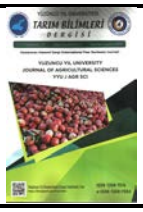
Yüzüncü Yıl Üniversitesi
Tarım Bilimleri Dergisi
(YYU Journal of Agricultural Science)

http://dergipark.gov.tr/yyutbd



Araştırma Makalesi (Research Article)

The Investigation Propolis Foraging Preference of Different Honey Bee Races

Nazife EROĞLU[1], Merve KAMBUR ACAR[2], Meral KEKEÇOĞLU*[3,4]

[1]The Scientific and Research Council of Turkey, Marmara Research Center, Food Institute, Kocaeli, Turkey
[2]Düzce University, Düzce Vocational School, Beekeeping Program,81010, Düzce, Turkey
[3] Düzce University, Faculty of Arts and Sciences, Department of Biology, 81620, Konuralp, Düzce, Turkey
[4]Düzce University, Beekeeping Research Development and Aplication Centre, 81620, Konuralp, Düzce, Turkey
[1]https://orcid.org/0000-0002-8618-2583 [2]https://orcid.org/0000-0001-9658-6584 [3]https://orcid.org/0000-0002-2564-8343
*Corresponding author e-mail: meralkekecoglu@gmail.com

Article Info

Received: 26.08.2020
Accepted: 15.09.2020
Online Published 30.03.2021
DOI: 10.29133/yyutbd.785911

Keywords

Anatolia,
Honeybee races,
Propolis.

Abstract: In this study, it was investigated whether different bee breeds prefer different plant sources to collect propolis. For this purpose four different honey bee race (*Apis mellifera caucasica, A. m. carnica, A. m. syriaca* and *A. m. anatoliaca*) naturally have been in Turkey were placed in the same isolated apiary; and Propolis was harvested from these races. chemical contents of alcoholic extractions of the harvested propolis were were analyzed by liquid chromatography-tandem mass spectrometry (LCMS / MS). In addition that, the pollen content of the same propolis samples were determined with a microscope. According to the LCMS / MS results the propolis samples collected by different honey bee race differed significantly in terms of quercetin and ferulic acid. Data obtained from polen analyses revealed that Fabaceae and Apiaceae (PD >45%) families were mostly detected in propolis samples obtained from different races. Although the polen from the Campanulaceae family was dedected only in the propolis samples from *A. m. anatoliaca* race, thr polen from Caryophyllaceae family was found in other propolis samples collected by *A. m caucasica* races. The results of this study showed that different honey bee races tend to different plant sources and the content of propolis may differ according to the bee races.

Farklı Bal Arısı Irklarının Propolis Toplama Tercihlerinin Araştırılması

Makale Bilgileri

Geliş: 26.08.2020
Kabul: 15.09.2020
Online Yayınlanma 30.03.2021
DOI: 10.29133/yyutbd.785911

Anahtar kelimeler

Anadolu,
Bal arısı ırkları,
Propolis.

Öz: Bu çalışmada farklı arı ırklarının propolis toplamak için farklı bitki kaynaklarını tercih edip etmediği araştırılmıştır. Bu amaçla Türkiye'de doğal olarak bulunduğu bilinen 4 farklı bal arısı ırkı (*Apis mellifera caucasica, A. m. carnica, A. m. syriaca and A. m. anatoliaca*) izole edilmiş aynı arılığa yerleştirilmiş ve bu ırklardan propolis hasadı yapılmıştır. Hasat edilen propolislerin alkolik ekstraksiyonları yapılarak sıvı kromatogram kütle spektrofotometrisi (LCMS/MS) ile kimyasal içerikleri analiz edilmiştir. Aynı zamanda aynı propolislerin mikroskop ile polen içeriği belirlenmiştir. Elde edilen sonuçlara göre farklı bal arısı ırklarının topladığı propolisler kuersetin ve ferulik asit bakımından önemli düzeyde farklılık göstermiştir. Farklı ırklardan elde edilen propolis örneklerinde en fazla Fabaceae and Apiaceae (PD >45%) familyasından polenlere reslanırken, Campanulaceae familyasından polene yalnızca *A. m. anatoliaca* ırkının topladığı polenlerde ve Caryophyllaceae familyasından polenlere ise sadece *A. m. caucasica* ırkının topladığı propolislerde raslanmıştır. Bu çalışmanın sonuçları farklı arı ırklarının farklı bitki kaynaklarına yönelme

davranışında bulunduğunu ve ırka göre propolis içeriğinin değişebileceğini göstermiştir.

1. Introduction

Propolis is a heterogeneous mixture of more than 300 compounds such as resin, balsam, bee secretions, pollen, and many other organic substances that vary widely according to their botanical and geographical origin, season, climate, flora, altitude, and even honeybee species (Ghisalberti, 1979; Kutluca, 2003; Şahinler and Aziz, 2005; Silici and Kutluca, 2005; Bankova et al., 2006; de Sousa et al., 2007; Sforcin, 2007; Popova et al., 2010; Miguel and Antunes 2011).

Very little information has been reported regarding why bees forage for a specific resin or propolis source in the field, this is probably due to difficulties to carry out foraging experiments which include, but are not limited to, relatively infrequent flights compared to pollen foraging, and choice of a single bee to use unobservable tree canopies (Simone and Spivak, 2012; Wilson et al., 2013). Therefore, we studied all honeybee races of *Apis mellifera* known to be exist in Anatolia, primarily at the same apiary and same season, to determine if there is any variation of chemical compound of collected propolis and possible pollen contamination, during their gathering to identify preferences of botanical sources.

Knowledge of the botanical origin and chemical composition of propolis is the most important subject to understand its structure, biological activity as well as beneficial properties. Among the six main chemical types of propolis based on botanical sources poplar propolis is most often identified with the botanical source of Populus nigra (Bankova, 2005). Birch propolis from Russia recorded with the plant source of Betula verrucosa Ehrh. Poplar and other species from temperate zones such as Europe were reported (Greenaway et al., 1987; Bankova et al., 1989; 1992; Marcucci, 1995; Bankova et al., 2002; Silici and Kutluca, 2005; Bankova et al., 2006; Moreira et al., 2008; Salatino et al., 2011). On the other hand, propolis sources in other parts of the world recorded Baccharis dracunculifolia D.C. from Brazil (Kumazawa et al., 2003; Park et al., 2004). Macranga ssp. from Japan and Taiwan, *Plumeria acuminata W. T. Aiton* and *P. acutifolia* Poir from Hawaii, *Myroxylon balsamum* (L.) Harms from El Salvador and Ambrosia deltoidea (Torr.) Payne from Mexico (Marcucci, 1995; Wollenweber and Buchmann, 1997; Bankova et al., 2006; Salatino et al., 2011; Wilson et al., 2013).

The main biologically active substances of poplar propolis are flavones, flavanones, phenolic acids, and their esters, while birch propolis contains flavones and flavonols predominantly (Bankova, 2005). In spite of this diversity of origin, the main sources of phenolic compounds determined in Turkish propolis were the poplar bud exudates (Silici and Kutluca, 2005; Bertrams et al., 2013). Previous investigations have shown that Turkish propolis samples from different regions may be categorized into four main groups depending on its chemical composition. The typical poplar samples from Middle and West Anatolia displayed very similar phenolic and flavonoid content. However, samples from Mediterranean, and Eastern Anatolia regions revealed different substances of low phenolic and very low flavonoid concentrations which were not present in *P. nigra* L. bud exudate, but possibly of *Populus euphratica* Oliv (Bertrams et al., 2013). On the other hand, Ankara propolis obtained from distant vicinities, like Kazan and Mamak, showed the presence of more than 24 compounds so far, including pinocembrin, pinostropin, isalpinin, pinobanksin, quercetin, naringenin, galangine, chrysin, and caffeic acid (Kartal et al., 2002; 2003; Popova et al., 2005; Uzel et al., 2005). The plant source of these compounds remains unknown and there were no signs of flavonoid aglycones which are typical compounds of poplar propolis, but the existence of steroid compounds and long-chain fatty alcohols may indicate new plant sources of propolis excluding *Pinus brutia* L. as an origin (Popova et al., 2005). Nevertheless, these early studies were not represented the different foraging preferences of bee colonies.

The present study aims to evaluate propolis collecting preferences of indigenous honeybees consisting of four subspecies (*A. m. anatoliaca, A. m. caucasia, A. m. syriaca, and A. m. carnica*), under controlled conditions in Central Anatolia. We investigated whether propolis collecting tendency of each honeybee species toward certain resinous plants. For this reason biologically active compounds and microscopic pollen analyses were used to describe the botanical origin of propolis samples collected by different races. A total of 20 phenolic compounds, which are most commonly found in poplar propolis, were analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

This research is the first study using both chemical and pallynological techniques to compare the different foraging preferences of honeybee subspecies and ecotypes of Turkey. Consequently, it contributes to future behaviour studies of honeybees and chemical standardization of Turkish propolis.

2. Materials and methods

The study was conducted using honeybee colonies under controlled conditions at the common Apiary in Anatolia, Turkey. This study was carried out under the same environmental conditions between April-July of 2015, and it included honeybees of the subspecies *A. m. anatoliaca, A. m. caucasia, A. m. carnica* and *A. m. syriaca.*

Plastic propolis traps with the dimensions of 420x500 mm were inserted in the early spring season of 2015, and collected by the end of the summer of the same year. Then, raw propolis samples were hand-collected from the traps by using a sharp blade and stored in deepfreeze (-25 ºC) until further processing. After cooling, all propolis samples were grinded with an electrical blender (Waring 8011EB) prior to extraction. Propolis samples of approximately 5 g were extracted by maceration with ethanol (1:10 ratio, 50 mL) at room temperature of 25-30°C employing three days of 150 rpm shaker agitation. The combined extracts were filtered on paper filters of the following grades: Whatman No:1 and Whatman No:4. The obtained solution was filtered through a 0.45 µm membrane syringe filter. The propolis extracts were run as duplicate and stored on a refrigerator after analysis. All chemicals (caffeic acid phenethyl ester, dimethylaminocinnamic acid, apigenin, caffeic acid, catechin, isorhamnetin, luteolin, myristic acid, naringenin, protocatechuic acid, pinobanksin, quercetin, syringic acid, biochanin, kaempferol, chalcone, coumaric acid, rosmarinic acid, chlorogenic acid, ferulic acid) were used as standards in LC–MS/MS analysis and obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). HPLC grade methanol was obtained from Sigma (Merck, Darmstadt, Germany).

This study followed a modified version of the methods given by Zohary (1973) and Yang et al. (2013). The mass-spectrometer measurements were performed on a hybrid triple quadrupole/linear ion trap mass spectrometer API 4000 QTRAP (Applied Biosystems, Darmstadt, Germany) with electrospray ionization (ESI). LC separations were performed in a C18 analytical column (Gemini® 5 µm particle size, 110 Å pore size, 50 mm x 2 mm, fully porous organo-silica LC Column). The run time for each injection was 5.5 min, the temperature of the column was 40 °C, and the injection volume was 10 µL. The mass-spectrometer worked with an electro-spray ion source (ESI) in positive mode under the selected ion monitoring (SIM) condition including a 0,70 amu width, a nebulizer pressure of 55 psi, a drying gas flow of 1 mL/min and a skimmer voltage of ~20-80 V. Data acquisition was carried out with the Workstation Method Builder.

The slightly modified methodology for pollen preparation was obtained from Warakomska and Maciejewicz (1992) and Pellati et al. (2011). Each 0.5 g powdered propolis samples were mixed with ethanol-ether-acetone (1:1:1) solution and shaken overnight. After filtering through a special filter paper with 20 µm holes, the suspension was centrifuged at 4100 rpm for 15 min. Then, the supernatant was poured on to two slides following preparation of the residues using basic fuchsine-glycerin gelatin. Pollen identification and counting were performed by microscope (Leica DM500). In accordance to melissopalynological criteria (Louveaux et al., 1970; Gençay and Sorkun, 2006), the common definition of pollen frequencies was used as PD for dominance (more than 45%), PA for accessory (15-45%), and PI for isolated (less than 15%).

SPSS-15.0.1 software package (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Kruskal-Wallis test was performed depending on the chemical composition of propolis collected by each honeybee subspecies and ecotypes.

3. Results

3.1. Chemical analyses of propolis according to honeybee subspecies

The mean amount of bioactive compounds retrieved from propolis samples by four different honeybee races in the same research apiary was revealed by the existence of analyses in variable degrees. The chemical analyses by LC-MS/MS were categorized by bee subspecies. Propolis collected by *A. m. carnica* displayed the highest values of caffeic acid phenethyl ester, dimethoxy cinnamic acid, apigenin,

isorhamnetin, luteolin, myristic acid, naringenin, pinobanksin and quercetin. *A. m. anatoliaca* retrieved the highest values of protocatheuic acid, syringic acid and kaempherol; while propolis of *A. m. caucasica* had the highest content of catechol and ferulic acid; and propolis collected by *A. m. syriaca* had the highest content of coumaric acid. On the other hand, rosmarin, chalcon and chlorogenic acid results seem to be either negligible or could not be detected.

Results of the Kruskal-Wallis test retrieved no statistical differences between honeybee groups with respect to chemical analyses. Surprisingly, only the quercetin compound showed significant differences among bee subspecies according to the Kruskal-Wallis test ($P<0.05$); and the amount of ferulic acid of propolis samples collected in the same environment vary between Syrian and Caucasian honeybee subspecies. Our chemical analyses revealed that the amount of quercetin and caffeic acid phenethyl ester were lower in the *A. m. anatoliaca* ecotype than in *A. m. caucasia, A. m. carnica*, and *A. m. syriaca* (Table 1).

Table 1. The chemical composition of propolis according to honey bee races and ecotypes

Chemical analytes	Mean bioactive substances of propolis ± Std. Dev, min/max values (ppm) by species and subspecies											
	A. m. caucasica(N=15)			*A. m.anatolica* (N=13)			*A. m. syriaca*(N=14)			*A. m. carnica*(N=12)		
	Mean ± Std.Dev.	Min	Max	Mean ± Std.Dev.	Min	Max	Mean ± Std.Dev.	Min	Max	Mean ± Std.Dev.	Min.	Max.
Caffeic acid phenethyl ester	567.80 ± 15.46	101	1005	174.50 ± 30.50	144	205	641.70 ± 17.09	113	858	771.50 ± 0.50*	771	772
Dimethoxy cinnamic acid	895.40 ± 25.99	149	1650	284.00 ± 83.00	201	367	1089.20 ± 38.10	212	1730	1326.00 ± 0.10	1326	1326
Apigenin	282.40 ± 42.92	141	388	128.00 ± 51.00	77	179	358.70 ± 95.02	92	528	391.00 ± 1.00	390	392
Caffeic acid	480.60 ± 13.86	69	799	78.00 ± 25.00	53	103	465.70 ± 13.18	57	652	563.50 ± 0.50	563	564
Catechin	128.60 ± 40.31	60	284	103.00 ± 51.00	52	154	95.00 ± 24.92	41	159	117.00 ± 1.00	116	118
Isorhamnetin	4277.00 ± 10.65	1109	7863	2267.50 ± 18.50	1081	3454	5528.00 ± 16.00	1039	8192	6062.50 ± 0.50	6062	6063
Luteolin	4214.80 ± 82.78	1543	6185	2023.00 ± 43	1590	2456	4116.50 ± 77.51	1804	5068	4374.50 ± 0.50	4374	4375
Myristic acid	149.20 ± 34.68	39	211	33.50 ± 7.50	26	41	339.20 ± 17.77	32	827	214.50 ± 0.50	214	215
Naringenin	344.40 ± 66.30	118	509	140.00 ± 49.00	91	189	483.50 ± 14.25	100	824	587.00 ± 1.00	586	588
Protocatheuic acid	41.60 ± 11.43	0	67	49.00 ± 13.00	36	62	46.70 ± 14.14	30	89	29.50 ± 0.50	29	30
Pinobanksin	1233.00 ± 29.30	245	1934	427.00 ± 18.00	242	612	805.70 ± 31.39	277	1583	1237.50 ± 0.50	1237	1238
Quercetin	38.40 ± 5.60	21	55	23.50 ± 0.50	23	24	50.20 ± 8.52	25	61	66.50 ± 0.50	66	67
Syringic acid	51.20 ± 8.74	33	75	110.50 ± 17.50	93	128	95.00 ± 31.68	51	189	103.50 ± 0.50	103	104
Biochanin	328.60 ± 20.69	0	1008	0	0	0	0	0	0	0	0	0
Kaempferol	50.60 ± 32.45	0	157	143.50 ± 44.50	99	188	63.00 ± 3.00	0	252	0	0	0
Chalcone	0.00	0	0	0	0	0	0.00	0	0	0	0	0
Coumaric acid	28.60 ± 7.22	12	52	0	0	0	63.50 ± 32.88	0	152	0	0	0
Rosmarinic acid	6.40 ± 2.87	0	16	0.50 ± 0.10	0	1	6.20 ± 3.66	0	14	0	0	0
Chlorogenic acid	0	0	0	0	0	0	0.00	0	0	0	0	0
Ferulic acid	359.80 ± 98.16	0	589	67.50 ± 48.50	19	116	109.00 ± 3.36	0	236	0	0	0

3.2. Botanical analyses of propolis according to honeybee subspecies

Data obtained from polen analyses revealed that Fabaceae (PD >45%) is the most common botanical family in all propolis samples. S1 also have high proportions of propolis from the botanical family Pinaceae. Meanwhile, *A. m. anatoliaca* held more Apiaceae, Betulacea, Fagaceae, and Graminae; and A4 exhibited Pinaceae in the level of PA. A2 samples were also rich on Fagaceae, and Pinaceae pollen. Interestingly, C1 held accessory Fagacea, and CA1, CA2, CA4 displayed more pollens from the botanical family Pinaceae (Table 2).

Table 2. The most frequent pollen types of collected propolis according to honey bee races *A. m. anatoliaca* (A1, A2, A3, A4), *A. m. caucasia* (CA1, CA2, CA3, CA4, CA5), *A. m. carnica* (C1, C2, C3), *A. m. syriaca* (S1, S2, S3, S4, S5) in research apiary.

Family	S1	S2	S3	S4	S5	CA1	CA2	CA3	CA4	CA5	A1	A2	A3	A4	C1	C2	C3
Apiaceae		▲	▲	▲	▲			▲	▲	▲	▲	▲		▲	▲	▲	▲
Asteraceae		▲					▲	▲	▲	▲	▲			▲		▲	
Betulaceae		▲		▲	▲	▲								▲	▲	▲	
Campanulaceae												▲	▲				
Caryophyllaceae						▲	▲							▲			
Chenopodiaceae	▲			▲	▲			▲		▲	▲	▲	▲		▲	▲	▲
Ericaceae			▲		▲												
Fabaceae	✸	✸	✸	✸	✸	✸	✸	✸	✸	✸	✸	✸	✸	✸	✸	✸	✸
Fagaceae			▲		▲	▲	▲	▲	▲	▲	▲		▲	⬣	⬣	⬣	⬣
Gramineae	▲	▲	▲	▲	▲	▲	▲		▲	▲	▲	▲	▲	▲	▲	▲	▲
Lamiaceae	▲																
Pinaceae	✸	▲	▲	▲	▲	⬣	⬣	▲	⬣	▲	⬣	▲	⬣	⬣	▲	▲	▲
Rosaceae											▲						
Salicaceae	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Tiliaceae		▲	▲		▲												
Malvaceae													▲				

▲ *PD=dominant (>45%), ⬣ PA=accessory (15-45%), ✸ PI = iso

4. Discussion and Conclusion

The factors affecting the composition and production of propolis by a honeybee are not truly known. Some researchers consider that seasonal factors are involved while others think inherent behavioural changes or the use of a resin (Ghisalberti, 1979; Wilson et al., 2013). Propolis collecting capability of honeybees is considered to be regulated by behavioural causes and differs from physiological properties (Barth, 1998). The foraging preferences of honeybees were investigated by the influence of pollen-based cues among six floral species, and three artificial substrates (pollen analogues). The responses of honeybees to the odours of different pollen species appear similar over those of analogue species, and individual honeybee foragers do not discriminate among sources based on intrinsic differences in quality, but, instead, efficiency of collection and recruitment (Ghisalberti, 1979). Recently, it was demonstrated that honeybees make discrete choices among many resinous plant species, even among closely related species. They use metabolomics methods after visiting for the first time like an environmental forensics to track an individual resin forager behaviour and metabolite patterns (Wilson, et al., 2013). The observed bees maintained fidelity to a single source for each foraging trip. The bees discriminately foraged for resin from eastern cottonwood (*Populus deltoides*), and balsam poplar (*Populus balsamifera*), among the many available, even closely related resinous plants which composition did not show significant seasonal or regional changes. In the present study LC-MS/MS analyses demonstrated that the pharmaceutical quality of the compounds constituting propolis may change slightly based on honeybee subspecies and pollen frequency, this also indicates different levels of abundance by subspecies. On the contrary of these outcomes, our study could not find significant differences in chemical analyses of propolis collected by the different races.

Plant secondary metabolites, like flavonoids and phenolic acid derivatives, were recorded in this study and may serve as chemotaxonomic markers (Dülger, 1997). Brazilian propolis obtained by Africanized A. mellifera, was classified into 12 groups based on physicochemical characteristics,

including five southern Brazilian groups, one southeastern Brazilian group and six northeastern Brazilian groups, the reported origin of these groups are resins of the poplar trees, plus Hyptis divaricata, and Baccharis dracunculifolia. It is noted that pinobanksin, pinocembrin, pinobanksin 3-acetate, chrysin, and galangin are the dominant flavonoids in propolis and poplar tree samples that were collected in southern Brazil (Pernal and Currie, 2002). Similarly, we found that propolis produced by the subspecies *A. m. carnica* had the highest amount of caffeic acid phenethyl ester, dimethoxy cinnamic acid, apigenin, caffeic acid, isorhamnetin, luteolin, myristic acid, naringenin, pinobanksin, and quercetin. Aliyazıcıoğlu et al. (2013) reported very low chlorogenic acid, epicathecin, syringic acid, and coumaric acid values with no amount of cathecin in Anatolian propolis.

The chemical composition of propolis samples collected from three different honeybee subspecies in Erzurum were identified by Gas chromatography-mass spectrometry (GC–MS) (Silici and Kutluca, 2005). It was found the differences between chemical content of propolis samples. Present study is compatible with (Silici and Kutluca, 2005)' results. In the present study propolis samples displayed the highest amount of catechin and ferulic acid gathered by *A. m. caucasia*; while protocatheuic acid, syringic acid, and kaempherol found the highest amount of propolis collected by *A. m. anatoliaca* and coumaric acid by *A. m. syriaca*.

In the recent time, the compound of propolis samples collected by different bee species was studied. The presence of phenolics was cited in 38 propolis samples produced in tropical Venezuela by imported Apis mellifera L., and five indigenous species of stingless bees (Chen et al., 2000). In general, no correlations between the composition of tropical propolis and the place of collection or the bee species were cited. Several chemical types of stingless bees' propolis were categorized (Silva et al., 2008), according to the prevailing type of compounds like: gallic acid, diterpenic and triterpenic types. Their study of chemical composition, and biological activity of propolis from Brazilian Meliponinae by GC-MS, showed that neither bee species nor the geographical location determine the chemical composition of Meliponinae propolis. Because of the Meliponinae forage behaviour over short distances (maximum 500 m), and use of the first plant exudate they encounter as main propolis source during their flights, these results may be expected. However, we would not compare these results with Apis mellifera subspecies and ecotypes used in this study due to their long flight range, assumed 2.5 km for drones Tomas-Barberan et al. (1993), and completely different collecting and hygiene behaviours considering hive antimicrobial cleaning provided mostly by propolis.

Pollen samples were examined under microscope to determine pollen contents which identified 16 plant families. The total number of pollen counted for each sample showed variations and also different organic materials and plant fragments complicating the analysis were observed in all samples. The most frequent pollen type detected was from Fabaceae family, which was found in the all samples as dominant pollen (> % 45). On the other hand, Malveaceae pollen type were in present just in A3 sample and Rosaceaea pollen type were in A1 sample. Pinaceae pollen types also were detected in all samples in different ratios. Similarly, Salicaceae pollen type were isolated in all samples as isolated pollen (< % 15). Graminaeae pollen type was also monitored in all samples except from CA3 samples. It could be said that bee preferences showed even differences among same bee types considering especially each of the beehive. Pinaceae pollen type, for instance, were determined in S1 sample dominantly, however, for S2, S3 and S4 samples detected as isolated Pinaceae pollen. In a different manner, Chenopodiaceae pollen type was only recorded in two *A. m. syriaca* bee type (S1 and S4). Regarding to these results bee preferences showed big variations. The moderately observed pollens were belong to the Apiaceae, Betulacea, Fagaceae, and Graminae families that density may differ in reference to each honeybee species. The preferences of each beehive both in the same subspecies suggested the variability of pollen abundance.

As a result, our findings of chemical composition reveal higher amount of compounds as isorhamnetin and luteolin in all bee species. Quercetin and ferulic acid showed slight differences among bee races. Maximum amount of quercetin was collected by *A. m. carnica* and ferulic acid by *A. m. cacucasica*. Biochanin found only at Anatolian and Caucasian honeybees as well as coumaric acid limited to propolis samples from *A. m. anatoliaca* and *A. m. carnica.* In the view of the *A. m. carnica,* only species among others, that does not have kaempherol, rosmarinic acid, chlorogenic acid, ferulic acid in collected propolis samples. Chlorogenic acid was not encountered at propolis samples.

Propolis is a natural product with great therapeutical properties though its composition remarkably diverse and applications particularly medical use got problems with its quality control and

lack of origination. The classifying propolis plant sources from different geographic regions are crucial which biologically active compounds may lead to the formulation of local types in respect to plant origin. Further studies need to concentrate on these issues before any apitherapy applications. Additionally, further work need to be larger scale of replicates considering this is a preliminary behavioural study for honeybees in Anatolia.

Acknowledgment

This study was fully supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK), BIDEB-2232 Project No: 113C036, Institution Code: 5134103 under the name of "Investigation of important honey bee, *Apis mellifera* L. (Hymenoptera, Apidae), subspecies and ecotypes in Turkey considering propolis collection capacity and chemical characterization of Turkish propolis".

References

Aliyazicioglu, R., Sahin, H., Erturk, O., Ulusoy, E., & Kolayli, S. (2013). Properties of phenolic composition and biological activity of propolis from Turkey. *International Journal of Food Properties 16*, 277-287.

Bankova, V.S., Popov, S. S., & Marekov, N. L. (1989). Isopentenyl cinnamates from poplar buds and propolis. *Phytochemistry 28*, 871-873.

Bankova, V., Dyulgerov, A., Popov, S., Evstatieva, L., Kuleva, L., Pureb, O., & Zamjansan, Z. (1992). Propolis produced in Bulgaria and Mongolia: phenolic compounds and plant origin. *Apidologie, 23*, 79-85.

Bankova, V., Popova, M., Bogdanov, S., & Sabatını, A.G. (2002). Chemical composition of European propolis: expected and unexpected results. *Zeitschrift für Naturforschung C 57*, 530-533.

Bankova, V. (2005). Recent trends and important developments in propolis research. Evid. Based Complement. *Alternative Medicine 2*, 29-32.

Bankova, V., Popova, M., & Trusheva, B. (2006). Plant sources of propolis: an update from a chemist's point of view. *Natural Product Communications: SAGE Journals, 1*, 1023-1028.

Barth, O. M. (1998). Pollen analysis of Brazilian propolis. *Grana 37*, 97-101.

Bertrams, J., Kunz, N., Muller, M., Kammerer, D., & Stintzing, F. C. (2013). Phenolic compounds as marker compounds for botanical origin determination of German propolis samples based on TLC and TLC-MS. *Journal of Applied Botany and Food Quality, 86*, 1.

Chen, C., Meyermans, H., & Burggreave, B. (2000). Cell-specific and conditional expression of caffeoyl-coenzyme A-3-O-methyltransferase in poplar. Plant Physiology 123, 853–868.

De Sousa, J. P., Bueno, P. C., Gregório, L.E., Da Silva Filho, A. A., Furtado, N. A., De Sousa, M. L., & Bastos, J. K. (2007). A reliable quantitative method for the analysis of phenolic compounds in Brazilian propolis by reverse phase high performance liquid chromatography. *Journal of Separation Science 30*, 2656-2665.

Dulger, C. (1997). *Kafkas, orta anadolu ve Erzurum balarısı (Apis mellifera L.) genotiplerinin Erzurum koşullarındaki performanslarının belirlenmesi ve morfolojik özellikleri.* Doctoral Dissertation, Ataturk University, Department of Zootechny, Erzurum, Turkey.

Gençay, O., & Sorkun, K. (2006). Microscopic analysis of propolis samples collected from east anatolia (Kemaliye, Erzincan). *FABAD Journal of Pharmaceutical Sciences 31*, 192.

Ghisalberti, E.L. (1979). Propolis: a review. *Bee world 60*, 59-84.

Greenaway, W., Scaysbrook, T., & Whatley, F. R. (1987). The analysis of bud exudate of populus euramericana, and of propolis, by Gas Chromatography-Mass Spectrometry. *Proceedings of the Royal society of London. Series B. Biological sciences 232*, 249-272.

Kartal, M., Kaya, S., & Kurucu, S. (2002). GC-MS analysis of propolis samples from two different regions of Turkey. *Zeitschrift für Naturforschung C, 57*, 905-909.

Kartal, M., Yildiz, S., Kaya, S., Kurucu, S., & Topcu, G. (2003). Antimicrobial activity of propolis samples from two different regions of Anatolia. *Journal of Ethnopharmacology 86*, 69-73.

Kumazawa, S., Yoneda, M., Shibata, I., Kanaeda, J., Hamasaka, T., & Nakayama, T. (2003). Direct evidence for the plant origin of Brazilian propolis by the observation of honeybee behavior and phytochemical analysis. *Chemical and Pharmaceutical Bulletin 51(6)*, 740-742.

Kutluca, S. (2003). *Propolis uretim yöntemlerinin koloni performansı ve propolisin kimyasal özellikleri üzerine etkileri.* Doctoral Dissertation, Department of Zootechny, Ataturk University, Erzurum, Turkey. Turkey YOK Dissertations and Theses database. p. 145.

Louveaux, J., Maurizio, A., & Vorwohl, G. (1970). Methodik der Melissopalynologie. *Apidologie 1,* 193-209.

Marcucci, M. C. (1995). Propolis: chemical composition, biological properties and therapeutic activity. *Apidologie 26,* 83-99.

Miguel, M. G., & Antunes, M. D. (2011). Is propolis safe as an alternative medicine? *Journal of Pharmacy & Bioallied Sciences 3,* 479.

Moreira, L., Dias, L. G., Pereira, J. A., & Estevinho, L. (2008). Antioxidant properties, total phenols and pollen analysis of propolis samples from Portugal. *Food Chemical Toxi. 46,* 3482-3485.

Park, Y. K., Paredes, Guzman, J. F., Aguiar, C. L., Alencar, S. M., & Fujiwara, F. Y. (2004). Chemical constituents in Baccharis dracunculifolia as the main botanical origin of southeastern Brazilian propolis. *Journal of Agricultural and Food Chemistry 52,* 1100-1103.

Pellati, F., Orlandini, G., Pinetti, D., & Benvenuti, S. (2011). HPLC-DAD and HPLC-ESI-MS/MS methods for metabolite profiling of propolis extracts. *Journal of Pharmaceutical and Biomedical Analysis 55,* 934-948.

Pernal, S. F., & Currie, R. W. (2002). Discrimination and preferences for pollen-based cues by foraging honeybees, Apis mellifera L. *Animal Behaviour 63,* 369-390.

Popova, M., Silici, S., Kaftanoglu, O., & Bankova, V. (2005). Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. *Phytomedicine 12,* 221-228.

Popova, M. P., Graikou, K., Chinou, I., & Bankova, V. S. (2010). GC-MS profiling of diterpene compounds in Mediterranean propolis from Greece. *Journal of Agricultural and Food Chemistry 58,* 3167-3176.

Sahinler, N., & Gul, A. (2005). The effects of propolis production methods and honeybee genotypes on propolis yield. *Pakistan Journal of Biological Sciences 8,* 1212-1214.

Salatino, A., Fernandes-Silva, C. C., Righi, A. A., & Salatino, M. L. F. (2011). Propolis research and the chemistry of plant products. *Natural Product Reports 28,* 925-936.

Sforcin, J.M. (2007). Propolis and the immune system: a review. *J. of Ethnopharmacology 113,* 1-14.

Silici, S., & Kutluca, S. (2005). Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. *Journal of Ethnop., 99,* 69-73.

Silva, B. B., Rosalen, P. L., Cury, J. A., Ikegaki, M., Souza, V. C., Esteves, A., & Alencar, S. M. (2008). Chemical composition and botanical origin of red propolis, a new type of Brazilian propolis. *Evidence-Based Complementary and Alternative Medicine 5,* 313-316.

Simone-Finstrom, M. D., & Spivak, M. (2012). Increased resin collection after parasite challenge: a case of self-medication in honey bees? *PLoS One 7,* 34601.

Tomas-Barberan, F. A., Garcia-Viguera, C., Vit-Olivier, P., Ferreres, F., & Tomás-Lorente, F. (1993). Phytochemical evidence for the botanical origin of tropical propolis from Venezuela. *Phytochemistry 34,* 191-196.

Uzel, A., Oncag, O., Cogulu, D., & Gencay, O. (2005). Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. *Microbiological Res. 160,* 189-195.

Warakomska, Z., & Maciejewicz, W. (1992). Microscopic analysis of propolis from Polish regions. *Apidologie 23,* 277-283.

Wilson, M. B., Spivak, M., Hegeman, A. D., Rendahl, A., & Cohen, J. D. (2013). Metabolomics reveals the origins of antimicrobial plant resins collected by honey bees. *PloS one 8,* 77512.

Wollenweber, E., & Buchmann, S. L. (1997). Feral honey bees in the Sonoran Desert: Propolis sources other than poplars (Populus spp.). *Zeitschrift für Naturforschung C 52,* 530-535.

Yang, W.Q., Xu, W., Yin, Y., Zhang, X. Y., Chen, H. L., Xin, Z. H., Shen, C. Y., & Zhang, R. (2013). Determination of nitrofuran metabolite residues in propolis by LC-MS/MS. *Chinese Journal of Analysis Laboratory 3,* 012.

Zohary, M. (1973). Geobotanical foundations of the Middle East. Stuttgart, Germany: *Fischer*.