products for 2 days and the final weight was assessed on 3rd day. The weight loss (%) assessment was done by calculating the difference between initial and final weight of the comb allowed for feeding by *G. mellonella*.



(a) Egg clusters (b) Eggs laid on filter paper bits (c) Eggs, Ready to hatch (d) Hatched eggs

Figure 1. Eggs of Greater wax moth, *G. mellonella*

(a) Neonate larva (b) Matured larva (Dorsal) (c) Matured larva (Ventral) (d) Different instars of larvae

Figure 2. Larvae of Greater wax moth, *G. mellonella*

(a) Pupa covered by Silken cocoon (b) Obtect type of pupa (c) Cluster of pupae

Figure 3. Pupa of Greater wax moth, *G. mellonella*

(a) Adult male (b) Adult female

Figure 4. Adult of Greater wax moth, *G. mellonella*

Figure 5. Combs affected by *G. mellonella*

Statistical Analysis

The experiments were laid in Completely Randomized Block Design (CRBD) with three replications and the data were statistically analyzed by Analysis of Variance (ANOVA) techniques (Khan and Khanum, 1994) and means were ordered by Duncan's Multiple Range Test (DMRT).

RESULTS

Efficacy of botanical extracts and essential oils against *G. mellonella*

According to the results (Table 2), an experiment on bioefficacy of medicinal and aromatic plant extracts against the greater wax moth, *G. mellonella* exhibited the effectiveness (Fig. 6) *O. basilicum* with 89.29% larval motality followed by, *A. calamus* (65.71%), *C. forskohlii* (56.66%) and *C. longa* (45.47%). Maximum reduction over control (%) was recorded in *O. basilicum* (88.47%) followed by *M. piperita* (78.70%), while least reduction (%) was recorded with *C. longa* (45.47%). Highest larval mortality (%) with *O. basilicum* might have done because of its insecticidal and anti-feedant properties against insects (Hussein and Abdelwahab, 2015)

Among the essential oils, *M. piperita* performed well against wax moth larvae with 80.24% kill of larvae, which is in line with the earlier findings (Hussein and Abdelwahab 2015). *M. piperita* was ranged from 29 to 79% which supports our research findings. Peppermint species have been reported to have potential insecticidal properties and act as an excellent repellent for insects (Kumar *et al*. 2011). Followed by *M. piperita*, *E. globulus* and *C. citratus* with 69.05% and 50.48% larval mortality respectively.

Per cent damage by *G. mellonella* with botanical extracts and essential oils

In another experiment on the comb weight loss due to larval damage in the botanical treatments (Table 3), the supremacy of *O. basilicum* extract which recorded a least weight loss of 21.81%. The order of efficacy (Fig. 7) of botanical treatments in terms of comb damage was, *O. basilicum* (21.81%), *M. piperita* (24.56%), *E. globulus* (29.95%), *A. calamus* (29.04%), *C. forskohlii* (31.34%), *C. citratus* (32.51%) and *C. longa* (33.47%) which is in conformity with the findings of Hussein and Abdelwahab (2015).

Table 2. Efficacy of medicinal and aromatic plant extracts and essential oils on different larval instars of Greater wax moth, *G. mellonella*

Treatment	% larval mortality of different larval instars*±S.D							Overall Mean	Reduction over control (%)
	I	II	III	IV	V	VI	VII		
Plant extracts									
Acorus calamus	86.67±2.89	75.00±5.0	70.00±5.12	66.67±7.64	58.33±5.77	53.33±5.73	50.00±8.66	65.71	63.07
	(68.67)^{bc}	(60.07)^{bc}	(56.84)^{cd}	(54.83)^{cd}	(49.82)^{cd}	(46.92)^{cd}	(45.01)^{c}		
Curcuma longa	68.33±7.64	58.33±5.77	48.33±5.73	48.33±5.77	40.00±5.00	31.67±7.64	23.33±2.89	45.47	41.28
	(55.85)^{d}	(49.82)^{d}	(44.04)^{f}	(44.04)^{e}	(39.21)^{f}	(34.15)^{e}	(28.86)^{f}		
Ocimum basilicum	100.00±0.0	98.33±2.89	98.33±2.81	88.33±2.89	86.67±2.88	81.67±2.84	71.67±2.89	89.29	88.47
	(89.71) ^{a}	(85.50) ^{a}	(85.50) ^{a}	(70.12) ^{a}	(68.67) ^{a}	(64.69) ^{a}	(57.86)^{a}		
Coleus forskohlii	78.33±2.88	68.33±2.84	60.00±5.00	58.33±7.64	53.33±5.77	38.33±5.70	40.00±5.00	56.66	53.33
	(62.29)^{cd}	(55.77)^{cd}	(50.79)^{de}	(49.83)^{de}	(46.92)^{de}	(38.22)^{d}	(39.21)^{de}		
Essential oils									
Eucalyptus globulus	91.67±2.89	85.00±5.0	76.66±2.89	73.33±7.64	63.33±2.89	46.67±7.60	46.67±7.64	69.05	66.67
	(73.40)^{b}	(67.41)^{b}	(61.15)^{c}	(59.06)^{bc}	(52.74)^{c}	(43.08)^{bc}	(43.08)^{cd}		
Mentha piperita	98.33±2.89	96.67±2.89	91.66±2.80	78.33±5.77	70.00±5.00	65.00±5.00	61.67±2.89	80.24	78.70
	(85.50)^{a}	(81.29)^{a}	(73.40)^{b}	(62.41)^{b}	(56.84)^{b}	(53.76)^{b}	(51.76)^{b}		
Cymbopogan citratus	58.33±5.77	66.67±5.77	53.33±2.89	55.00±5.00	51.67±7.64	36.67±5.77	31.67±5.77	50.48	46.67
	(62.40)^{cd}	(54.79)^{cd}	(46.91)^{ef}	(47.88)^{e}	(45.97)^{ef}	(37.22)^{d}	(34.18)^{ef}		
Control (Water)	11.67±2.89	6.67±2.89	6.67±2.89	8.33±2.45	6.67±2.36	5.00±0.0	5.00±0.0	7.14	-
	(19.89)^{e}	(14.76)^{e}	(14.76)^{g}	(16.60)^{f}	(14.76)^{g}	(12.92)^{f}	(12.92)^{g}		
SEm±	14.90	21.30	13.69	14.91	9.96	11.22	9.52	-	-
C.D (p= 0.05)	6.62	8.00	6.41	6.68	5.46	5.80	5.34	-	-

Note: * Mean of three replications for each instar. Figures in parentheses are arc sine transformed values; S.D: Standard Deviation; SEm: Standard Error of mean; C.D: Critical Difference. Figures are not having the same alphabetical letters in a same column differ significantly at $p < 0.05$.

Table 3. Weight reduction of comb (%) by Greater wax moth, *G. mellonella*

Treatment	(%) weight reduction of comb by different larval intars of *Galleria mellonella***							Overall Mean
	I	II	III	IV	V	VI	VII	
Plant extracts								
Acorus calamus	3.30	5.87	6.60	19.73	47.77	56.43	63.60	29.04
	(1.82)^{cd}	(2.42)^{cd}	(14.89)^{d}	(26.37)^{d}	(43.71)^{d}	(48.70)^{e}	(52.89)^{e}	
Curcuma longa	6.17	7.97	9.97	22.53	55.70	61.53	70.43	33.47
	(2.48)^{b}	(2.82)^{b}	(18.38)^{b}	(28.34)^{b}	(48.27)^{b}	(51.67)^{b}	(57.06)^{b}	
Ocimum basilicum	0.83	2.90	2.37	15.87	29.57	43.43	57.70	21.81
	(0.91)^{f}	(1.70)^{f}	(8.78)^{e}	(23.47)^{e}	(32.93)^{f}	(41.22)^{g}	(49.43)^{f}	
Coleus forskohlii	3.80	6.00	7.50	21.93	52.10	59.50	68.53	31.34
	(1.95)^{c}	(2.45)^{cd}	(7.50)^{cd}	(27.92)^{bc}	(46.20)^{bcd}	(50.48)^{c}	(55.88)^{c}	
Essential oils								
Eucalyptus globulus	2.77	5.77	6.47	20.57	50.33	58.30	65.47	29.95
	(1.66)^{d}	(2.40)^{d}	(14.73)^{d}	(26.96)^{cd}	(45.19)^{cd}	(49.78)^{d}	(54.01)^{d}	
Mentha piperita	1.77	3.50	3.43	17.20	39.63	47.87	58.53	24.56
	(1.30)^{e}	(1.87)^{e}	(10.65)^{e}	(24.50)^{e}	(39.01)^{e}	(43.78)^{f}	(49.91)^{f}	
Cymbopogan citratus	5.47	6.47	8.93	22.27	53.20	61.90	69.30	32.51
	(2.34)^{b}	(2.54)^{c}	(17.38)^{bc}	(28.15)^{b}	(46.84)^{bc}	(51.89)^{b}	(56.35)^{bc}	
Control (Water)	8.80	11.70	33.27	57.53	73.93	78.83	82.13	49.46
	(2.96)^{a}	(3.42)^{a}	(35.18)^{a}	(49.33)^{a}	(59.32)^{a}	(62.61)^{a}	(65.01)^{a}	
SEm±	0.01	0.01	1.87	0.47	2.52	0.14	0.25	-
C.D (p= 0.05)	0.16	0.13	2.36	1.19	2.75	0.64	0.87	-

Note: ** Mean of three replications for each instar. Figures in parentheses are arc sine transformed values; SEm: Standard error mean; C.D: Critical difference.

Figures are not having the same alphabetical letters in a same column differ significantly at $p < 0.05$.

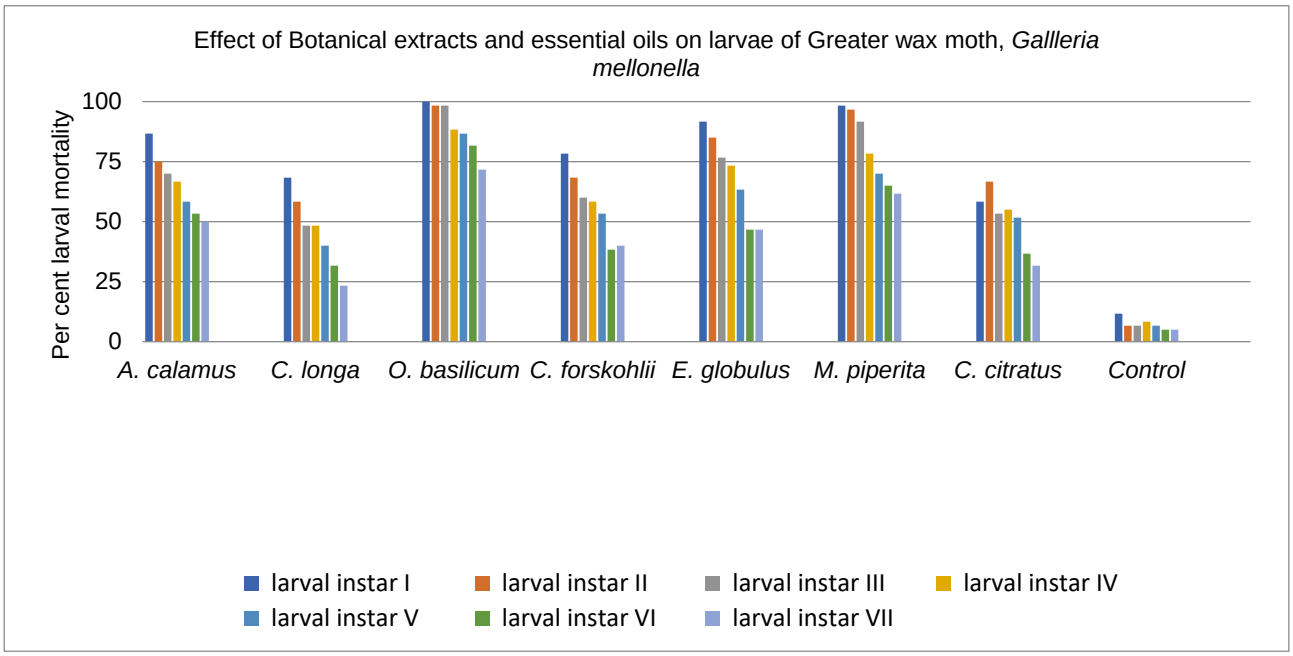


Figure 6. Per cent larval mortality of *G. mellonella* with different botanical treatments

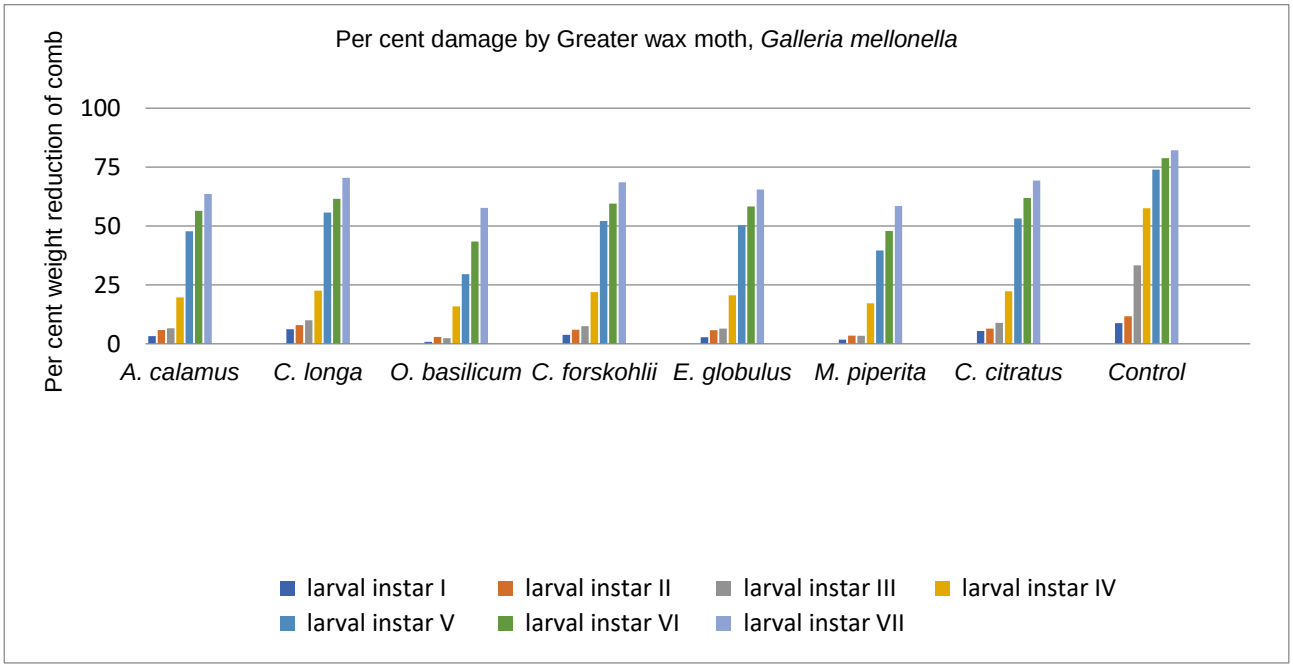


Figure 7. Per cent damage by *G. mellonella* with different botanical treatments

Per cent damage by G. mellonella with botanical extracts and essential oils

In another experiment on the comb weight loss due to larval damage in the botanical treatments (Table 3), the supremacy of *O. basilicum* extract which recorded a least weight loss of 21.81%. The order of efficacy of botanical treatments in terms of comb damage was, *O. basilicum* (21.81%), *M. piperita* (24.56%), *E. globulus* (29.95%), *A. calamus* (29.04%), *C. forskohlii* (31.34%), *C. citratus* (32.51%) and *C. longa* (33.47%) which is in conformity with the findings of Hussein and Abdelwahab (2015).

DISCUSSION

The botanical extracts *viz*., *O. basilicum* and *A. calamus* at 5% were performed better than other plant extracts of *C. longa* and *C. forskohlii* for the management of wax moth under stored conditions. These finding are in accordance with that of Taye and Mekonen (2019) who concluded that leaf extracts of *Azadirachta indica* and *Ocimum basilicum* were more effective against greater wax moth, *G. mellonella* within 48 hours and this might be attributed to their insecticidal, growth regulatory and anti- feedant properties against insects. Among essential oils, the effectiveness of Peppermint oil, *M. piperita* was found promising with 80.24% mortality of wax moth larvae compared with Eucalyptus and Lemongrass oil. The natural plant based botanicals are more economical and eco-friendly when compared to synthetic chemical pestiicdes. Therefore, leaf extract of *Ocimum basilicum* and essential oil from *Mentha piperita* can be exploited as sources for bio-pesticides to control *G. mellonella*. Considering the loss of cells due to larval attack in the treated combs, Sweet basil, *O. basilicum* showed a promising performance due to least comb damage of 21.81%. In the light of inferences obtained from this present investigation, the *O. basilicum* plant extract could be utilized for the eco-friendly management of wax moth under storage conditions.

Conclusion

Tested Botanical extracts exhibited the greatful response to the different larval instars of greater wax moth, *G. mellonella* as compared with control. According to the results, *O. basilicum* (Sweet basil) 5% was found to be effective against greater *G. mellonella* followed by *M. piperita* (Peppermint) 5% within 48hrs as they have potential insecticidal properties (Hussein and Abdelwahab, 2015). These natural plant products never cause any health hazards to non-target organisms as well as human beings and also cost effective. Hence, these botanical extracts can be used for the management of greater wax moth, *G. mellonella*.

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Conflict of interest: The authors have no conflict of interest to declare.

Ethical issues: Not applicable

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DERLEME / REVIEW

THE POSSIBLE EFFECTS OF HEAVY METALS IN HONEY AS TOXIC AND CARCINOGENIC SUBSTANCES ON HUMAN HEALTH: A SYSTEMATIC REVIEW

Toksik ve Kanserojen bir Madde Olarak Baldaki Ağır Metallerin İnsan Sağlığına Olası Etkileri: Sistematik bir Inceleme

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ABSTRACT

Heavy metals are widely known through natural resources, natural resources such as soil, dust in the atmosphere, snow and rain. Soil contaminants, especially heavy metals, can be absorbed by plants and enter the food cycle. Heavy metal contamination causes environmental concerns, such as entering the food chain and contaminating food, which can be harmful to human health. Consumption of food contaminated with heavy metals can cause several disorders including genetic toxicity, carcinogenicity, mutagenicity, teratogenicity, neurotoxicity, endocrine disorders, immune problems and impaired psychosocial function. Bees also absorb heavy metals through the consumption of contaminated water, pollen, and nectar, inhalation of particles during flight, and adhesion of particles to their hairy body as they move on plant and soil surfaces while searching for food. For this review study, keywords such as heavy metals and honey were used. The databases searched in those articles were Google Scholar, SID, Scopus, PubMed, Science Direct, and ISI. The searched articles were reviewed. Given that honey is a valuable and widely consumed food in the diet of most people in different nations, so the study of the quality of honey in the consumer market in order to maintain the health of consumers seems necessary.

Keywords: Bee, Honey, Heavy metals, Toxic, Carcinogenic agent, Carcinogen

ÖZ

Toprak kirleticileri, özellikle ağır metaller, bitkiler tarafından emilebilir ve besin döngüsüne girebilir. Arılar ayrıca yiyecek ararken bitki ve toprak yüzeylerinde hareket ederken kirli su, polen ve nektar tüketimi, uçuş sırasında partiküllerin solunması ve partiküllerin tüylü vücutlarına yapışması yoluyla

ağır metalleri emer. Bu derleme çalışması için ağır metaller ve bal gibi anahtar kelimeler kullanılmıştır. Bu makalelerde aranan veri tabanları Google scholar, SID, Scopus, PubMed, Science direct ve ISI idi. Aranan makaleler incelendi. Balın, farklı uluslardaki çoğu insanın diyetinde değerli ve yaygın olarak tüketilen bir gıda olduğu göz önüne alındığında, tüketicilerin sağlığını korumak için tüketici pazarında bal kalitesinin araştırılması gerekli görünmektedir. Bu konuda kamuoyunu bilgilendirmemiz gerekiyor, bu yüzden bu derleme balda ağır metallerin varlığını açıklıyor.

Anahtar Kelimeler: Arı, Bal, Ağır metaller, Toksik, Kanserojen madde, Kanserojenler

Abbreviations

Fe Iron, Zn Zinc, Cu Copper, As Arsenic, Pb Lead, Cd Cadmium, Ni Nickel, Cr Chromium, Al Aluminium, Mn Manganese, ICP-OES Inductively coupled plasma-optical emission spectrometry, EU European Union, FDA Food and Drug Administration, WHO World Health Organization

GENIŞLETILMIŞ ÖZET

Giriş: Bal, arılar tarafından üretilen çiçek ve bitkilerin nektarıdır. Bal, fruktoz, glikoz, maltoz, sakaroz, protein, mineraller ve su içeren değerli bir besindir. Toprak kirleticileri, özellikle ağır metaller, bitkiler tarafından emilebilir ve besin döngüsüne girebilir. Arılar ayrıca yiyecek ararken bitki ve toprak yüzeylerinde hareket ederken kirli su, polen ve nektar tüketimi, uçuş sırasında partiküllerin solunması ve partiküllerin tüylü vücutlarına yapışması yoluyla ağır metalleri emer. Genel olarak ağır metaller nörolojik bozukluklara, kanserlere, besin eksikliklerine, obeziteye, solunum ve kardiyovasküler bozukluklara, karaciğer, böbrek ve beyin hasarına, alerji ve astıma, endokrin bozukluklara, kronik viral enfeksiyonlara neden olabilir. Enzim disfonksiyonu, kansızlık, yorgunluk, baş ağrısı ve baş dönmesi, zayıflamış bağışıklık sistemi, gen hasarı, erken yaşlanma, cilt bozuklukları, hafıza ve iştah kaybı, artrit, osteoporoz ve akut durumlarda ölüme neden olur.

Amaç: Balın ağır metaller açısından kirlenmesinin araştırılması

Yöntemler: Bu derleme çalışması için ağır metaller ve bal gibi anahtar kelimeler kullanılmıştır. Bu makalelerde aranan veri tabanları Google bilim, SID, Scopus, PubMed, Science direct ve ISI idi. Aranan makaleler incelendi. Bal uzun zamandır sadece gıda olarak değil, aynı zamanda birçok hastalık ve sağlıkla ilgili sorunları tedavi etmek için de yaygın olarak kullanılmaktadır.

Tartışma ve Sonuç: Türkiye'de yapılan bir çalışmanın sonuçları da balın ağır metallerle kirlenme oranı ile sanayi merkezlerinin sayısı ile bölgedeki kirlilik oranı arasında doğrudan bir ilişki olduğunu göstermiştir (Al-Khalifa ve Al-Arify, 1999). Bu rapor, ülkede incelenen bal örneklerinde (özellikle kadmiyum ve cıva) ağır metal miktarlarının izin verilen oranın üzerinde olduğunu göstermiştir. Saveh City'de (Markazi Eyaleti, İran) balın kadmiyum ve arsenik ağır metalleri tarafından kontaminasyonunun ölçülmesine ilişkin sonuçlar, Türkiye, Arjantin, Nijerya ve Pakistan gibi ülkelerde yapılan ve Türkiye'deki bal örneklerinde çok yüksek kontaminasyon gösteren araştırmalara benzerdi (Samimi ve ark., 2001). Bu, bu bölgede sanayi alanlarının varlığına bağlanabilir.

Hırvatistan ve Kosova'da yapılan çalışmaların sonuçları, bal örneklerindeki kurşun içeriğinin diğer Avrupa ülkelerinin rapor edilen miktarından daha yüksek olduğunu gösterdi ki bu endişe vericidir. Bu bulgular, karayolları ve demiryollarından uzak alanlarda bal üretim kovanlarının bulunması ihtiyacını vurgulamaktadır (Bilandžić ve diğerleri, 2011; Fadil ve diğerleri, 2020).

Toma et al. Nijerya'da yapılan bir çalışmada, bal örneklerindeki demir, bakır, manganez ve çinko miktarının dünya sağlık örgütü (WHO) ve gıda ve tarım örgütü (FAO) tarafından belirlenen izin verilen maksimum konsantrasyondan ve ortalama konsantrasyondan daha yüksek olduğu ortaya konmuştur. Endüstriyel şehirlerdeki ağır metallerin oranı kırsal alanlara göre daha yüksektir (Toma et al., 2020).

Altekin ve ark., Piven ve ark. Cs137 ve K40 aktivitelerinin arılar tarafından çevreden bala taşındığını belirtmiştir. Ayrıca bal örneklerinde tespit edilen Cr, Zn, Fe, Ni, Mn, Pb, Cu, Cd ve Co konsantrasyonları FAO/WHO tarafından insan sağlığının korunması için belirlenen limitlerin altındaydı ve herhangi bir risk oluşturmadı (Altekin ve diğerleri, 2015; Piven ve diğerleri, 2020). Bal kontaminasyonunun diğer bir kaynağı, kirletici

maddeler, aletler, arıcılık uygulamaları, çiçek kaynakları, mutfak eşyaları, böcekler, hayvanlar ve su ile teması içerir (Mahmoudi ve diğerleri, 2014). Balın ortalama asidik pH'ı (pH = 3,9) olduğundan metal kaplar ve ağır metal bileşikleri içeren paketler yoluyla bal bulaşabilir, bu nedenle zamanla kutunun metalini aşındırabilir. Bu nedenle, lehimsiz kutular kullanılarak baldaki kurşun ve kalay gibi ağır metallerin miktarını azaltmak için saklama kutularında uygun kaplamalar kullanılabilir (Bonyadian vd., 2011). Bonyadian ve diğerleri, mumlu balın diğer numunelerden daha fazla kurşun içerdiğini bulmuşlardır, bu da balın metal kaplarda depolanmasına ve mumun kimyasal bileşimine atfedilebilir (Bonyadian ve diğerleri, 2011).

Çoğu çalışmada İran balının ağır metallerle kontaminasyonu düşük bulunmuş ve yetişkin arıların vücutlarının polen ve baldan daha fazla kontamine olduğu bildirilmiştir. Görünüşe göre arılar bal üretirken bulaşmalarını azaltıyor. Balın farklı ülkelerdeki çoğu insanın diyetinde değerli ve yaygın olarak tüketilen bir gıda olduğu göz önüne alındığında, tüketicilerin sağlığını korumak için tüketici pazarında balın kalitesinin araştırılması gerekli görünmektedir. Bu konuda kamuoyunu bilgilendirmemiz gerekmektedir. bu yüzden bu derleme yazı balda ağır metallerin varlığını açıklamaktadır.

INTRODUCTION

Honey is the nectar of flowers and plants produced by bees. Honey is a nutrient that has valuable healing properties (Bilandžić et al., 2011). It contains fructose, glucose, maltose, sucrose, protein, minerals, and water (Ioannidou et al., 2005). The composition of honey varies according to plant species, climate, geographical conditions, environmental conditions as well as the method of beekeepers in honey production (Azeredo et al., 2003). Each year, about 30 percent of people in developed countries develop foodborne illnesses. It has been proven that the main way heavy metals enter the body is through food chains (Sobhanardakani & Kianpour, 2016). Heavy metals are the most important sources of contaminants in water, soil, and food (Duruibe et al., 2007; Pirhadi et al., 2021). Heavy metals are toxic and cause various diseases such as cancer, disorders of hemoglobin biosynthesis and anemia, gastrointestinal bleeding, inflammation and renal, pulmonary, gastrointestinal and heart failure. Heavy metals are carcinogenic and endanger a person's health (Duruibe et al., 2007). Today, the role of heavy metals in environmental pollution and their adverse effects on human health have been identified (Malakootian et al., 2011; Pirhadi et al., 2021). Soil contaminants, especially heavy metals, can be absorbed by plants and enter the food cycle. Because heavy metals have a long half-life, they are likely to accumulate in the tissues of living organisms (Hegazi & El-Kay; Rezaei Raja et al., 2016).

Bees can also absorb heavy metals through the consumption of contaminated water, pollen, and nectar, inhalation of particles during flight, and the adhesion of particles to their hairy bodies as they move on plant and soil surfaces while searching for food (Hegazi & El-Kay., 2010). In general, heavy metals can cause neurological disorders, cancers, nutrient deficiencies, obesity, respiratory and cardiovascular disorders, liver, kidney, and brain damage, allergies and asthma, endocrine disorders, chronic viral infections. Enzyme dysfunction, anemia, fatigue, headache and dizziness, weakened immune system, gene damage, premature aging, skin disorders, loss of memory and appetites, arthritis, osteoporosis, and in acute cases cause death (Khaneghah et al., 2020; Negahdari et al., 2021; Singh et al., 2010).

Honey is used as an indicator for measuring environmental pollution such as heavy metal pollution (Celli & Maccagnani, 2003). Heavy metals in honey can be worrisome and dangerous, toxic and carcinogenic for consumers (Leblebici & Aksoy, 2008). Excessive and permanent discharge of pollutants into the environment increases the contamination of honey as one of the important food products with heavy metals. Therefore, in this review study, honey contamination with heavy metals has been investigated.

METHOD FOR REVIEW

For this review study, keywords such as heavy metals and honey were used. The databases searched for in those articles were Google Scholar, SID, Scopus, PubMed, Science Direct, and ISI.

RESULTS

The results of reviewing articles on heavy metals in honey are shown in Table 1.

Table 1. Prevalence of Heavy metals in Honey samples from various countries during 2011–2021 (mg/Kg).

Country	Year	Sample	Cadmium	Chrome	Copper	Manganese	Zinc	Lead	Arsenic	Nickle	Aluminium	Iron	Unit	Method	Ref.
Iran	2015	15	63.18± 43.39	58.05± 30.32			684.43± 190.43			56.15± 54.32			µg/kg	ICP-OES	(Sobhanard akani & Kianpour, 2016)
Iran	2013-2014	72					4.4±3.40	0.08±0.04	0.11± 0.04				ppm	Atomic absorption spectrometry	(Mahmoudi et al., 2018)
Iran	2010	89		7.09±9.4			9.99±26.5	0.04±0.1	0.0008± 0.0011	0.003± 0.005		0.6±0.9	ppm	Atomic absorption spectrometry	(Saghaei et al., 2012)
Iran	2013	27							0.005± 0.002				ppm	Graphite Furnace Atomic Absorption	(Piran et al., 2015)
Iran	2010	10	0.39 ± 0.08		0.13 ± 0.08	0.42 ± 0.16	2.53 ± 2.93	0.11 ± 0.05	0.16 ± 0.13		9.62 ± 6.7	5.31 ± 2.29	mg/kg	ICP-AES	(Akbari et al., 2012)
Iran	2013	25	27.62±32	899.75± 184.03	243± 559.3		1481.64 ± 1709.81	507.58± 402.14	<11.87	651.78± 173.29			µg/kg	ICP-OES	(Aghamirlou et al., 2015)
Turkey	2013	20	0.011±0.0002	0.007±0.0004	0.064± 0.0086	0.603±0.0084	3.976±0.0416	0.078±0.0036		0.041± 0.0014		0.424± 0.0026	µg/l	ICP-OES	(Altekin et al., 2015)
Turkey	2018	3	0.64±0.08	1.05±0.00	0.87± 0.01	21.58±0.07	2.59±0.00	0		0.10± 0.01	10.41 0.00	20.52± 0.09	mg/kg	ICP-OES	(Temizer et al., 2018)
Bangladesh	2015		0.01 ± 0.00	0.39 ± 0.32	0.15 ± 0.06	2.69 ± 1.66		0.16 ± 0.04					mg/kg	Atomic Absorption Spectroscopy	(Sarker et al., 2015)
Turkey	2015	180				4,636 ± 3.943					124.863 ± 313.44	67.352 ± 34.636	ppb	ICP-OES	(EKİCİ, 2018)
Iraq	2015		0.108-0.8200			0.0392±0.0481		0.100-0.730	0.0104-0.035	0.210-0.894		0.117-0.440	mg/kg	FAAS and GFAAS	(Dhahir & Hemed, 2015)
Turkey	2015	100	0.343±0.205		0.06± 0.028		6.76±3.88	1101±1.277			1.490	41.13	mg/kg, wet weight	atomic absorption spectrometry	(UT)
Ghana	1017	20	0.625 ± 1.667	2.655 ± 4.773	13.855 ± 10.213	8.215 ± 4.452	0.615 ± 1.996	79.815 ± 16.796	0.665 ± 0.108	15.785 ± 10.968			mg/Kg	atomic absorption spectrometry	(Magna et al., 2018)
Italia	2016	72	0.61±0.66	12.8±10.7	220±134	664	1072±1315	32.8±47.9	1.27±2.88		1400±2300	2080± 1060	µg/kg	ICP-MS	(Quinto et al., 2016)
Ukraine	2019	60	0.02±0.01					0.185±0.01					mg/Kg	atomic absorption spectrometry	(Piven et al., 2020)
Kosovo	2018-2019	80	0.05	0.04	0.36		1.150	0.88				1.670	mg/Kg	atomic absorption spectrometry	(Fadil et al.)
Poland	2019	50	0.02±0.01			3.39±2.89		0.05±0.10		0.45±0.54	11.64±19.88		mg/kg d.w	ICP-OES	(Tomczyk et al., 2020)
Turkey	2020	146	0.09±0.07	0.01±0.01	0.37±0.37	11.05±1.21	1.58±1.11	0.18±0.05		0.11±0.06	2.55±2.55	4.21± 1.15	mg/Kg	ICP-OES	(Kanbur et al., 2021)
Ethiopia	2018	12	0.46±0.04	3.16±0.25	0.250±0.03	0.46±0.03	2.85±0.24	ND		2.61±0.16		9.65± 0.75	µg/g	flame atomic absorption spectrometry	(Yohannes et al., 2018)
Nigeria	2020	5	0.041±0.01	0.037±0.01	0.368±0.126	9.79±0.37	2.88±0.15	0.013±0.01				1.55± 0.27	µg/kg	Atomic Absorption Spectrophotometer	(Toma et al.)

Nigeria	2015 - 2017	40	0.109±0.046	6.03±0.78	51.84±6.7		38.98±8.46	0.26±0.055					µg/g	inductively coupled plasma mass spectrometer	(Ernest et al., 2018)
Romania	2015	52	2.19	41.57	228.26				7.82			22708.25	µg/kg	ICP-MS	(Oroian et al., 2016)
Brazil	2014		<2 -8	83-94				141 -228					ng/g	atomic absorption spectrometry	(de Andrade et al., 2014)
India	2012	70	0.008±0.005	0.0561±0.008	0.008±0.004		0.3725±0.615	0.002±0.001	0.319±0.20	0.011±0.003		10109±0.74	mg/Kg	Flame Atomic absorption spectrometer	(Bhalchandra & Baviskar)
Saudi Arabia	2017		0	0	0.001	0	0	0		0.001		0.003	mg/L	y Hydride generation atomic absorption spectroscopy (HGAAS)	(Aljedani, 2017)
Tadla-Azilal	2017	10	<0.015					<0.07					mg/Kg	atomic absorption spectrophotometry	(Moujanni et al., 2017)
Poland	2020	49	0.025±0.023					0.19±0.179					mg/Kg	ICP-OES	(Winiarska-Mieczan et al.)
Croatia	2019	244	0.013	0.29	5.81	53.1	12.6	0.458	0.037	0.88	288.5	82.5	mg/Kg	ICP-MS	(Bilandžić et al., 2011)
Hungary	2014	187				3.32±3.11		0.51±0.2			2.53±4.67		mg/Kg	ICP-OES	(Sajtos et al., 2019)

DISCUSSION

Honey has long been used not only as a food but also it has been widely applied to treat many diseases and health-related problems (Ediriweera & Premarathna, 2012). Honey can be contaminated by various sources. These sources can be classified into two important categories: contaminants of environmental origin and those related to beekeeping and maintenance (Mahmoudi et al., 2014). According to rules and regulations of the institute of standards and industrial research of Iran (ISIRI), provisional tolerable daily intake (PTDI) for lead, cadmium, mercury, and arsenic is equal to 0.0036, 0.001, 0.007, and 0.0021 in milligrams per kilogram of temporary bodyweight, respectively (Mahmoudi & Emami, 2015). Studies have shown that there is a direct relationship between contamination of honey with heavy metals in honey samples and industry-related environmental pollution in the area (Demirezen & Aksoy, 2005).

The results of a study conducted in Turkey also demonstrated a direct relationship between rate of honey contamination with heavy metals and number of industrial centers and rate of contamination in the area (Al-Khalifa & Al-Arify, 1999). This report indicated that amounts of heavy metals in honey samples studied in the country (especially cadmium and mercury) were higher than permissible rate. The results regarding measuring contamination of honey by heavy metals of cadmium and arsenic in Saveh City (Markazi Province, Iran) were similar to studies done in countries, such as Turkey, Argentina, Nigeria, and Pakistan, showing very high contamination of honey samples in this region (Samimi et al., 2001). This can be attributed to the presence of industrial areas in this region. The results of studies conducted in Croatia and Kosovo showed that content of lead in honey samples was higher than the reported amount of other European countries, which is worrying. These findings highlight the need for locating honey production hives in areas far from highways and railways (Bilandžić et al., 2011; Fadil et al., 2020).

Toma et al. in a study conducted in Nigeria demonstrated that the amount of iron, copper, manganese, and zinc in honey samples was higher than the maximum allowable concentration set by the world health organization (WHO) and food and

agriculture organization (FAO), and average concentration of heavy metals in industrial cities was higher than rural areas (Toma et al., 2020). Berinde and Michnea reported a positive relationship between metal content in honey samples, respiration of the contaminated particles in the air, and contaminated surface water (Berinde & Michnea, 2013). Cimino et al., showed the effects of volcanic activity on honey (Cimino et al., 1984). In this regard, the results of a study done in Chile showed that aluminum at the concentration of 6.15 ± 4.53 mg/kg and cadmium with the concentration between 0.01 - 0.05 mg/kg were the highest and lowest amounts of heavy metals in honey, respectively (Fredes & Montenegro, 2006). High levels of aluminum were attributed to the presence of colonies in the lands with volcanic soils. Radioactivity is naturally present in the environment and contaminates leaves and flowers and also has negative effects on human health.

The results of studies performed by Altekin et al., and Piven et al. indicated that Cs137 and K40 activities were transported by bees from the environment into honey. In addition, concentrations of Cr, Zn, Fe, Ni, Mn, Pb, Cu, Cd, and Co detected in the honey samples were lower than the limits established by FAO/WHO for protection of human health, and they had no risk to public health (Altekin et al., 2015; Piven et al., 2020). Another source of honey contamination includes having contact with contaminants, tools, beekeeping practices, floral sources, utensils, insects, animals, and water (Mahmoudi et al., 2014).

Honey can be contaminated through metal containers and heavy metal compounds-containing packages because honey has an average acidic pH (pH = 3.9), so over time, it can corrode metal of the can. Therefore, suitable coatings can be used in storage cans to reduce the amount of heavy metals, such as lead and tin in honey using solderless cans (Bonyadian et al., 2011). Bonyadian et al., found that the waxed honey contained more lead than other samples, which could be attributed to storage of honey in metal containers and chemical composition of wax (Bonyadian et al., 2011).

In most studies, contamination of the Iranian honey with heavy metals was low, and it has been reported that adult bees' bodies are more contaminated than pollen and honey. It seems that bees reduce their contamination while producing honey (Samimi et al., 2001).

In general, low amount of heavy metals in honey is not considered a problem and the amount of heavy metals in honey is significantly lower than in bees due to filtering done by bees (Bogdanov, 2006). Akbari et al., and Saghaei et al., in their studies conducted in Iran demonstrated that lead levels in honey measured in samples obtained from the markets in Iran, Ardabil, and Urmia cities were equal to 0/11, 0/935, and 0/04 mg/kg, respectively. The honey samples obtained from the market of Urmia City showed good quality in terms of the amount of heavy metals (Saghaei et al., 2012). Also, it has been indicated that average consumption of honey in Poland, Romania, and Ethiopia is nutritionally safe for health of children and adults (Winiarska-Mieczan et al., 2021). Bhalchandra in a study showed the presence of toxic metals including As, Cd, Pb, Hg, and Ni in all honey samples. Average concentration of heavy metals in honey samples was lower than the standard Indian limit (Bhalchandra & Baviskar, 2015).

The results of a study conducted in Saudi Arabia indicated the highest amount of potassium and cadmium in only one of the samples (0.008 ±0.008) (Saghaei et al., 2012). The results of a study done in China showed that the amounts of cadmium, lead, arsenic, and mercury were equal to 1.34, 33.98, 13.44, and 1.65 µg/kg, respectively (Tuzen et al., 2007).

There are various techniques to determine chemical elements, such as heavy metals and minerals in honey samples. Various studies have been performed on determination of heavy metals in honey samples. The most commonly used techniques are inductively coupled plasma optical emission spectrometry (ICP-OES), flame emission spectrometry (FES), flame atomic absorption spectrometry (FAAS), inductively coupled plasma mass spectrometry (ICP-MS), high-performance liquid chromatography (HPLC), Atomic absorption spectroscopy (AAS), total reflection x-ray fluorescence spectroscopy (TXRF), Graphite furnace atomic absorption spectroscopy (GFAAS), and hydride generation atomic absorption spectroscopy (HGAAS). ICP-OES that are used as analytical techniques for detection of chemical elements with excellent sensitivity (Fakhri et al., 2019). This method has been applied in most studies to determine heavy metals in various samples. In

any case, food contamination with heavy metals and other environmental chemicals may occur (Manouchehri et al., 2021; Pirhadi et al., 2021) and any medicinal plants used by bees may contain active compounds or heavy metals (Manouchehri et al., 2021; Abbasi et al., 2020; Aidy et al., 2020; Karimi et al., 2019; Abbasi et al., 2016; Abbaszadeh et al., 2018; Sedighi et al., 2019; Nouri et al., 2019; Abbasi et al., 2021) and be transmitted to bees.

Conclusion

Honey has long been used not only as a food but also it has been widely applied to treat many diseases and health-related problems. In most studies, contamination of the Iranian honey with heavy metals was low, and it has been reported that adult bees' bodies are more contaminated than pollen and honey. It seems that bees reduce their contamination while producing honey. Given that honey is a valuable and widely consumed food in the diet of most people in different nations, so the study of the quality of honey in the consumer market to maintain the health of consumers seems necessary. We need to inform the public about this, so this review article explains the presence of heavy metals in honey.

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DERLEME / REVIEW

ARI POLENİ PROTEİNLERİ VE FONKSİYONEL ÖZELLİKLERİ

Bee Pollen Proteins and Their Functional Properties

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ÖZ

Bal ve arı ürünlerinden biri olan polen arılar için önemli besin kaynaklarındandır. Polen özellikle ergin, yaşlı ve larva dönemindeki arıların beslenmesinde protein, vitamin ve mineral madde gibi ihtiyaçlarının karşılanması için kullanılmaktadır. Polen içerisindeki protein oranı ile arıların beslenmesinde temel protein ihtiyacını karşıladığı için kovana yeterli düzeyde alınmalı ve uygun şartlarda depolanmalıdır. Arı poleni insan sağlığı için besleyici ve terapötik özelliklere sahiptir. Apiterapide, ilaç sanayinde, gıda endüstrisinde, arı yetiştiriciliğinde, hayvan yemi olarak, kozmetik sanayinde ve polinasyon çalışmaları gibi geniş bir kullanıma sahip arı poleninin fonksiyonel etkileri henüz tam olarak bilinememektedir. Arı poleninin teknik açıdan fonksiyonel gıda maddesi olarak olası kullanımı, fiziksel, kimyasal ve teknofonksiyonel özelliklerinin bilinmesine bağlıdır. Ancak arı polenlerinin çeşitli alanlarda kullanımında bu etkileri gözardı edilmektedir. Bunun nedeni arı polenlerinin toplandığı mevsim ve bölgeye bağlı olarak protein içeriğinin değişmesi ve bu değişimin polen proteinlerinin fonksiyonel özellikleri üzerine etkilerinin net şekilde ortaya konmamasıdır. Bu derleme ile literatür bilgileri ışığında, arı poleni proteinleri ve sahip oldukları fonksiyonel özelliklere temel oluşturulmuş ve daha fazla araştırma yapılmasına dikkat çekilmesi hedeflenmiştir.

Anahtar Kelimeler: Polen, Polen proteini, Fonksiyonel özellikler

ABSTRACT

Consumption of bee products has increased significantly due to the their therapeutic and antiviral activity. Moreover it could offer nutrition improvement and reducing the risk of developing serious infectious diseases. Pollen is a bee product which used especially in the nutrition of adult, old and larval honeybees to meet their needs such as protein, vitamins and minerals. Depending on the botanical and geographical origins, bee pollen can be included different composition. Additionally, the protein content and composition of bee pollen are the major protein source of honeybees pollen. Although, the functional properties of bee pollen which have generally used in apitherapy, pharmaceutical industry, food industry, beekeeping, animal feed, cosmetic industry and pollination studies, the functional properties of bee pollen proteins have not been clearly revealed yet. Moreover, the using of bee pollen proteins as a functional food, it depends on physical, chemical, nutritional and technofunctional properties of its. In the literature, bee pollen proteins and their tecnofunctional using has not determined with all details yet. Therefore, in this review provides to determine the functional properties of bee pollen proteins and an overview opportunities for their use in various applications.

Keywords: Pollen, Pollen proteins, functional properties

EXTENDED ABSTRACT

Goal: Bee proteins, one of the beekeeping products, have been consumed by people for years due to their antifungal, antimicrobial, antioxidant, anti-inflammatory, antitumor, chemopreventive/ chemoprotective, and antiradiation benefits. Bee pollen, which can be consumed as human food, is very rich in protein. However, this protein content of the bee product has not been clearly investigated. In this study, first of all, bee pollen will be evaluated in terms of its physical, chemical and technofunctional properties such as protein solubility, emulsification properties, water and oil absorbtion capacity, foaming capacity and stability. Thus, the use of bee pollen proteins is not only for human health, but also in industry. The study also drew attention to further research for the functional properties of bee pollen proteins in order to contribute to the development of new functional products.

Discussion: For the technofunctional properties of bee pollen, the chemical ingredients of its such as carbohydrates, lipids and ash are as significant as its protein content and composition. Thus, when evaluating the technofunctional properties of bee pollen, it is necessary to consider the effects of all surfactant substances in bee pollen, as well as their increasing and decreasing interactions with other compounds individually and collectively.

The most important of the technofunctional properties is considered to be protein solubility, because it affects emulsification properties, gelation properties and foaming capacity and stability. Although, bee pollen contains low protein compared to other protein sources, it has high protein solubility.

However, bee pollen proteins have better oil absorbing capacity than water absorbing capacity, good emulsification properties and foam suppression activity compared the other protein sources (soy flour, black gram kidney bean flour). All these technofunctional properties are mainly due to the presence of surfactants and interactions with other pollen components.

Although the nutritional value and therapeutic effects of bee pollen proteins are known, in the literature studies have not include enough data about technofunctional properties of bee pollen proteins. Therefore, further research should be conducted on its possible use in functional food products for their technofunctional properties.

Conclusion: Bee pollen proteins and their technical use as a functional food depends on its physicochemical and technofunctional properties. However, past studies in this field are very limited in the literature. For this reason, more studies should be studied on the proteins of bee pollen and its technological properties. Especially, the using of bee pollen proteins in the food industry, it needs to some new technofunctional properties are determined. Thus, it is possible to predict the interaction of bee pollen with food and the changes during processing with the findings obtained. In conclusion, this review offers an opportunity to the food industry to determine the functional properties of bee pollen proteins and identify novel food uses to develop ingredients.

GİRİŞ

Polen, kovana bal getiren işçi arılar tarafından çiçeğin stamenlerinden toplanmakta, nektar veya bal ile nemlendirilerek arının arka bacaklarında biriktirilmektedir (Krell 1996). Bal arıları, bal özlerini toplarken vücutlarına farkında olmadan yapışan polenler ile bitkilerde tozlaşmayı ve bal arılarının beslenmesi için gerekli olan polenin toplanmasını sağlarlar (Erdoğan ve Dodoloğlu 2005). Bir arı, vücut ağırlığı ile orantılı olarak, kovana tek seferde 15-45 mg polen taşıyabilmektedir. Kolonilerin yıllık polen üretimi ise koloni büyüklüğü ve çevresel şartlara (iklim, bitki örtüsü vb.) göre farklılaşmaktadır (Dreller ve Tarpy 2000). Kovanda depolanan polenler koyuldukları gözlerde üzerine bal veya sıvı eklenerek muhafaza edilir.

Endüstriyel anlamda insan tüketimine sunulması istenen polenin, toplanması kadar kurutulması ve saklama koşulları da besin bileşimi açısından önemlidir. Kurutulacak olan polenlerin önceki yıllarda güneşte kurutulması kabul edilirken bu kurutma yönteminin polenin sahip olduğu besin içeriğinde azalmaya neden olduğu belirlenerek yöntemin kullanımından vazgeçilmiştir (Aydın 2016).

Kurutulmuş halde %6-8 nem içeriğine sahip olan polenlerde bu oranın üstüne çıkılması veya altına inilmesi halinde bozulma ve besin değerinde kayıplar meydana gelmektedir (İnci 1999). Bu nedenle tüketilecek olan polenin hem protein içeriğinin hem de diğer besin bileşenlerinin zarar görmemesi için uygun kurutma teknikleri tercih edilmelidir. Polen kurutma işleminin, sıcaklık (36-

45°C) kontrolünün sağlandığı kurutma dolaplarında yapılması alternatif kurutma yöntemlerinden biridir, ancak kurutma işlemi sonrasında polenler yabancı maddelerden temizlenmelidir.

Paketlenen polenlerin içerisinde kalan hava boşluklarının CO_2 (karbondioksit) ile doldurulması ve polen içindeki havanın vakumlanarak çıkarılması ile güve kelebeği ve benzeri zararlılara ait yumurtalarının vereceği zarar önlenebilir; polenler bu şekilde uzun süre bozulmadan saklanabilir (Aydın 2016).

Kurutulan ve temizlenen polenlerin raf ömrü sıcaklıkla ters orantılı olarak değişmektedir. Örneğin oda sıcaklığında 1-2 ay, 5°C'da 1 yıl, -15°C'da ise besin değerinde önemli bir kayıp yaşanmadan uzun süre depolanabilmektedir. Ayrıca raf ömrünü arttırmak için polenlerin koyu renkli cam kaplara konulması, ışıksız ve serin yerlerde muhafaza edilmesi avantaj sağlamaktadır. Geliştirilen teknolojiler ve yeni yöntemlerle polen farklı şekillerde muhafaza edilmektedir. Örneğin; polen toz şekerle karıştırılarak (Genç ve Dodoloğlu 2002), mikrodalgada kurutularak (Kanar 2017), vakum altında dondurularak (Kumova ve Korkmaz 1998), dondurularak (Kutlu vd. 2015) ve sıcak hava ile kurutularak (Aydın 2016) muhafaza edilmektedir.

Polenin sahip olduğu besin bileşenleri toplandığı bölgeye bağlı olarak farklılık göstermektedir. Bu nedenle polenin besin değerleri için tam bir standart olmasa da polen, yaklaşık %4-15 su, %7,5-40 protein, %15-82 şeker, %1,3-7 lipid, %1-3,5 vitamin ve mineral içermektedir (Almeida-Muradian vd. 2005, Stanciu vd. 2011, Kostić vd. 2015). Arı poleni besin değeri yanında sahip olduğu biyolojik aktivite sayesinde; antitümör, hemopreventif/kemoprotektif, antimikrobiyal, antifungal, antioksidan, anti-radyasyon ve anti-inflamatuar özelliklerle insan sağlığı üzerine olumlu etkiler göstermiştir (Kinsella ve Melachouris 1976, Pascoal vd. 2014).

Arı poleni tek başına tüketilebildiği gibi diğer gıda ürünlerinin içerisine katılarak da tüketicilerin tercihine sunulmaktadır. Ancak arı poleninin diğer gıdalarla olan etkileşimlerine ait fazla veri bulunmamaktadır. Bu nedenle, endüstride kullanımının yaygınlaşmasını ve yeni teknofonksiyonel özelliklerinin tespit edilmesini sağlamak amacı ile arı poleninin proteinleri ve teknolojik özellikleri hakkında daha fazla çalışma yapılmalıdır. Bu çalışmada öncelikle arı poleni proteinlerinin çözünürlük, emülsifiye etme, köpürme özellikleri, su ve yağ tutma kapasitesi gibi teknofonksiyonel özellikleri değerlendirilecektir. Böylelikle elde edilen bulgularla arı poleninin gıdalarla etkileşimi ve işleme sırasındaki değişikliklerin öngörülmesine olanak sağlanabileceği düşünülmektedir.

Arı Poleni Proteinleri

Arı poleninin içeriğinde bulunan aminoasitlerdeki farklılıklar, arıcılığın yapıldığı bölgeye, mevsime ve botanik çeşitliliğe göre değişiklik göstermektedir. Genel olarak arı polenlerinin çeşitli kombinasyonlarda esansiyel olan ve olmayan aminoasit içeriğine sahip olduğu bilinmektedir (Tablo 1) (Paramas vd. 2006, Mărgăoan vd. 2010, Yang vd. 2013, Gardana vd. 2018, Negrao ve Orsi, 2018, Thakur ve Nanda 2018, Taha vd. 2019, Zuluaga-Domínguez vd. 2019, Al-Kahtani vd. 2020, Sommano vd. 2020, Thakur ve Nanda 2020, Bayram 2021, Bayram vd. 2021). Alınan polen örnekleri üzerinde yapılan çalışmalarda İspanya, İtalya ve Kolombiya'dan (Gardana vd. 2018) prolin ve arjinin; Hindistan'da (Thakur ve Nanda 2018) glutamik asit, aspartik asit, prolin, lösin, alanin, lizin; Brezilya'da (Negrao ve Orsi, 2018) prolin, glutamik asit, aspartik asit, lizin ve Türkiye'de (Bayram 2021, Bayram vd. 2021) prolin, asparajin ve aspartik asit başlıca aminoasit kaynaklarını oluşturmaktadır. Ancak mevsimsel değişiklikler polenlerin bileşimindeki aminoasit kombinasyonunun farklılaşmasına sebep olduğu gibi bu kombinasyondaki baskın aminoasitlerin değişmesine de yol açmaktadır.

Esansiyel olmayan bir aminoasit olan prolin, bal arıları için uçuş enerjisi kaynağı olmasından dolayı, Dünya üzerinde toplanan polenlerde çoğu zaman baskın aminoasit olarak karşımıza çıkmaktadır. Prolinin baskın olarak görülmediği polenlerin toplandığı bölgelerde, Türkiye'de yetiştirilen deli baldan elde edilen kovanlardan toplanan *Rhododendron ponticum* polenlerde olduğu gibi, botanik özelliklerden dolayı baskın aminoasit (asparajin) değişebilmektedir (Bayram 2021). Ancak prolin baskınlığını kaybetse de polen bileşiminin önemli bir kısmını oluşturmaktadır.

İçeriğindeki kıymetli bileşenler nedeniyle arı poleni genellikle besin takviyesi olarak tüketilmektedir. Günümüzde yapılan araştırmalarla, arı poleni sadece besleyici ve tedavi edici özellikleri ile gıda takviyesi olarak kullanılmamakta, aynı zamanda ürün kalite özelliklerini geliştirmek için fonksiyonel bir bileşen olarak geliştirilmeye çalışılmaktadır.

Tablo 1 Arı poleni içeriğinde bulunan esansiyel ve esansiyel olmayan aminoasitler

Esansiyel aminoasitler	*Esansiyel olmayan aminoasitler*	
Histidin	Alanin	Gama-aminobütirik asit
İzolözin	Arjinin	3-Amino izobütirik asit
Lözin	Aspartik asit	Aminoadipik asit
Lizin	Asparajin	Etanolamin
Metionin	Sistein	Glutamin
Fenilalanin	Glisin	Sarkozin
Treonin	Glutamik asit	Homositrülin
Triptofan	Prolin	Sitrülin
Valin	Serin	Ornitin
	Taurin	Norlösin
	Tirozin	Homoserin
	g-Aminobütirik asit	Sarkozin

Yapılan çalışmalarda arı poleni, fermente sütlü içeceklere eklendiğinde antimikrobiyal aktivite sergilemiştir. Benzer şekilde asidofilus sütü ve probiyotik yoğurda arı poleni eklenmesi, duyusal özellikleri etkilemeden probiyotik canlılığı ve içecek viskozitesini ve yağ seviyesinden bağımsız olarak laktik asit üretimini arttırmıştır (Glušac vd. 2015).

Fırın ürünlerinde arı poleninin kullanımı, Krystyjan vd. (2015) tarafından bisküvi şeklindeyken ve Conte ve ark. (2018) tarafından glutensiz ekmek şeklindedir. Hazırlanan bisküvilerde artan arı poleni oranı ile birlikte protein, şeker, kül, lif, polifenoller ve antioksidan potansiyeli önemli ölçüde yükselmiştir, ancak lezzet bu durumdan olumsuz etkilenmiştir (Krystyjan vd. 2015). Ekmekte ise tekno-fonksiyonel özelliklerde bir gelişme, bayatlama oranında azalma ve ekmeğin genel organoleptik kabul edilebilirliğinde bir artış elde edilmiştir.

Ayrıca yapılan son araştırmalarda, arı poleni yüksek antioksidan potansiyeli ve fenolik bileşik içeriğine sahip olması nedeniyle pudinglerde (Anjos vd. 2019), yağ oksidasyonunu engellemek amacıyla domuz sosislerinde (de Florio Almeida vd. 2017), diğer et ürünlerinde (Novaković vd. 2021) ve içeceklerde (Zuluaga vd. 2016) kullanılmasıyla olumlu sonuçlar elde edilmiştir.

Arı poleninin gıdalarda kullanımına ek olarak bileşiminde yer alan aminoasitlerle yapılan bir çalışmada kışlık buğday ununa prolin ve glutamin ilavesinin ekmek hamurunun fonksiyonel özellikleri üzerine etkileri incelenmiştir. Prolin veya glutamin ilavesi, yumuşak buğday hamuru ve ekmek özelliklerinde önemli gelişmeler sağlamazken, glutamin ve prolin kombinasyonu sinerjik etki göstererek, hamur ve ekmek özelliklerini iyileştirmiştir (Fermin vd. 2005).

Bu çalışmalar, diğer ürünlerde arı poleni kullanımı içi umut vermekte ve daha fazla araştırılması için bir temel sağlamaktadır. Ancak arı poleninin gıda ürünlerindeki uygulamalarında, içerisine ilave edileceği ürünün besin değeri, biyoaktif bileşen içeriği, tekno-fonksiyonellikleri, organoleptik özellikleri ve gıda güvenliği açısından kapsamlı bir şekilde incelemelidir.

Arı poleninin teknik açıdan fonksiyonel gıda maddesi olarak kullanımı; fiziksel, kimyasal ve teknofonksiyonel özelliklerine bağlıdır. İşlenmiş gıda ürünlerinin çoğu, gıda biyopolimeri, proteinler, polisakaritler ve çeşitli partikül türleri içeren çok bileşenli koloidal sistemlerdir (Schimidt 1997). Bu nedenle gıda işlemede en çok ilgi gören teknofonksiyonel özellikler arasında çözünürlük, emülsiyon oluşumu ve stabilizasyonu, köpük oluşumu ve stabilizasyonun yanı sıra su ve yağ tutma kapasitesi de bulunur. Arı polenlerinin bu özellikleri üzerine yapılan çalışmalar sınırlı kalsa da

yapılan çalışma ile konuya dikkat çekilmesi amaçlanmıştır.

Arı Poleninin Fonksiyonel Özellikleri

Protein çözünürlüğü

Protein çözünürlüğü, emülsifikasyon, köpürme ve jelasyon gibi diğer özellikleri etkilediğinden, teknofonksiyonel özelliklerden en önemlisi olarak kabul edilmektedir (Lee vd. 2003, Kanar 2017). Protein çözünürlüğü, protein kompozisyonu ve konformasyonun yanı sıra proteinler ve diğer bileşenler (tuzlar, lipidler, karbonhidratlar ve fenolik bileşikler) arasındaki etkileşimler vasıtasıyla belirlenmektedir. Protein çözünürlüğünü etkileyen faktörlerden bir diğeri de proteinlerin moleküler ağırlıklarıdır. Düşük (10-25 kDa) ve orta (25-50 kDa) molekül ağırlıklı proteinlerin, yüksek molekül ağırlıklı proteinlere (50-80 kDa) göre protein çözünürlüğü daha yüksektir.

Arı poleni protein çözünürlüğü ortalama 11,22±6 g/100 g (84.91 to 87.56%)'dır (Kostić vd. 2015). Bu değerler, pH 7'deki düşük çözünürlüklü ticari soya proteini ürünleri (unlar, konsantreler ve izolatlar) için elde edilen değerlerle karşılaştırıldığında (Lee vd. 2003) ticari soya proteini ürünlerinin yaklaşık 20 g/100 g veya daha düşük çözünürlüğe sahip olduğu görülmektedir. Çözünürlüğü etkileyen bir diğer önemli faktör de ıslanabilirlik ve dağılabilirliktir. Bu konuda arı poleni ile ilgili çalışmalarda ıslanabilirlik 285.67-1909.46s ve dağılabilirlik 34.10 ve 51.06% olarak değişirkenlik göstermektedir. Bu değerler keçi sütünden elde edilen süt tozu (ıslanabilirlik, 418.80-594.00s; dağılabilirlik, %78.38-84.11) ile kıyaslandığında arı poleninin daha yüksek ıslanabilirlik ve daha düşük dağılabilirlik özelliğine sahip olduğunu göstermektedir (Reddy ve diğerleri, 2014).

Emülsiyon yapıcı özellikler

Gıdalardaki yapı ve yapıları oluşturan birimler arasındaki emülsiyonlar önemli bir rol oynamaktadır. Emülsifiyer bu bileşenler gıdaya ağızda arzu edilen hissi özellikleri kazandırmakta ve mayonez, kahve, krema likörleri, bazı meyve içecekleri ve et ürünlerinin birçoğunda ve diğer birçok gıda ürününde yapının oluşumunda anahtar bileşenler olarak karşımıza çıkmaktadır (Dalgleish 2004).

Proteinler, temel gıda emülgatörlerinden biri olarak kabul edilmesine rağmen, lipidler gibi diğer bileşenler de iyi emülsiyon yapıcılar gibi davranır. Ayrıca arı poleni; potasyum ve kalsiyum gibi mineralleri, pektin ve nişasta gibi dengelenmiş veya arayüzey malzeme ile etkileşime girebilen yüklü veya yüklenmemiş polisakaritleri içermektedir (Kostić vd. 2015). Bu nedenle, emülsiyon kararlılığı ve aktivitesi ile protein çözünürlüğü veya çözünebilir protein içeriği arasındaki ilişki arı poleni numunelerindeki birçok yüzey aktif bileşen arasındaki varlığın ve etkileşimlerin sonucunda ortaya çıkabilmektedir.

Sırbistanda yapılan bir çalışmada arı poleni proteinlerinin emülsiyon yapıcı özellikleri; ESI (emülsiyon stabilite indeksi) ve EAI (emülsiyon aktivite indeksi) araştırılmış, EAI ortalama 15,71±3,29 m^2g^{-1}, ESI ise ortalama 31,85±8,94 dakika değerinde bulunmuştur. (Kostić vd. 2015). Yapılan diğer bir çalışmada arı polen çeşitliliği emülsiyon özellikleri açısından değerlendirilmiş ve hindistan cevizi poleni emülsiyon aktivitesi %46.76±0.83 m^2g-1, stabilitesi %26.32±0.31 dakika, kolza poleni emülsiyon aktivitesi %44.83±0.53 m^2g^{-1} ve stabilitesi %21.62±0.37 dakika olarak belirlenmiştir (Thakur ve Nanda, 2020). Bu sonuçlara bakıldığında, arı poleninin emülsiyon aktivitesi, karnabahar unu (%50.948 ±2.06), şalgam unu (%46.108±2.18), buğday unu (%43.141 ±2.25), pirinç unu (%41.48 ±1.842), maş fasülyesi (%41.17±1.021) gibi ürünlerle yaklaşık aynı değer aralığındayken, patates unu (%39.05±4.984), börülce unu (%25.213 ±1.37) ve meksika fasulyesi unu (%21.749 ±1.70) gibi protein içerikli ürünler için elde edilen sonuçlardan daha iyi düzeydedir (Kumar vd. 2017, Chandra ve Samsher 2013).

Arı poleninin emülsiyon stabilitesine bakıldığında; şalgam unu (%42.460±2.12), patates unu (%41.92±1.824), karnabahar unu (%40.346±0.79), buğday unu (%38.38±4.785), maş fasülyesi (%37.95±2.362) ve pirinç unu (%37.31±5.407) gibi ürünlerden daha düşük meksika fasulyesi unu (%19.140±1.67) ve börülce unu (%6.949 ±1.46) gibi protein içerikli ürünlerden daha yüksek bir değere sahip olduğu görülmektedir (Kumar vd. 2017, Chandra ve Samsher 2013). Bu değerler arı polenlerinin iyi emülsifiye edici özelliğe sahip olduğunu göstermektedir (Heywood vd. 2002). Ayrıca emülsifiye edici özelliklerdeki bu farklılıklar; sıvı damlacık boyutu ve dağılımına, protein özelliklerine (hidrofobiklik, konformasyon, konsantrasyon ve çözünürlük), çözücü koşullarına (tuzlar, pH ve sıcaklık), faz hacim oranına ve sürekli faz viskozitesine göre değişkenlik göstermektedir (Avramenko vd. 2013).

Köpürme özellikleri

Ekmek, kek, dondurma gibi gıda ürünlerinin işleme sırasında veya sonrasında, yapısının ve dokusunun oluşması ve korunması için havaya ihtiyaç duyulmaktadır. Arı poleni köpük stabilitesi (%17.50-20.00) ve kapasitesi (%6.21-8.69) açısından düşük oranlara sahiptir. Bu nedenle köpük yapıcı özellik gerektiren ürünlerde tercih edilmezler (Kostić vd. 2015). Arı poleni, fındık unu proteinleri ile karşılaştırıldığında kapasitesinin (%8) aynı değerlerde, fakat stabilitesinin fındık proteinlerine göre daha yüksek olduğu gözlenmiştir (Tatar vd. 2015). Ancak yumurta albümini, kajudan elde edilen protein izolatları ile kıyaslandığında bu değerler oldukça düşük kalmaktadır (Neto vd. 2001).

Köpük yapıcı özelliklerin bulunmaması, arı poleni bileşiminde bulunan yüzey aktif lipidlerden kaynaklanabilir. Lipidler proteinlerden daha yüksek yüzey aktivitesi nedeniyle köpük stabilitesinde azalmaya neden olmaktadır (Phillips 2013). Ayrıca fosfolipidlerin ve lipoproteinlerin köpük baskılayıcı ajanlar olduğu bilinmektedir (Lee vd. 2003). Liang vd.'nin yaptığı çalışmada (2013), polen tanelerinin lipid profilinin analiz edilmesiyle polar lipidlerin büyük kısmının membrana bağlı fosfolipitler, fosfatidilkolin ve fosfatidilserin olduğu tespit edilmiştir. Böylelikle ortaya çıkan düşük köpürme ve köpük baskılayıcı özelliği ile arı poleni köpürme özelliklerinin istenmediği gıda bileşeni olarak kullanılabilir.

Su tutma kapasitesi (STK)

Su tutma kapasitesi, ürünün nemli olması gibi belirli ürün özellikleri için önemlidir. Yüksek su tutma kapasitesi olan gıdalar, özellikle depolama sırasında gıda ürünlerini kırılgan ve kuru hale getirebilir. Arı poleninin su tutma kapasitesi; yapılan çalışmalarda (Kostić vd. 2015) ortalama 1,43±0,29 gg^{-1} iken polen cinsine göre kişniş poleninde 0.47±0.05 gg^{-1} ve hindistan cevizi poleninde 0,72±0,08 gg^{-1} olarak değişmektedir. Bu değerler, meksika fasülyesi için elde edilen değerlere göre daha düşüktür (Wani vd. 2013). Ancak soya fasülyesi proteininkiyle benzer şekildedir (Acuna vd. 2012).

Arı poleninin su tutma kapasitesini etkileyen başlıca bileşenler, kutup grupları içerdikleri için polar veya yüklü yan zincir ve çözünmeyen karbonhidratlar gibi hidrofilik kısımlar içeren çözünmeyen proteinlerdir. Ayrıca, bu moleküller, kılcal eylemler yoluyla su moleküllerini bağlayabilen üç boyutlu yapılara sahiptir. Bu nedenle, kutupsal ve yüklü bölgelerdeki lipidlerin daha iyi su tutma kapasitesi kazanmada katkıda bulunabileceği belirtilmelidir.

Yağ tutma kapasitesi (YTK)

Herhangi bir gıda bileşiminin yağ tutma kapasitesi, gıda uygulamaları için önemlidir. Çünkü lipidler, lezzet tutucu ve ağızdaki tadı artırıcı etkiye sahiptir (Ötles 1995). Çözünmeyen moleküllerin hidrofobik kısımlarının bulunması YTK için oldukça önemlidir. Arı poleninin YTK'sı için ortalama değeri 2,49±0,56 gg^{-1} (Kostić vd. 2015) iken, yapılan diğer çalışmalarda kişniş poleninde 1.31± 0.11 g/g ve hindistan cevizi poleninde 2.13 ±0,20 g/g olarak değişmektedir. Bu değerler meksika fasulyesi (2,2-2,3 gg^{-1}) (Wani vd. 2013) ve soya fasulyesi (1,43 mlg^{-1}) (Acuna vd. 2012) için elde edilen değerlerden daha yüksektir.

Arı polenlerinin YTK'sına önemli oranda katkıda bulunan ana bileşen, polenin ana bileşenini oluşturan sporopollenin olabilir. Sporopollenin, polen kuru ağırlığının %20'sinden fazlasını oluşturan yağ asidi-lignin benzeri kompleks bir polimerdir (Stanley ve Linskens 2012). Sporopolleninin hidrofobik kısmı ve hidrofobik kalıntılara sahip olan diğer çözülmeyen bileşenler arı polenlerinin YTK'sını artırabilir. Yapılan çalışmalar bu anlamda incelendiğinde arı poleni proteinlerinin, hidrasyon özelliklerine göre daha iyi lipofilik özellik gösterdiği belirlenmiştir (Kostić vd. 2015).

SONUÇ VE ÖNERILER

Eski çağlardan beri çiçek poleni besin yararları için insanlar tarafından kullanılmıştır. Günümüzde besin takviyesi olarak sıkça tüketilen arı poleninden gıda işlemede de fonksiyonel özelliklerinden faydalanılabilir. Arı poleni diğer protein kaynakları ile kıyaslandığında düşük protein içermesine karşın yüksek protein çözünürlüğü, su tutma kapasitesinden daha iyi yağ tutma kapasitesi, iyi emülsiyonlaşma özellikleri ve köpük baskılama aktivitesine sahiptir. Bütün bu teknofonksiyonel özellikler temel olarak yüzey aktif bileşenlerin varlığı ve diğer polen bileşenleriyle olan etkileşimlere bağlı olarak ortaya çıkmaktadır.

Arı poleninin teknofonksiyonel özellikleri için protein içeriği ve bileşimi kadar karbonhidratların, lipitlerin, külün içeriği ve bileşimi de önemlidir. Böylece arı poleninin teknofonksiyonel özellikleri değerlendirilirken arı polenlerindeki tüm yüzey aktif

bileşenlerin özelliklerini, bireysel ve toplu olarak diğer bileşiklerle olan artırıcı ve azaltıcı etkileşimlerini de göz önünde bulundurmak gerekmektedir.

Arı poleni, besleyici değerinin yüksek olmasından dolayı insan sağlığının korunması ve iyileştirilmesi amacıyla yaygın olarak tüketilmeye başlanmıştır. İnsan sağlığı üzerindeki takviye edici etkileri bilinse de teknolojik açıdan gıda ürünlerinde, olası kullanımıyla ilgili daha ileri araştırmalar yapılmalıdır. Bu araştırmalar yapılırken arı poleninin; besleyici değeri, sağlık etkileri ve belirli teknofonksiyonel özelliklerin kombinasyonunun çalışılmasının gıda sektöründe büyük bir potansiyele kapı aralayacağı düşünülmektedir.

Mali kaynak: Mali kaynak sağlayan bir kurum bulunmamaktadır.

Etik belgesi: Etik belgesi gerekli değildir.

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