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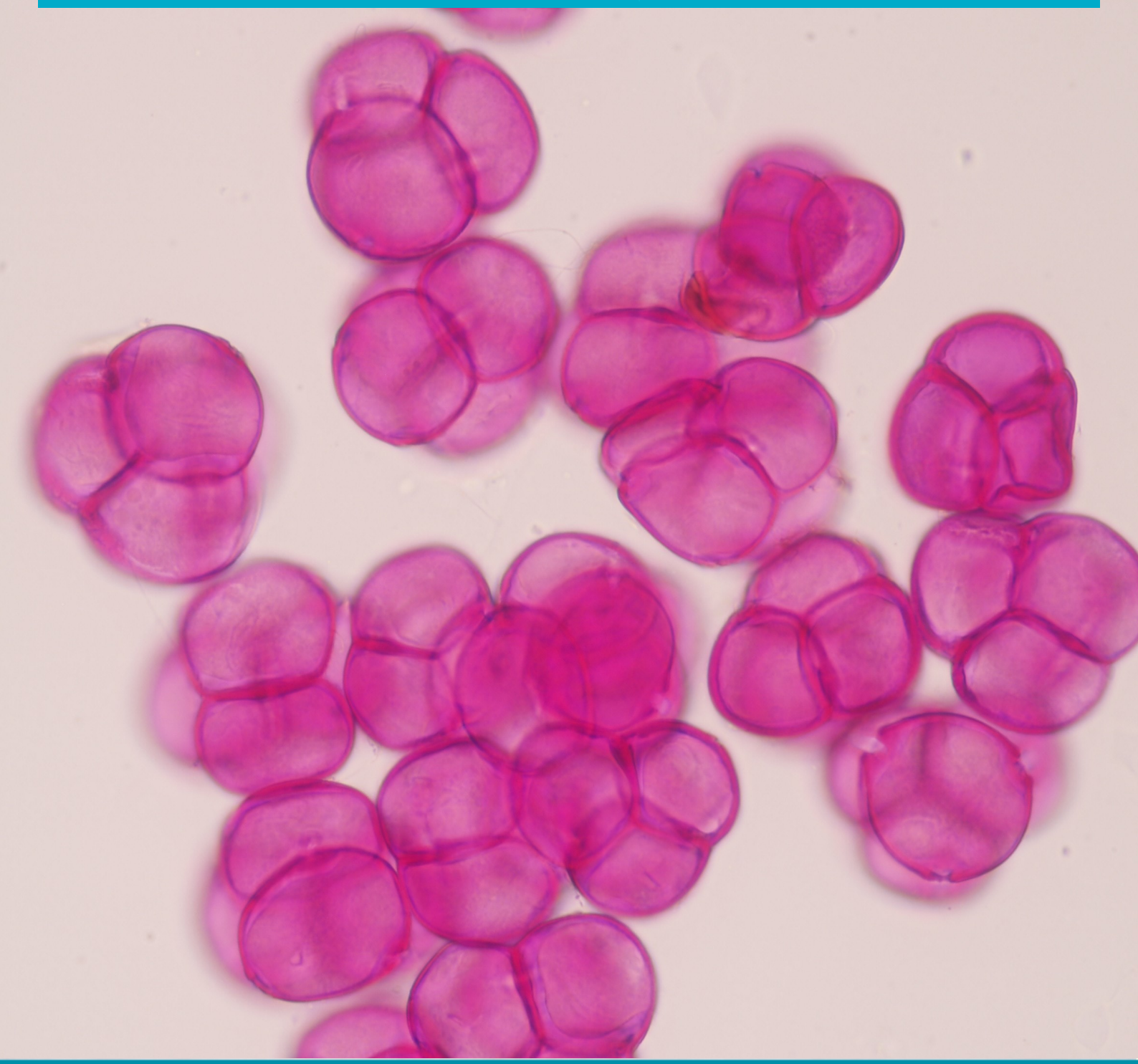

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MELLIFERA RESEARCH ARTICLE

Seasonal Variation in Forage Plant Selection, Foraging Time, Duration and Preference of *Apis mellifera adansonii* in the Rainforest and Semi-savannah Ecosystems of Nigeria

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ABSTRACT

The foraging behaviour such as plant selection, time, duration and floral preference of colonies of *Apis mellifera adansonii* were studied during the dry and wet seasons in 2012 -2014. Colony observations were made from 6:00 am - 7:30 am, 11:00 am - 1:00 pm and 6:00 pm - 7:30 pm during the period, meteorological data on sunset and sunrise, moonrise and moonset were collected for the time of observations of the foraging workers. All the time recorded for the start and end of foraging were local time for the study sites. The floral plant species distributed in 3 kilometer radius of the colony sites were sampled. Honey and pollen combs were also collected from all the experimental colonies for mellisopalynological investigations. There was a difference between the abundance of foraging plants in the savannah and rainforest zones in the tropics as indicated by Jacard's (Cj) and Sorenson's (CS) similarity coefficient of 0.37 and 45.9% respectively. The bees average starting and ending times of foraging were 07:01 h and 18.20 h respectively and the average duration of foraging was 11:30 h per day. During the dry and wet seasons, the foraging time and duration was not significantly different ($P < 0.05$). Pollen analyses showed that the bees in the savannah ecosystem visited more flowers than those in the forest ecosystem; the former had a significantly higher pollen sum of 254 and 543 during dry and wet seasons respectively ($P < 0.05$). The colonies in both forest and savannah areas showed preference for Elaeis *guineensis* Jacq.(Arecaceae). Results from this study have given insights into the "bee plants" of the two ecosystems as well as providing information to bee keepers about the behaviour of bees for improved apiary management.

Keywords: Foraging, floral preference, pollen, nectar, *Apis mellifera adansonii*

Introduction

Apis mellifera adansonii Latreille, 1804 compared to the European honey bee (Apis mellifera mellifera L.) have high foraging power but low productivity because it consumes more of the honey hoards in the period of nectar and pollen dearth [1]. The basic foods of honey bee colony are nectar and pollen [2,3,4]. Worker bees during foraging, gather and collect pollen and nectar from flowers of plants. The pollens are used by the bees to make pollen bread and nectar is used for honey. The nectars are the derivatives of phloem sap which are formed from specialized group of cells

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called nectaries [5]. The nectaries are termed floral part, if they occur as part of a flower or extrafloral, if they occur elsewhere on a plant [6,7,8]. Nectary contains nectar which is an aqueous solution of sugars [9]. Similarly, pollen grains are usually present in floral part called anther suspended by a structure called filament, both collectively referred to as stamen [10].

During foraging, *A. mellifera adansonii* visit diverse and selective plant species for nectar and pollen. These foraging workers have two types: the scout bees and reticent bees. The latter constitute 40–90% of the total foragers' population [11]. Foraging activity is initiated by the scout bees that go to the field, return and display a dance communication and odour plume to alert the reticent bees about the food source, location and distance [12]. The foragers select their foraging plants for nectar, pollen and resin; they collect water, and can also collect wax from scale insects, *Ceroplastes* sp. [13].

The foraging activity starts in tandem with the sunrise and sunset. However, this activity is greatly affected by the regional and climatic conditions. For example, Joshi and Joshi [14] and Alqarni [15] claimed under a desert condition, a higher number of foragers left the colonies at 10 am. The foraging activities whether for pollen or nectar is impacted by many factors. These factors can be divided into two major groups: in-colony factors and out-colony factors [16]. The in-colony factors include genetic factors -: colony-level trait [17], and the genotype of bee strain (e.g. high and low pollen-hoarding bees) strongly affected foraging behaviour for nectar or for pollen [18], the pollen demand of the colonies [4], the specific position of one flower over another [19]; colony strength and brood rearing activity [20,21], mated or virgin queen [22]; types of bee hive; and the hygienic status of foraging bees which may halt their return to their colonies or increased time to return when on forage [23]. The out-colony factors include: availability of suitable plant resources and other environmental factors such as temperature, humidity and time of the year [16,24].

Foraging worker bees may change from water foragers to pollen foragers in relation to the colony conditions. In this case, foragers use their experience in trophallactic contacts to assess the pollen need of their colonies [4]. Similarly, where there are shortages of pollen or in conditions of pollen dearth, honey bee colonies may increase the proportion of pollen foragers without increasing foraging rate [25].

Honey bees show floral constancy during foraging. There are many examples of foraging preference; nectar and pollen preference were observed due to the specific position of one flower over another [19]. According to Fohouo et al. [26], highest number of foraging workers were attracted to *Syzygium guineense* var. *guineense* (Myrtaceae) and the lowest number on *Psorospermum febrifugum* (Hypericaceae) because of abundance and availability of the target vegetation.

The pollen content of the honey not only reflects agricultural practices and surrounding vegetation but also the floral diversity and species composition of the melliferous plant species nearby the apiary [27]. Abou-Shaara [16] suggested and cited some standard methods for monitoring the foraging activity of the honey bees :- caging in net conditions[19]; marking and recapturing foraging workers [1]; self-marking devices for studying the foraging range [28]; video recording in insect-proof tunnels [29]; pollen traps [30] and

harmonic radar to record flight paths of foraging honey bee workers [31,32]. Foraging activity can be measured by employing different parameters including, the commencement or/and cessation time [14].

Therefore, to improve on current knowledge of our understanding of honeybee floral preference and foraging behaviour, colonies of *Apis mellifera adansonii* in apiaries located in the rainforest and semi-savanna ecosystems of Nigeria were studied during the wet and dry seasons. The objectives were to compare the relative abundance of foraging plants, foraging time, duration and floral preference of the freely foraging colonies of *Apis mellifera adansonii*. Results from this study will give insight into the activities of this bee species and therefore improve hive management for greater profits for beekeepers.

Material and Methods

Study sites

Field investigations were carried out in two large apiaries located in Oyan, Odo-Otin and Ife South Local Government Areas, in Osun State, Nigeria. The farm locations are in the geographical coordinates of 8°2'0"N, 4°42'0"E and 7°31'3"N and 4° 31'34"E respectively, Odo-Otin belongs to a semi-savannah and Ife South belongs to a rainforest ecological zone (Figure 1). Foraging activities

Observations of foraging time were done by using a Time Lapse Video Camera (TLC200 pro HDR) during the dry and wet seasons of 2012-2014, in eight randomly selected free-foraging colonies, four from each farm sites for fifteen days in

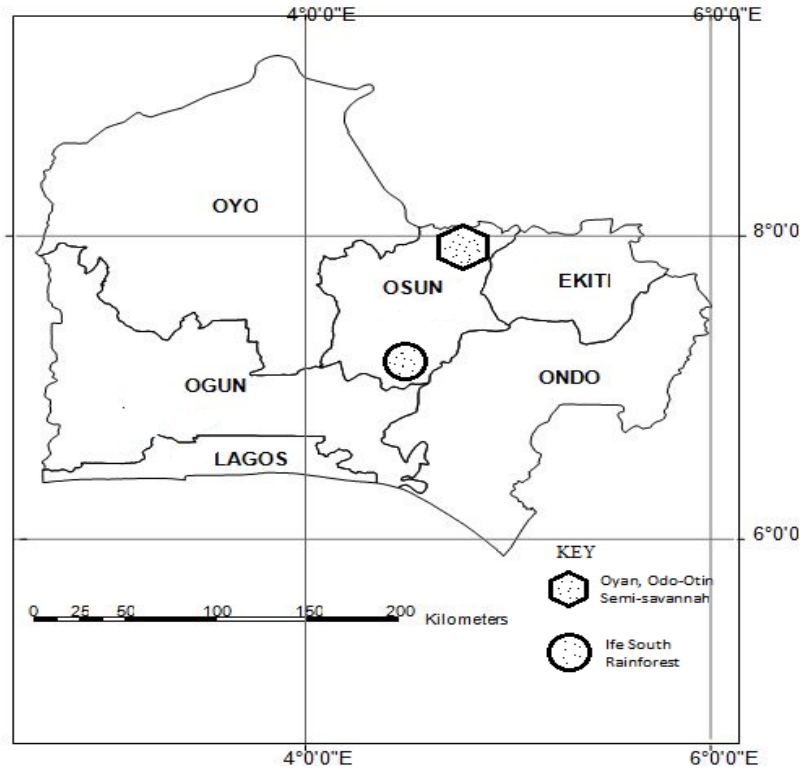

Figure 1. Map showing the locations of the two apiaries, Oyan,Odo-Otin and Ife South LGAs

December (dry season) and fifteen days in June (wet season). The camera was set to record activities in the time lapse of 5:40-7:20 am, 11:00 am-1:00 pm and 5:40-7:20 pm and information downloaded thereafter. We collected the meteorological data on sunset and sunrise, moonrise and moonset during each time we observed the workers' foraging time and duration. The foraging time was taken as the time a bee/bees took the first outbound flight from the hives and ending time was taken when all the bees that have left the hive returned to assemble for the night. All the time recorded for the start and end of foraging were local time for the study sites. Colony foraging duration was the length of time recorded from start to end of foraging, while the day length was calculated from meteorological data obtained.

Palynological analysis

Plant, Honey and Pollen combs sampling

Honey combs and pollen combs were sampled from each of the eight selected colonies. The surrounding vegetation within 3 kilometers radius of each apiary were sampled. Afterwards all the plant samples were pressed and brought to the University of Lagos Herbarium for identification. The identification of the plant specimens were accomplished to the family, genus and where possible to species levels. Those that could not be identified were regarded as unidentified.

Isolation of pollen grains from honey and pollen combs

Microscopic pollen analyses of each honey and pollen comb samples were done by using the methods given by the International Commission for Bee-Botany [33,27] and Laboratory work included acetolysis treatment of samples according to Erdtman [34] and Shubharani [35], microscopic analysis of prepared residues and preparation of reference pollen slides from plant specimens collected during sampling in the field. Two fields of view each were microscopically studied per sample.

Identification of recovered pollen grains was done with the aid of pollen atlases, and other published floral catalogues and journals such as Sowunmi [36]; Agwu and Akanbi [37]. Photomicrographs of some important pollen grains were taken with a Motic 2000 digital camera. (See Plate 1)

Statistical Analysis

Data collected from mellissopalynological analyses were analysed using descriptive statistical measures of frequency, classification of frequencies according to Louveaux et al. [27] and relative abundance. Inferential statistical techniques of sample t-test were used to evaluate significant differences between different relative abundance of pollen, foraging time and duration. Bee foraging plant species were identified and Jaccard (Cj) and Sorenson (Cs) Similarity Coefficients were used to compare the abundance of the foraging plant species in semi-savannah and rainforest ecological zones.

Results

We observed many varieties of honey bee foraging plants in both the semi-savannah and rainforest ecological zones in the tropics. In the rainforest, during the dry and wet seasons, the honey bees *Apis mellifera adansonii* were found to forage on 15 plant and 17 plant families respectively having a total of 28 plant species (Table 2). Jacard's (Cj) and Sorenson's (CS) similarity coefficient of 0.52 and 50% were obtained respectively, showing similarity between the abundances of the foraging plants during the wet and dry seasons (Tables 2

and 3). On the other hand 22 and 18 plant families having a total of 46 plant species (Table 1) were analysed as foraging plants in the semi-savannah ecological zone during the dry and wet seasons; Jacard's (Cj) and Sorenson's (CS) similarity coefficient of 0.46 and 51.2% were obtained respectively, showing similarity between the abundances of the foraging plants during the wet and dry seasons (Table 2). According to these analyses there is no similarity (Jacard's (Cj) and Sorenson's (CS) similarity coefficient of 0.37 and 45.9% respectively) between the abundances of foraging plants in the semi-savannah and rainforest zones in the tropics (Table 2).

The bees showed varying degree of preference for pollen types of different foraging plants. In both the rainforest and semi-savannah ecological zones, foraging worker bees showed low constancy for pollen from *Elaeis guineensis* Jacq. (Araceceae) and occasional frequency for pollen from *Alchornea cordifolia* Schum & Thonn

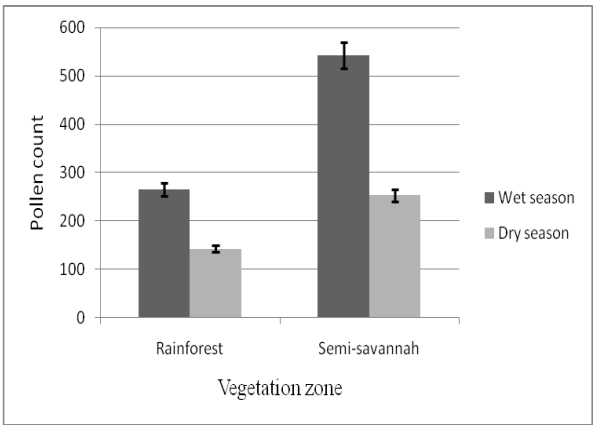

Figure 2. Comparison of pollen count in the honey and pollen comb samples in the semi-savannah and rainforest vegetation

(Euphorbiaceae). In the savannah, the bees show occasional frequency for pollen from *Sporobolus pyramidalis* P.Beauv. (Poaceae). In the rainforest, the bees occasionally frequent more pollens from *Syzygium guinensis* Willd Conocarpus eracta Linn. (Combretaceae), *Anacardium occidentale* Linn.(Anacardiaceae), *Amaranthus viridis* Linn. (Amaranthaceae), *Gomphreria celosioides* Mart (Amaranthaceae), *Albizia glaberrina* Schumach. & Thonn (Mimosoideae), *Manihot esculenta* Crantz, *Securinega virosa* Roxb. ex Willd and *Euphorbia hirta* Linn (Euphorbiaceae) (Table 1, Figure 3a, b and c). In the rainforest zone with a diverse and rich vegetation, the total pollen recovered during the wet season was 266, and during the dry season it decreased to 143 (Figure 2). Similarly, in the semi-savannah ecosystem, the pollen foraged during the wet season was 543 and during the dry season it decreased to 253 (Table 1 and Figure 2). The extensive human interference of this derived savannah had impacted its biodiversity, the rainforest, though equally impacted but was able to provide more diversed flora for the bees use. The reported importance of *Elaeis guinensis* in honey production by several workers in Nigeria is also exhibited in this study. This is reflected by the pollen of *Elaeis guinensis* (Table 1) with a record of 257 counts out of a total recovered pollen of 543 in the honey and pollen comb samples in the semi-savannah area. Its relative abundance of 47.33 % during the wet season assigns "very frequent" classification according to Louveaux et al. [27]. In the rainforest, *Elaeis guinensis* pollen recorded 96 out of a total of 266 recovered pollen from the honey and pollen comb samples. This pollen type also showed a "frequent" classification with its relative abundance of 36.09 % (Table 1) during same wet season. Apart from *E. guineensis*, another important pollen types recorded in this study were *Syzygium guineense* with a "frequent" classification in the rainforest and Euphorbiaceae pollen type with also "frequent" classification in the semi-savannah. Other pollen

Table 1. Pollen count, relative abundance and frequency class from honey comb samples during the wet season from rainforest and semi-savannah

Vegetation	Rainforest			Savannah		
Plant family	Pollen count	Relative abundance	Frequency class	Pollen count	Relative abundance	Frequency class
Asteraceae	10	3.76	Rare	26	4.78	Rare
Malvaceae	22	8.27	Occasional	7	1.28	Rare
Myrtaceae	55	20.68	Occasional	3	0.54	Rare
Sapotaceae	5	1.90	Rare	-	-	-
Mimosaceae	1	0.38	Rare	-	-	-
Euphorbiaceae	22	8.28	Occasional	113	20.81	Occasional
Poaceae	11	4.14	Rare	56	10.31	Occasional
Solanaceae	7	2.63	Rare	3	0.54	Rare
Amaranthaceae	2	0.76	Rare	8	1.47	Rare
Arecaceae	96	36.09	Low constancy	257	47.33	Low constancy
Fabaceae	1	0.38	Rare	-	-	-
Cyperaceae	1	0.38	Rare	-	-	-
Rutaceae	4	1.50	Rare	-	-	-
Meliaceae	1	0.38	Rare	-	-	-
Onagraceae	3	1.12	Rare	-	-	-
Rubiaceae	-	-	-	11	2.02	Rare
Myricaceae	-	-	-	1	0.18	Rare
Schrophulariaceae		-	-	1	0.18	Rare
Bignoneaceae	-	-	-	1	0.18	Rare
Sapindaceae	-	-	-	2	0.36	Rare
Portulaceae	-	-	-	1	0.18	Rare
Rubiaceae	-	-	-	11	2.02	Rare
Combretaceae	22	8.27	Occasional	3	0.55	Rare
Anacardiaceae	3	1.12	Rare	5	0.92	Rare
Steculiaceae	-	-	-	34	6.26	Occasional
POLLEN SUM	266	100		543	100	

(*Plant families identification using floral catalogues and journals* [36, 37]. *Frequency class is according to Louveaux et al. [27], Relative abundance (%)*

types in both the dry and wet seasons' recoveries recorded "rare" classification.

The pollen count and abundance from the samples in the wet and dry seasons in the rainforest ecosystem significantly reduced ($t = 3.630$, $df = 29$, $p = 0.001$) (Table 3, Figure 2). Likewise, in the semi-savannah ecosystems, the pollen count and abundance from wet to dry seasons reduced significantly ($t = 2.877$, $df = 27$, $p = 0.008$ ($p < 0.050$) (Table 3, Figure 2). The pollen counts in the honeys and pollen combs of the bee colonies

Table 2. Jaccard and Sorenson Similarity Coefficient of relative abundances of bee forage plant species

	Forest	**Savannah**	
Similarity coefficient	Dry/Wet	Dry/Wet	Savannah/Forest
Jaccard's similarity coefficient (C_j)	0.52	0.46	0.37
Sorenson's similarity coefficient (C_s)	50.0%	51.6%	45.9%
Result	Similar	Similar	Dissimilar

from the forest and the savannah were significantly different (t = 4.603, df = 57, P < 0.001 (Table 3).

The colonies were found to commence foraging early morning 07:02 ± 0:03AM and ended in the evening between 18:20 ± 0:00 to 18:56 ± 0:00PM. The individual bees were not observed for foraging time. The mean duration of foraging in the wet and dry season in the forest and savannah were not significantly different (p < 0.001). Though, the mean duration of day length was longer in the wet than dry seasons (Table 4).

vation was reported also by many authors in their works. Ayodele *et al* [38] recorded 49 melliferous plant species in the forest and 5 out of the 49 species were dominant in the derived savannah zones of southwestern Nigeria. Mbah and Amao [39] also recorded a total of 28 plant taxa visited by honey bees *Apis mellifera adansonii* in Zaria, in the savannah belt of Nigeria and 61 plant taxa were also visited by honey bees in Sudan savannah zone in northeast Nigeria.

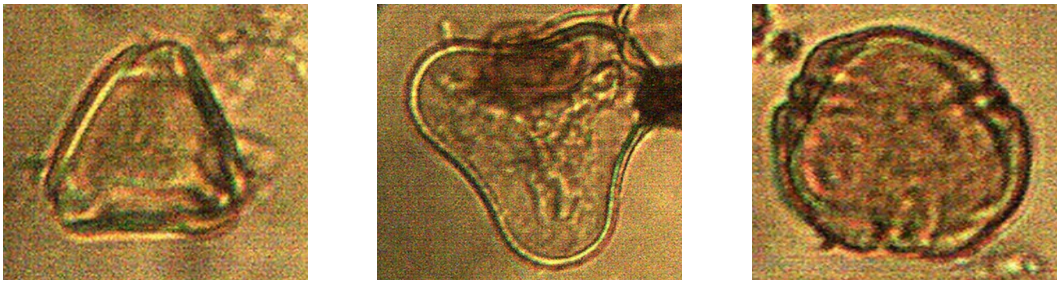

Figure 3:. Photomicrographs of pollens from

(a) *Syzygium guineense* **(b)** *Elaeis guineensis* **(c)** *Alchornea cordifolia* **(**Mag. X400)

Discussion

The wide range of pollen isolated from the honey and pollen comb samples from the two ecological zones, apparently, showed that the honey bees selected many plants for foraging among the different plants present in both the semi-savannah and rainforest ecosystems. This obser-

The very frequent and frequent classifications of *Elaeis guineensis* Jacq. (Araceceae) pollen type and rare classification of *Alchornea cordifolia* Schum & Thonn (Euphorbiaceae), Syzygium guineense Willd (Myrtaceae) and Asteraceae pollen types etc (Table 1) in the two ecological areas were at variance with the observations of

Table 3. t-test analysis of pollen counts in the honey samples from rainforest and semi-savannah.

	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Savannah	2.877	27	0.008	6.60321	1.8939	11.3125
Forest	3.630	29	0.001	6.91533	3.0191	10.8116
Forest/Savanna	4.603	57	0.000	6.76466	3.8216	9.7077

many authors in the savannah area in Nigeria. Fohouo et al.[26] and Mbah and Amao [39] recorded pollen types of *Tridax procumbens* , *Tectonia grandis* and Malvaceae as the most foraged. Ayansola and Davies [40] also recorded *Aspilia Africana, Chromolaena odorata, Manihot esculenta, Talinum triangulare and Amaranthus viridis* were frequently foraged taxa in the south-western Nigeria. In the Sudan Savanna zone in North-eastern Nigeria, Dukku [41] recorded *Azadiracta indica* (Meliaceae), *Tectona grandis* (Verbenaceae) and *Tridax procumbens* (Asteraceae) pollen types as mostly foraged plants species. With these different dominant pollen recorded by all these authors, it is clear that bees search for pollen among plants that are available in each ecological area as long as the pollen are good for them as food. Again, the result of Ige and Modupe [42] in the analysis of 20 honey samples from two different localities in two states in the North Central, Nigeria, a savannah zone, showed that *Elaeis guineensis* (Araceceae) and *Poaceae* were dominant in their honey samples, this is an indication that the pollen and/or nectar of these plant species were important bee foods. Our findings on bee forage preference further agreed with Abou-Shaara [16] conception that bees are plant specific in their foraging due to some resources present in their pollens and or nectaries. Similarly, the pollen preference of the honey bee foragers reflected the climate and vegetation of the hive areas. This finding was rightly stated by Aycan et al [43] that pollen preference of honey bee foragers reflected the flora of the area and its climate. Ige and Apo [44], Adeonipekun [45], Aina and Owonibi [46] remarked that high species diversity is characteristic of vegetation in the tropics, where they recovered 36, 43, and 45 plant taxa respectively from Nigerian honeys, pollen pellets and pollen load samples and this high species diversity of the vegetation in the tropics truly influenced the foraging preference of bees. In spite of these high species diversity, bees are still specific in their foraging.

Again, our finding that seasonal changes also influence bee foraging activities supported [47,48,49] who had earlier reported this in their field work. This was further confirmed by Tirado et al [50] who studied foraging activities of social insects and reported that their activities were influenced by not only climate but also seasonal changes, timing and location of food. The earlier works on timing of foraging by bees have indicated that higher activities started early in the morning to midday and decreased in the evening. For example, Alqarni [15] observed in the desert a higher number of forag-ers leaving their colonies at 8.00 am more than at 10.00 am, Joshi and Joshi [14], observed that honey bee workers started foraging by 6.17 am in the sub-tropics while Haftom

et al [51] recorded the highest numbers of honeybees commencing foraging at 10:30 - 11:30 am, with the least number recorded at 16:30 - 17:30 pm. From our findings, average starting time was 7.00 am in the rainforest and 7.03 am in the semi-savanna and ceasation time in both the rainforest and semi-savanna was 18.38 pm. The average duration of bee foraging activity was 11.36 h in both ecosystems. This again agreed with earlier works that bees foraging started early in the day and by evening; activities are brought to a halt. Of course, most plants open their flowers in the morning to afternoon during heavy sunlight and by evening some flowers are closed. It was possible to conclude that season, length and time of the day, sunset and sunrise and region had significant effect on pollen abundance and bee foraging.

Conclusion

The rainforest and semi-savannah ecosystems have rich and diverse bee foraging plants. West African honey bee, *Apis mellifera adansonii* foraged during the wet and dry seasons, and foraged many plant species. In the savannah, the honeybees most frequently visited *Elaeis guineensis* Jacq.(Araceceae) while in the rainforest, the honeybees frequently visited *Elaeis guineensis*. Jacq (Araceceae) and *Syzygium guinensis*. Willd. (Myrtaceae). Foraging activities of *Apis mellifera adansonii* colonies either in the savannah or forest or during the dry or wet seasons; started nearly at the same time of the day. Floral preference and foraging behaviour were regulated by floral availability, abundance and season, to an extent duration and time of the day. The discovered bee preference for some plants as pollen sources has further supported their significance in honey bee farming in Nigeria and also encourages and assures migrant beekeepers of the availability of good forage when moving from the forest to the savannah part of the country.

Table 4. Summary of 15 days observation of foraging time and duration during the dry in all colonies

Season	Apiary	Mean day length HR	Av. starting time AM (15 days)	Av. ending time PM(15 days)	Mean duration of foraging	Asymp. Sig.
Wet[a]	Rain Forest	12:31± 0:00	7:00 ± 0:02	18:56 ± 0:00	11:55 ± 0:01	0.000
Dry[b]	Rain Forest	11:42 ± 0:00	7:03 ± 0:03	18:20 ± 0:00	11:17 ± 0:03	(p<0.05)
Wet	Semi - savannah	12:32 ± 0:00	7:03 ± 0:03	18:56 ± 0:00	11:53 ± 0:03	0.000
Dry	Semi - savannah	11:42 ± 0:00	7:02 ± 0:03	18:20 ± 0:00	11:17 ± 0:03	(p<0.05)

Nijerya'nın Yağmur Ormanları ve Yarı-Savan Ekosistemlerindeki *Apis mellifera adansonii*'nin Nektarlı Bitki Seçimi, Tarlacı Dönemi ile Süresi ve Tercihindeki Mevsimsel Değişim

ÖZ

Apis mellifera adansonii kolonilerinin bitki seçimi, zamanı, süresi ve çiçek tercihi gibi tarlacı davranışları 2012-2014 yılları süresince kurak ve yağışlı mevsimlerde incelenmiştir. Koloni gözlemleri dönem boyunca sabah 06: 00-7: 30, 11:00-13:00 ve 18:00-19:30 saatleri arasında yapılırken tarlacı işçi arıların gözlemleri sırasında gün batımı ile gün doğumu, ay doğumu ile ay batımı arasındaki meteorolojik veriler toplanmıştır. Tarlacı davranışlarının başlangıcı ve bitişi için kaydedilen bütün zaman tanımları için çalışmaların yapıldığı alanın yerel saati kullanılmıştır. Koloni bölgelerinin 3 km yarıçapına yayılmış olan floral bitki türleri örneklenmiştir. Mellisopalinojik araştırmalar için tüm deneysel kolonilerden bal ve polen petekleri toplanmıştır. , savan ve tropik yağmur orman bölgeleri arasında nektarlı bitkiler bolluğu açısından fark olduğu Jacard'ın (Cj) ve Sorenson'ın (CS) sırasıyla %0.37 ve %45.9 benzerlik katsayı-

sı oranı ile belirtildiği şekilde gözlenmiştir. Arıların, tarlacı davranışlarının ortalama başlangıç ve bitiş saatleri sırasıyla 07:01 ve 18:20 arasında olup ve günlük ortalama tarlacı süresi 11:30saattir. Kurak ve yağışlı mevsimlerdeki tarlacılık zamanı ve uzunluğu önemli ölçüde farklı değildir (P<0.05).Polen analizleri, savan ekosistemindeki arıların orman ekosistemdekilerine göre daha fazla çiçeği ziyaret ettiğini göstermiştir. Savan ekosistemindekilerin toplam polen sayısı, kurak mevsimde 254 iken yağışlı mevsimde 543'tür (P<0.05). Hem orman hem de savan bölgesindeki koloniler, *Elaeis guineensis* Jacq.(Arecaceae)'i tercih etmişlerdir. Bu çalışmanın sonuçları, iki ekosistemin "arı bitkileri" hakkında bilgi vermenin yanı sıra gelişmiş arıcılık için bilinmesi gerekli olan arı davranışları hakkında arıcıları da bilgilendirmektedir.

Anahtar Kelimeler: Polen toplama, çiçek tercihi, nektar, *Apis mellifera adonsonii*

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MELLIFERA RESEARCH ARTICLE

Chemical Composition of Ecuadorian (Hymenoptera: Apidae: Meliponini) Commercial Pot-honeys: *Trigona fuscipennis* "abeja de tierra", *Melipona mimetica* "bermejo", and *Scaptotrigona ederi* "catiana

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ABSTRACT

The quality of fifteen commercial pot-honeys produced by "abeja de tierra" *Trigona fuscipennis*, "bermejo" *Melipona mimetica*, and "catiana" *Scaptotrigona ederi* stingless bees in Ecuador was evaluated for ash, free acidity, hydroxymethylfurfural, reducing sugars, sucrose and water contents. These pot-honeys were also described for their visual viscosity, color, smell, aroma, dominant taste and other physiological sensations in the mouth. Fifteen pot-honeys were purchased in El Oro, Loja and Manabí Ecuadorian provinces. Acceptance was done on six honeys with 40 assessors using a 10-cm unstructured line scale anchored with the expressions 'like' expressions. Sensory dominant taste, visual appearance, smell, and aroma (using the odor-aroma table for pot-honey) and other physiological sensations were described. Measurements of ash content were done by gravimetric method, free acidity by potentiometric method, hydroxymethylfurfural by spectrophotometric method, reducing sugars and sucrose by cuprimetric method, and moisture by the refractometric method.Pot-honey produced by Trigona is the most different from Apis mellifera with free acidity some 12-20 times higher than the maximum of 40 meq/kg, double water content of the maximum 20 g/100 g, and a third of the minimum 65 g/100g of reducing sugars. Pot-honey produced by Melipona and Scaptotrigona may fulfill Apis mellifera standards, with a slightly higher moisture up to 27.88 g/100 g and free acidity up to 76.77g/100 g, but lower contents of reducing sugars [50.75-63.38] g/100 g. Sucrose content of pot-honey produced by Trigona, Melipona and Scaptotrigona is lower than 5 g/100g as in the Apis mellifera honey standards. Smell and aroma were more "floral" for Melipona, "citrusy" for Trigona and "pollen" for Scaptotrigona pot-honey.Compositional and sensory data on pot-honey is a contribution to the database of the revised Ecuadorian honey standards NTE INEN 1572, and will eventually support the inclusion of standards in a new pot-honey norm.

Keywords: Ecuador, entomological origin, Meliponini, chemical analysis, pot-honey, quality standards, sensory descriptors

Introduction

Approximately 500 species of stingless bees belong to the Meliponini tribe [1], and live in tropical and subtropical regions [2]. These bees store honey in cerumen pots, therefore the term "pot-honey" was coined [3] to differentiate them from honey produced in beeswax combs by *Apis mellif-*

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era and and other *Apis* spp. In Latin America stingless bee keeping is known as meliponiculture, the origin of the term is uncertain, and could be linked to the *Melipona* genus or to the subfamily Meliponini. The traditional stingless beekeeping or meliponiculture should be protected to prevent its extinction [4], and paradoxically, stingless bees should be protected from stingless beekeepers for a sustainable instead of predatory practice. The decline of forest and plant species diversity, increase competition for food in large meliponaries [5], and reduce pot-honey yields. Therefore, the traditional practice needs input from current knowledge on stingless beekeeping and environmental protection, to pinpoint an ultimate philosophy "caring gentle bees to protect forests" [6]. As an indicator of the great biodiversity of stingles bees, 89 species of Meliponini are reported in the Southern region of Ecuador [7].

The medicinal use of honey or pollen produced in cerumen pots by eight taxa of Brazilian stingless bees was investigated in the zootherapy study of Costa-Neto [8]. These medicinal properties need to be demonstrated, and one approach is to study bioactive compounds such as flavonoids [9]. Honey alone or combined with conventional therapy was recently reviewed as a novel antioxidant [10]. The antioxidant activity of honey varies according to the entomological source of honey [11]. In the research on Ecuadorian pot-honey, a comprehensive biopharmaceutical approach was done in a *Scaptotrigona* mixture collected by Achuars in Morona Santiago province [12].

Although the oldest fossil of a bee in our planet is a stingless bee [13], and Precolumbian honey was produced only by stingless bees; pot-honey is not included in the honey regulations because they are currently devoted to *Apis mellifera* which was a species introduced after the discovery of America [14].

The first draft for a norm of honey produced by stingless bees was presented by Vit during the 1999 Annual Meeting of the International Honey Commission held in the European Center of Taste Science in Dijon, France, with scientific representatives of 18 countries [15]. Since the standards suggested by Guatemala, Mexico and Venezuela in 2004 [16], and the review done to set quality standards in 2006 [17], the proposal of a norm for pot-honeys of the world is now supported by new data, e.g. from Argentina [18], Australia [19], Bolivia [20], Brazil [21], Colombia [22], Guatemala [23] and Venezuela [24].

In this work, Ecuadorian pot-honeys produced by *Trigona fuscipennis* Friese, 1900. named "abeja de tierra", and *Melipona* (*Michmelia*) *mimetica* Cockerell, 1914 named "bermejo", and *Scaptotrigona ederi (*Schwarz, n.p.) named "catiana" were studied to contribute for the proposal of its inclusion in the Ecuadorian honey norm [25] either in the same table of standards for *Apis mellifera* –as suggested for the most abundant pot-honey in Venezuela produced by *Melipona favosa* known as "erica" [26], in the annex like in the Colombian regulations [27], or in a new norm for pot-honey.

Material and Methods

Pot-honeys

Fifteen pot-honeys were purchased from local stingless bee keepers or in markets from El Oro, Loja and Manabí Ecuadorian provinces, and kept frozen until analysis. Stingless bees were collected from the entrances of nests in logs or hives using isopropyl alcohol, dried and kept in plastic boxes before sending them to Dr. Silvia R.M. Pedro for

Table 1. Entomological origin of pot-honeys

Ethnic name of stingless bees	Scientific name of stingless bees
"abeja de tierra"	*Trigona fuscipennis*
"bermejo"	*Melipona mimetica*
"catiana" or "catana"	*Scaptotrigona ederi*

identification at Universidade de São Paulo, Ribeirão Preto, Brazil; and using the book on stingless bees from the South of Ecuador [7] for "abeja de tierra" that is referred to few species of *Geotrigona* spp. Duplicates of some bees are deposited in the Entomology Laboratory of Universidad Nacional de Loja, Ecuador, with Professor José Ramírez; Pontificia Universidad Católica de Ecuador, Quito, with Professor Clifford Keil; Kansas University, USA, with Professor Charles D. Michener†; and Smithsonian Tropical Research Institute, Panama, with Dr. David W. Roubik. Ethnic and scientific names of stingless bees producing the Ecuadorian pot-honey samples analyzed here are given in Table 1.

Chemical analyses

Only the honey produced in combs by *Apis mellifera* is considered for the ten quality standards in the Ecuadorian honey norm NTE INEN 1572 [25] and corresponding analytical methods: relative density and moisture, reducing sugars, sucrose, ratio fructose: glucose, free acidity, insoluble solids, ash, hydroxymethylfurfural (HMF), and diastase number.

Sensory analyses

Sensory analysis was done to describe the dominant taste [31] visual appearance, smell, and aroma, using the odor-aroma table for pot-honey [32]. Other physiological sensations were also observed. An acceptance test was done with 40 assessors for honeys of *Trigona, Melipona, Scaptotrigona*, two commercial *Apis mellifera* one amber and the other light amber using a 10-cm unstructured line scale anchored with the expressions 'like it a little' and 'like it a lot', in the left (1 cm) and right ends (9 cm), respectively. The acceptance scores were measured.

Statistical analyses

Chemical results and acceptance scores were statistically processed with SPSS [33] to compare means of *Trigona, Melipona* and *Scaptotrigona* pot-honeys with ANOVA, post-hoc Tukey test.

Results

Chemical analyses

Fifteen commercial pot-honeys were collected during field work in El Oro, Loja and Manabí Ecuadorian provinces, and analyzed for six chemical parameters currently done in routine analysis by the Ecuadorian sanitary authority ARCSA, namely ash, free acidity, hydroxymethylfurfural (HMF), moisture, reducing sugars and apparent sucrose. Raw data, averages and *Apis mellifera* Ecuadorian standards are given in Table 2. The following tendencies of pot-honey contrasted with the NTE INEN 1572 *Apis mellifera* honey were observed: 1. Moisture is generally higher in pot-honey [18.77-38.74] g/100g, compared to the *Apis mellifera* standard, maximum 20 g/100g. 2. Free acidity is variable, *Melipona* and *Scaptotrigona* pot-honeys contents of 25.23-76.77 meq/kg are more similar to the *Apis mellifera* standard, maximum 40 mg/kg; whereas *Trigona* has contents 12-20 times higher with a range of 497.03-810.01 meq/kg. 3. Reducing sugars are lower in pot-

Table 2. Chemical analysis of *Melipona*, *Geotrigona* and *Scaptotrigona* pot-honey, and *Apis mellifera* honey standards

Bee taxa	Number of pot-honey	Ash (g/100g)	Free acidity (meq/kg)	HMF (mg/kg)	Moisture (g/100 g)	Reducing sugars (g/100 g)	Apparent sucrose (g/100 g)
Trigona fuscipennis	n=5	0.12[c] (0.02) [0.10-0.14]	631.77[b] (108.54) [497.03-810.01]	41.48[c] (9.92) [31.40-60.03]	37.12[b] (1.52) [34.53-38.74]	23.97[a] (7.70) [16.21-33.90]	2.89[a] (1.00) [1.42-4.15]
Melipona mimetica	n=5	0.03[a] (0,01) [0.01-0.04]	49.02[a] (15.05) [38.92-76.77]	11.81[a] (12.91) [0.30-28.00]	22.27[a] (2.15) [18.77-24.89]	58.71[b] (4.56 [50.75-63.38]	2.01[a] (1.14) [0.99-4.01]
Scaptotrigona ederi	n=5	0.08[b] (0.04) [0.04-0.15]	40.95[a] (9.52) [25.23-48.93]	25.88[b] (10.88) [5.75-35.05]	21.97[a] (3.22) [19.43-27.88]	42.01[b] (5.82) [36.33-51.82]	2.66[a] (1.08) [1.35-4.34]
Apis mellifera	NTE INEN 1572	Max 0.5	Max 40	Max 50	Max 20	Min 65	Max 5

Averages ± (SD), [minimum-maximum] values are given. Different superscripts indicate significant difference in honey composition between the tree groups. $P < 0.05$.

honey (16.24-63.38 g/100 g) than the *Apis mellifera* standards, minimum 65 g/100 g. 4. Sucrose content of *Trigona, Melipona* and *Scaptotrigona* pot-honeys studied here is lower than the maximum 5 g/100 g permitted for *Apis mellifera* honey.

Sensory analyses

All the pot-honeys analyzed here were liquid, and few of them developed tiny crystals after freezing, causing a visual milky viscosity in three *Trigona* honeys. The color varied from light to dark amber. The smell varied in the bee, candy, caramel, menthol, fermented, floral, fruity notes. The aromas were similar with bee, citrusy, floral, lemon zest, fermented, fruity, menthol, pollen, and resinous. A *Trigona* honey had book glue off-odor and stable off-aroma. Dominat flavors are sweet for *Melipona* honeys and sour for *Trigona* honeys, *Scaptotrigona* honeys are more variable sweet, sour sweet, sour astringent and even bitter. Four honey samples –two of *Trigona* and two of Scaptotrigona– caused salivation while tasting. Floral for *Melipona* and citrusy for Trigona were the most frequent descriptors perceived in the smell and aroma; pollen was frequently perceived in the smell and aroma of Scaptotrigona.

The acceptance of the honeys varied as follows: *Apis mellifera* light amber 6.5 ± 2.8, *Apis mellifera* amber 5.1 ± 2.8, *Trigona* amber 3.9 ± 3.0, *Melipona* light amber 6.7 ± 2.5, *Scaptotrigona* light amber 5.8 ± 2.8. There were no significant differences between the acceptance in the five honey types tested here $P < 0.05$. The highest acceptances were assessed for the light amber amber honeys, both *Apis mellifera* and *Melipona*, and slightly lower *Scaptotrigona*. For a group of 16 over 40 assessors, the light amber *A. mellifera* was the best honey, while the *Melipona* honey was the best for 10 assessors, both rated with values

from 5.9 to 10.0; the *Scaptotrigona* honey was chosen as the best honey by 9/40 assessors who rated it with acceptances from 8.2 to 10.0.

Discussion

Although pot-honeys are major honeys in the forests –as stated by Dr. D.W. Roubik, they are still minor honeys in the market. Therefore, they need promotion, protection and development of their infant industry [34]. Educational initiatives from Australia [35] and pot-honey shows [36] do expand the knowledge of stingless bees, meliponiculture, pot-honey, sensory appeal, composition, and understanding of medicinal uses by the public.

Melipona stingless bee species build bigger cerumen pots to process their honey [37] and have higher honey yields. Therefore their honeys have been studied more frequently. The chemical composition of *Melipona* honeys varies according to the species. Average water contents (g/100 g) are 24.9 for *M. brachychaeta* and 24.1 for *M. grandis* from Bolivia [20], 28.02 *M. quinquefasciata* [38], 28.84 *M. scutellaris* [39] and 24.8 *M. subnitida* [40] from Brazil, 25.8 *M. compressipes*, 27.6 *M. eburnea*, and 24.8 *M. favosa* from Colombia [22], 17.32 for *M. beecheii*, 19.66 for *M. solani* and 20.37 for *M. aff. yucatanica* from Guatemala [41], and 28.0 *M. favosa* from Venezuela [42]; with a range of 17.32 to 28.84 g water/100 g. Moisture varied from 18.85 to 22.80 g/100 g for the Ecuadorian *Melipona* honeys, within the moisture range of honey from eleven *Melipona* species from Bolovia, Brazil, Colombia, Guatemala, and Venezuela [20,22,38,40,41,42]. Average free acidities (meq/kg) are 10.4 for *M. brachychaeta* and 16.0 for *M. grandis* from Bolivia [20], 28.02 for *M. quinquefasciata* [38], and 32.49 for *M. subnitida* [40] from Brazil, 23.23 for *M. beecheii*, 4.95 for *M. solani* and 10.59 for *M. aff. yucatanica* from Guatemala [41], and 51.7 for *M. favosa* from Venezuela [42]; with a range of 4.95 to 51.7 meq/kg. In our study with five *Melipona* honeys, the variation of free acidity from 38.92 to 76.77 meq/kg is within the free acidity range of honey produced by eight Melipona species from Bolivia, Brazil, Guatemala, and Venezuela [20, 38, 40, 41, 42].

Scaptotrigona mexicana honey from Guatemala has a free acidity of 12.68 meq/kg and water content of 18.74 g/100 g, 57.22 g reducing sugars/100 g, and 0.06 g apparent sucrose/100 g [41]; the water content for the Ecuadorian *Scaptotrigona* honey in Table 2 is also 18.74 g/100g, but the free acidity 40.1 meq/kg is higher than that found in Guatemala, and consequently the content of 42.25 g/100 g reducing sugars is lower than the minimum 65% of the *Apis mellifera* standard. The low sucrose has no problem because the standard establishes a maximum of 5%, perhaps a more refined limit could be suggested with a lower maximum value for sucrose of pot-honeys.

Dardón and Enríquez [41] reported a free acidity of 85.53 meq/kg and a water content of 32.09 g/100g for the underground honey produced by *Geotrigona acapulconis*; these were the highest acidity and moisture between the honeys produced by nine species of Meliponini in Guatemala. Also in Table 3, the Ecuadorian underground *Trigona* honey shows the highest free acidity and moisture. The fact that average of free acidity in the Ecuadorian *Trigona* is 609.33 meq/kg should be explained by the species and the interactions of the underground nest with the soil. Behavioral observations of the underground bees and their nests are needed to understand

Table 3. Suggested pot-honey standards for *Geotrigona*, *Melipona*, and *Scaptotrigona*

Bee taxa	Ash (mg/100g)	Free acidity (meq/kg)	HMF (mg/kg)	Moisture (g/100 g)	Reducing sugars (g/100 g)	Apparent sucrose (g/100 g)
Trigona	0.12 [0.10-0.14] Max. 0.5	631.77 [497.03-810.01] Max. 800	41.48 [31.40-60.03] Max. 60	37.12 [34.53-38.74] Max. 40	23.97 [16.21-33.90] Min. 16	2.89 [1.42-4.15] Max. 5
Melipona	0.03 [0.01-0.04] Max. 0.5	49.02 [38.92-76.77] Max. 50	11.81[a] [0.30-28.00] Max. 30	22.27 [18.77-24.89] Max. 25	56.71[b] [50.60-63.38] Min. 50	2.01 [0.99-4.01] Max. 5
Scaptotrigona	0.08 [0.04-0.15] Max. 0.5	40.95 [25.23-48.93] Max. 50	25.88[b] [5.75-35.05] Max. 40	21.79 [19.43-27.88] Max. 30	42.01 [36.33-51.82] Min. 35	2.66 [1.35-4.34] Max. 5
Apis mellifera Honey Type 1 INEN 1572	Max 0.5	Max 40	Max 50	Max 20	Min 65	Max 5

Averages and [minimum-maximum] values are given.

such a different pot-honey [43], and hypothesize the origin of such chemical array. This is a very thin honey with 37.06 g/100 g water content, almost double of the 20% maximum permitted in the Ecuadorian honey norm [25]. These elevated compositional values of water and acids, explain a decrease on reducing sugars to a 24.53 g/100 g average which is lower than the minimum of 65% established in the norm [25].

The statistical analysis from Table 2 show that honey produced by *Melipona* and *Scaptotrigona* are more similar between each other in free acidity, moisture, and reducing sugar contents than honey produced by *Trigona* in Ecuador. However apparent sucrose is not different in the three honey types. Similarly, these observations was done with Venezuelan *Melipona*, *Scaptotrigona* and *Trigona* honeys from Venezuela in 1998 [44], although pot-honey produced by *Trigona* are different. Quality standards needed by pot-honey were substantiated in 1999 [15] during the Annual Meeting of the International Honey Commission, and later proposed for Guatemala, Mexico, and Venezuela in 2004 [45], and later also for Brazil in 2006 [46] and 2013 [39]. The first official insert in the Annex of the Colombian honey norm ICONTEC [27] derived from the comprehensive review done in 2006 [16]. The proposal to expand the Ecuadorian honey norm NTE INEN 1572 [25] to other honey bee species, namely stingless honey bees was one reason for the current revision; during the third meeting the *M. favosa* model [26] was presented as an option for the crucial decisions to be taken, and the best option for further development of meliponiculture.

A provisional idea for pot-honey standards in Ecuador is given in Table 3, with concerns that 30-40 honey samples of each group should be analyzed for a sensible and solid database. Averages and [minimum-maximum] values from Table 2

are retained in Table 3 to visualize the proposal of quality standard for each parameter (free acidity, moisture, reducing sugars and apparent sucrose) for the three pot-honey groups based on their entomological origin *Trigona*, *Melipona* and *Scaptotrigona*. The column of apparent sucrose is highlighted in grey because the standard of a permitted maximum of 5 g/100 g remains the same for the honey produced by the three genera of Meliponini. Conservative proposals are made when the *Apis mellifera* honey standard is met by pot-honey. Compared to the free acidity maximum value 40 meq/kg in the *Apis mellifera* honey standards, reference values should be increased up to 800 for *Trigona* and 50 for *Melipona* and *Scaptotrigona* honey. The maximum moisture of 20% also needs to be increased up to 25% for *Melipona*, 30% for *Scaptotrigona* and 40% for the *Trigona*. On the other hand, the 65% minimum of reducing sugars requires a reduction to 16% for *Trigona*, 50% for *Melipona* and 35% for *Scaptotrigona*. This proposal is a trend that needs to be validated with more pot-honeys from Ecuador. This is not the first proposal, and therefore it is supported by research done in Brazil, Guatemala, Mexico, and Venezuela [45]. Ecuador is about to start a contribution in regulatory promotion of pot-honey. The final outcome would have consensual value by the Technical Committee TC-NTE INEN 1572 revising and updating the honey norm, with the agreement to create the pot-honey norm in 2016.

Sensory characteristics and defects of honey from *A. mellifera* were based on the wine sensory experience adapted to the perception of honey bee keepers and consumers by Gonnet and Vache (1984) [47]. A leading research with *Melipona quadrifasciata* by French and Brazilian scientists made the observations of her delicious thin and sour honeys [48] –always re-discovered by young melittologists and endlessly communicated in seminars, workshops and papers of pot-honey produced by stingless bees– as already stated by Schwarz since 1948 [49] for the widely relished honeys in tropical America before Columbus. An ayurvedic observation on dominant flavor of food to prepare balanced meals [50] was incorporated in early sensory approach of commercial honeys from Venezuela [32], where the sugary matrix does not always convey to the sweet perception collectively assigned to honey, even in botanical types with more than 65% reducing sugars, such as the bitter *Castanea* and *Arbutus unedo* European honeys. Comparing honey extracted from beeswax combs and cerumen pots, needs adaptations to keep the similarities and to insert differences. For instance, the fermentive off-odor is a defect for a matured *Apis mellifera* honey [47], but it is a feature in some types of pot-honeys [51], and therefore this descriptor was included as a family in the odor-aroma table [33] because Meliponini process their honey diversely.

Bee products have healing properties because they improve circulation, decrease inflammation and boost immune protection [52, 53]. Pot-honey has been studied for the antibacterial [54], antioxidant [10, 17] and anticancer activity [55]. A vast research is needed to demonstrate putative medicinal properties and the active components derived from stingless bees, their diet or perhaps originated with their interactive microbiota [56]. Discoveries such as the C-glycosides in honeys [9] are possible due to the great biodiversity of Meliponini [57].

In the Ecuadorian coast *Geotrigona, Trigona* and *Scaptotrigona* honeys are more abundant. According to meliponicultors the two *Melipona* species known as “bermejo” and “cananambo”

were easily kept in the past, some 10-20 years ago, but now feral nests of *Melipona* are scarce and these bees abandon the hive more easily than *Scaptotrigona* named "catiana", as if the bees are more sensitive to management and climate change. In the Ecuadorian Amazon rainy forest, *Melipona* honey is harvested by pot-honey hunters and sold by Kichwa and Shuar nationalities in native Indian fairs from Puyo, Pastaza province, Tena, Napo province and El Coca, Orellana province with a top price of almost 10 $/125 g, whereas in El Oro province the cost is 20 $/750 g. Ecuadorian Kichwas and Shuars, like Brazilian Enawene-Nawe [58] do not breed bees, but other Brazilian indigenous societies do, like the Guarani [59] and the Pankararé [60]. Native knowledge about stingless bees connect social groups with nature, in a sort of ecological cosmovision of ancestral awareness.

Government enterprises are needed for producers, possibly to subsidize the quality control procedures, to generate scientific research needed to demonstrate putative medicinal properties, and to open marketing channels. Ministries of Health, and of Agriculture and Livestock could support a strategical planning for ancestral knowledge embracing traditional stingless beekeeping and pot-honey-hunting in Ecuador, among other agricultural practices to participate in the Change of Productive Matrix as envisaged by the Knowledge Sharing Program (KSP) from South Korea [61] and the Good Living (originally from Kichwa language Sumak Kawsay, translated into Spanish as Buen Vivir). Ecuador will join the seminal initiative Route of Living Museums of Meliponini in the World, launched by Costa Rica and Venezuela in 2013 [37], with a leading role for the regulatory concepts, with the current systematic Prometeo Project location of stations for meliponaries and stingless bee nests (Vit, unpublished data). Besides their great biodiversity, the identified stingless bees keep changing their names [62], therefore a specialized entomologist is mandatory in any scientific team investigating pot-honey. The unifloral Italian honeys were carefully filed in a book [63] that could be imitated to illustrate the tropical meliponine honeys for a better knowledge of the public and the scientists. However, this year the melissopalynological analysis were excluded from the first revision of the Ecuadorian honey norm [64], as well as the Annex with reference values for pot-honey standards as in the Colombian honey norm reviewed in 2007 [27], only the scientific references were left to inform on pot-honey produced by stingless bees from the tribe Meliponini.

Conclusion

Pot-honeys produced by Ecuadorian *Trigona fuscipennis* "abeja de tierra", *Melipona mimetica* "bermejo" and *Scaptotrigona ederi* "catiana" where characterized, and suggested chemical quality standards were compared with those of *Apis mellifera* honey. Sensory analysis were useful to describe the diversity and also to assess the acceptance of pot-honey. Further data is needed to reduce a standard, as is the case for the *Melipona* honey standard of the State of Bahia, Brazil, with a lower HMF limit, up to 10 mg/kg [65].

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Ekvator (Hymenoptera: Apidae: Meliponini) ticari pot ballarının kimyasal bileşimleri: *Trigona fuscipennis* "abeja de tierra", *Melipona mimetica* "bermejo" ve *Scaptotrigona ederi* "catiana"

ÖZ

"Abeja de tierra" *Trigona fuscipennis*, "bermejo" *Melipona mimetica* ve "catiana" *Scaptotrigona ederi* iğnesiz arıları tarafından üretilen on beş ticari pot balının kalitesi, kül, serbest asitlik, hidroksimetilfurfural, indirgen şekerler, sukroz ve su kriterleri bazında değerlendirilmiştir. Bu ballar, görsel viskozite, renk, koku, aroma, hakim tat ve diğer organoleptik analizlere de tabi tutulmuştur. *Geotrigona* tarafından üretilen pot-bal, Apis mellifera ballarına kıyasla serbest asitlik derecesi maksimum değeri olan 40 meq / kg'dan 12-20 misli fazla, maksimum su içeriği olan 20 g / 100 g'lık değerin iki katı, ve maksimum 65 g/100g olan indirgenmiş şeker değerinin üçte biri değerlerine sahiptir.

Melipona ve *Scaptotrigona* tarafından üretilen pot-bal, *Apis mellifera* standartlarına yakın özelliktedir. Nem değerleri 27.88 g / 100 g , serbest asitlik 76.77 g/100 g ve indirgenmiş şekerler [36.33-55.38] g / 100 gram civarında tayin edilmiştir.

Anahtar Kelimeler: Ekvator, entomolojik köken, Meliponini, kimyasal analiz, pot-bal, kalite standartları, duyusal tanımlayıcılar

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MELLIFERA RESEARCH ARTICLE

Investigation of Pollinator Species of Order Hymenoptera in Kastamonu University Campus

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ABSTRACT

Pollination is defined as the transfer of male gametophyte, called pollen to the stigma's of the female organ of seeded plants . The pollination has vital importance for the formation of fruit and the development of fertile seeds. While some plants have the ability to self-pollination, the vast majority of plants require a carrier vector for pollination. In this study, it was aimed to determine the pollinator diversity in the campus area of Kastamonu University and to determine pollen diversity on pollinator vectors. For this purpose, a field study was carried out in the campus area in May and October 2016, the collected samples were identified and the pollen on them was determined. Among the samples collected in May as a result of the study, 7 species / subspecies [*Xylocopa violacea* (Linnaeus, 1758), *Apis mellifera anatolica* (Maa, 1953), *Ceratina sp.*, *Bombus terrestris* (Linnaeus, 1758), *Megachile sp.*, *Polistes dominula*, *Phygadeuon sp.*] Belonging to 4 families of the Hymenoptera family (Apidae, Ichnemonidae, Megachilidae, Vespidae). Five species/subspecies [*Ceratina* sp., *Sphecodes* sp., *Symmorphus* sp., *Apis mellifera anatolica* (Maa, 1953), *Bombus terrestris* (Linnaeus, 1758)] belonging to 3 family members of the Hymenoptera family (Apidae, Halictidae, Vespidae) . To determine pollen variety collected taxa were washed with alcohol and the pollen and spores slides prepared. Pollen grain of 40 taxa and spores of 6 taxa identified and amount of they counted from this slides.

Keywords: Pollination, Hymenoptera, pollen, entemogami

Introduction

Pollination, which is one of the conditions required for fruit and seed formation in flowering plants, is defined as the transfer of pollen grains from the male to the stigma of the female organ in different ways, primarily insects [1,2,3]. While some plants have the ability of self-pollination, the vast majority of plants require a carrier vector for pollination. As a carrier vector sometimes water and wind are used, while the vast majority of flowering plants are pollinating with animals. Mollusk, birds, mammals (bats, some apes, etc.) and insects are the most important pollinator vectors. Pollinators are a very important contributor to natural and agricultural ecosystems by providing both biological diversity and increasing yield and quality in crop production [3,4]. The vast majority of bees who act as pollinators in natural and cultural areas are social and solitary bees [4].

The Hymenoptera group is one of the most important insect order, including all of the most known insect as ants, bees, bugs, leaves and woody weevils. Hymenoptera is an insect group

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that contains at least 115000 defined species and shows a holometabol metamorphosis. They take their names because of they have two pairs of membranous wings. Of more than 250 thousands flowering plant species spreading around the world, it is known that about 20 thousand were visited by bees [5, 6]. The bees visit the flowers to collect nectar and pollen. They use nectar for source of carbohydrates and pollen is more for a source of protein [7].

They need carbohydrate, protein, fat, minerals, vitamins and water for development, growth, maintenance-nutrition and incubation activity. While flowers and secretory nectars are used for source of honey carbohydrate needs, pollen meets all the remaining nutritional needs of bees. While carbohydrates and water are sufficient for the survival of adult honey bees, larvae needs to pollen, which is the source of the proteins, lipids, minerals and vitamins needed for the growth and development of its [8,9].

According to Güler and Çağatay [10], the use of honey bees and bombus bees in organic farming and alternative production methods has been increased in recent years and some species belonging to the family Megachilidae have been found to be more effective in the pollination of some plants. As results of this studies *Creightonella* Cockerell 1908, *Chalicodoma* Lepeletier 1841, *Megachile* Latreille 1802, *Coelioxys* Latreille 1809, *Osmia* Panzer 1806, *Chelostoma* Latreille 1809, *Hoplitis* Klug 1807, *Heriades* Spinola 1808, *Anthocopa* Lepeletier 1825, *Archianthidium* Mavromoustakis 1939, *Paraanthidium* Friese 1898, *Anthidiellum* Cockerell, 1904, *Rhodanthidium* Isensee, 1927, *Pseudoanthidium* Friese 1898, *Icteranthidium* Michener 1948 and *Anthidium* Nurse 1902, bees species determined as pollinator.

About 65% of the world's crop production which including wheat, corn, rice pollinated with wind while 35% of agricultural production need to animal pollination [11]. It is stated that more than ¼ of the world's agricultural production depends on the pollinating functions of bees for better seed and fruit formation [12]. It is stated that the yield of many fruits and vegetables such as oranges, apples, peaches, apricot, plums, cherries, tomatoes, melons, squashes, grapes, olives and cucumbers which are estimated to be worth over 100 billion dollars worldwide is related to the pollination by bees [4].

It is estimated that more than 20,000 bee species with insects and vertebrates pollinator number on the earth over 400,000. Plants provide many awards for pollinators, and a special relationship between plant and pollinator is developed. It is stated that sometimes a special and dependent relationship is established between plant and pollinator, as in the case of the genus of *Yucca* (Agavaceae) and their pollinator moth species *Tegeticula* Zeller 1873, while many plant species are attracted to a large number of pollinators to make them become pollinated [4]. Kandori [13] determined that 45 insect species belongs to 5 order visited *Geranium thunbergii* Siebold ex Lindl. & Paxton flowers in the 2-year study and 11 of them were primer pollinators.

In this study, it was aimed to determine the species of Hymenoptera which is play a role as pollinator in the campus area of Kastamonu University, to determine the pollen type and amount of the pollinators.

Material and Methods

Collection of Pollinator Samples

In May and October 2016, a field study was carried out at Kastamonu University Kuzeykent campus in order to determine the types of pollinators. On the campus, flowering plants grown naturally and grown for landscaping were examined and insects seen on the flowers were catched and picked in 50 ml tubes. On the tubes, the date, collection location and label information of the host plant are written. Insects with falcon tubes were brought to the laboratory and kept in 10 ml of 70% alcohol.

Identification of Pollinator Species

The identification of the Hymenoptera specimens was carried out on specimens taken in 70% alcohol for pollen analysis and on samples which killed with ethyl acetate and prepared as museum material. The wing venation, mouthparts, compound eyes, antennae, legs, colors of the samples to be diagnosed were examined under the Leica APO S8 stereomicroscope Identification made according to previous literature [14, 15, 16, 17]

Determination of Pollen Variety and Amount

To determine pollen type and amount pollinator samples were shaken and the pollen they carried on them was passed through to the alcohol and the alcohol was transferred in to into 15 ml tubes than centrifuged for 10 min at 4000 rpm. After centrifugation, the top alcohol is removed and the bottom sediment is mixed with the vortex, then dropped onto the clean slides, the alcohol is slightly heated until it is evaporated. On dried sample, glycerin-gelatin with safranin stain was dropped and covered with coverslip. Determination of amount and variety of pollen was examined by Leica DM3000 Digital Imaging system after 1 day incubation of slides for staining. The pollen type and their amount on the specimens was determined by scanning the entire area of the slides and the literature on pollen morphology was used for identification of pollen type [18, 19, 20, 21].

Results

According to the study conducted in May and October 2016, 32 samples of Hymenoptera were collected and 9 taxa belonging to 9 genre of 5 families (Apidae, Halictidae, Ichnemonidae, Megachilidae, Vespidae) were determined (Figure 1). As a result of investigated slides, prepared from collected bee taxa, pollen of 40 taxa and spores of 6 fungi taxa identified and their amount is demonstrated in Table 1 and 2.

APIDAE

APIS Linnaeus, 1758

***Apis mellifera anatolica* Maa, 1953**

Samples of *A. melifera* bee collected on *Taraxacum officinalis* in May.

Pollen grain of *Acer* sp., Anthemidae, Boraginaceae, Caryophyllaceae, Cupressaceae, *Cyperus* sp., Graminaceae, Lamiaceae, *Lapsana* sp., *Morus* sp., *Pinus* sp., *Plantago* sp., Rosaceae sp., *Rumex* sp., *Salix* sp., *Senecio* sp., *Scirpus* sp., *Syringia* sp., *Taraxacum* sp., *Trapogon* sp., *Tussilago* sp. and *Urospermum* sp. taxa were identified and counted on collected *A. mellifera anatolica* speciemen in May. Fungi spores of *Alternaria* sp. and *Puccinia* sp. were also observed on the same pollinator.

Pollen grains of *Brassica* sp., *Lapsana* sp. and *Daucus* sp. taxa were observed from the specimens of *A. mellifera anatolica* collected in October and fungi spore belonging to *Puccinia* sp. was identified (Figure 2, Table 1 and 2)

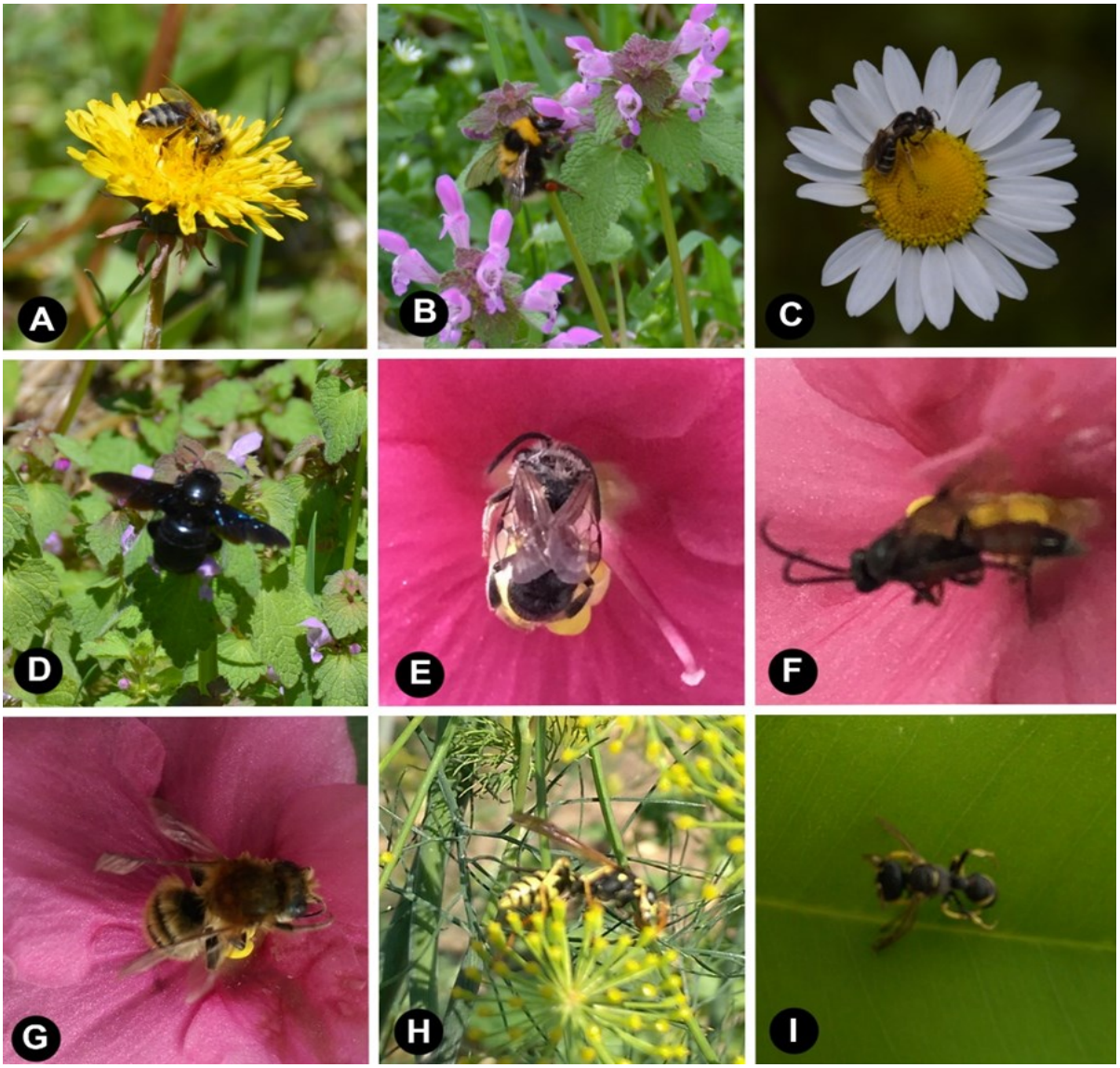


Figure 1. Identified bee pollinator collected from Kastamonu University campus. **A;** *Apis mellifera anatolica,* **B;** *Bombus terrestris,* **C;** *Ceratina sp.*, **D;** *Xylocopa violacea,* **E;** *Sphecodes sp.*, **F;** *Phygadeuon sp.*, **G;** *Megachile sp.*, **H;** *Polistes dominula,* **I;** *Symmorphus sp.* (Photo: Talip Çeter)

BOMBUS Latreille, 1802

***Bombus terrestris* (Linnaeus, 1758)**

B. terrestris specimens were collected from *Lamium* sp. in May, and from *Trifolium* sp. in October.

Pollen of Cupressaceae, *Lamium* sp., Liliaceae, Pinaceae, *Populus* sp., Rosaceae and *Syringia* sp. taxa were observed from the specimens of *B. terrestris* collected in May. The pollen of *Lactuca* sp., *Trifolium* sp. and spores of *Pleospora* taxa were counted from the specimens collected in October (Figure 3, Table 1).

***CERATINA* Latreille, 1802**

Ceratina sp.

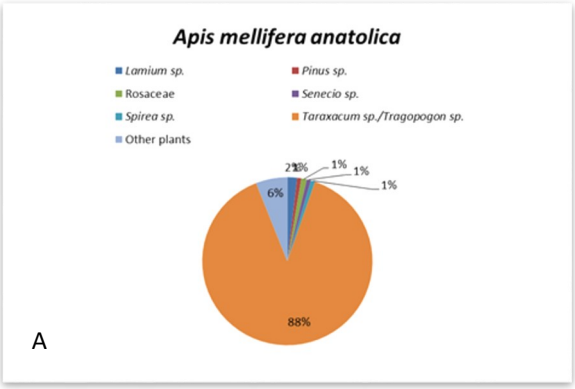
Pollen of *Lamium* sp., *Taraxacum* sp., *Anthemis* sp. and spores of *Alternaria* were observed from specimens of *Ceratina sp.* collected in May, while *Brassica* sp., *Lapsana* sp., *Plantago* sp. pollen were observed on specimen collected in October (Figure 4, Table 1 and 2).

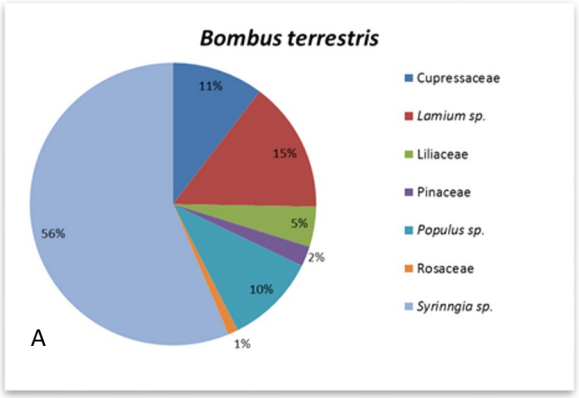
Table 1. Pollen types and amount of plant taxa observed on pollinator bee taxa collected from Kastamonu University campus.

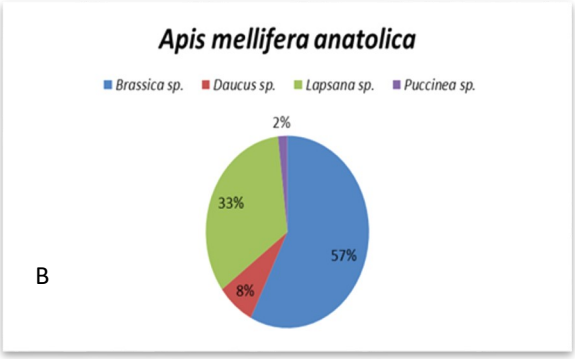
	May 2016							**October 2016**				
	Xylocopa violacea	*Ceratina* sp.	*Megachile* sp.	*Polistes dominula*	*Phygadeuon* sp.	*Apis mellifera anatolica*	*Bombus terrestris*	*Ceratina* sp.	*Sphecodes* sp.	*Symmorphus* sp.	*Apis mellifera anatolica*	*Bombus terrestris*
Aesculus sp.	2											
Acer sp.						3						
Alnus sp.	1											
Anthemidae			10			5						
Betula sp.	4											
Brassicaceae										1		
Brassica sp.								5			31	
Boraginaceae						7						
Caryophyllaceae						3						
Chenopodiaceae										4		
Cupressaceae	15		5		6	13	9					
Cyperus sp.						5						
Daucus sp.											4	
Echium sp.	3		4									
Fabaceae										2		
Graminaceae	7		6		3	9						
Lactuca sp.												5
Lamium sp.	280	89	75			35	13					
Lapsana sp.						8		63			18	
Liliaceae	2				1		4					
Malva sp.									4			
Morus sp.						7						
Pinaceae							2					
Pinus sp.	9			1		15						
Plantago sp.	3					6		1	1			
Populus sp.	8						9					
Quercus sp.	65											
Rosaceae	120					20	1					
Rumex sp.				2		4						
Salix sp.	3				1	10						
Senecio sp.					9	15						
Scirpus sp.						4						
Spirea sp.	40					15						
Syringa sp.	7					11	49					
Taraxacum /Tragopogon sp.	20	2			3	1687						
Tilia sp.	1											
Trifolium sp.												65
Tussilago sp.						5						
Urospermum sp.						2						

Table 2. Fungi spore types and amount observed on pollinator bee taxa collected from Kastamonu University campus.

	May 2016						October 2016			
	Xylocopa violacea)Linnaeus, 1758)	*Ceratina sp.*	*Megachile sp.*	*Phygadeuon sp.*	*Apis mellifera anatolica*	*Bombus terrestris*	*Sphecodes sp.*	*Symmorphus sp.*	*Apis mellifera anatolica*	*Bombus terrestris*
Alternaria sp.	6	1		1	8			1		
Drechslera sp.							1			
Epicoccum sp.			1							
Pleospora sp.	3		1							1
Puccinea sp.					11				1	
Venturia sp.	2									







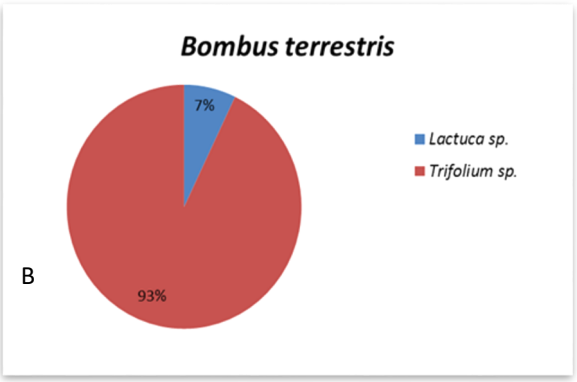
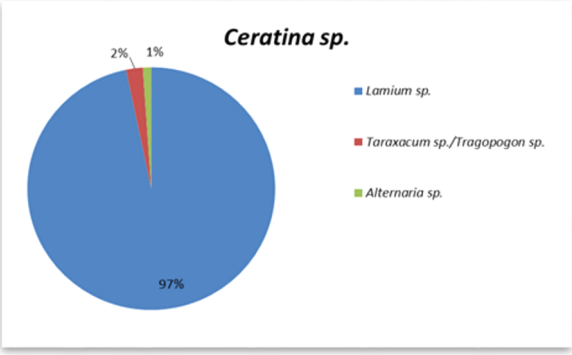


Figure 2. Pie graphs of pollen observed on *A. mellifera anatolica*. **A;** May, **B;** October.

Figure 3. Pie graphs of pollen observed on *B.terrestris*. **A;** May, **B;** October.



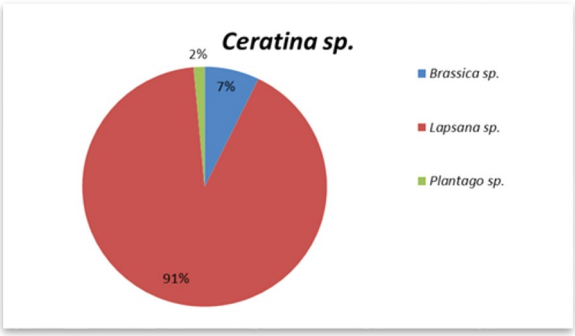


Figure 4. Pie graphs of pollen observed on *Ceratina sp.* **A;** May, **B;** October.

XYLOCOPA Latreille, 1802

***Xylocopa violacea* (Linnaeus, 1758)**

The specimen of *X. violacea* were collected from *Lamium* sp.

Pollen grain of *Populus* sp., *Quercus* sp., *Pinus* sp., Liliaceae, *Pinus* sp., *Plantago* sp., *Alnus* sp., *Echium* sp., Graminaceae, Cupressaceae, *Lamium* sp. Rosaceae, *Salix* sp., *Spirea* sp., *Syringia* sp., *Taraxacum* sp. and *Tilia* s p. plant taxa and spores of *Alternaria* sp., *Pleospora* sp. and *Venturia* sp fungi taxa were observed from the specimens collected in May (Figure 5, Table 1 and 2). In the field study of October, the specimen of this pollinator was not collected.

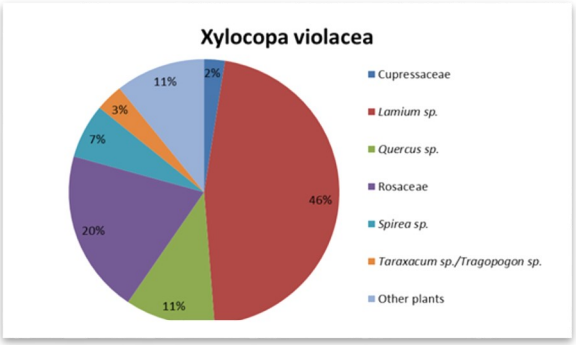


Figure 5. Pie graph of pollen observed on *X. violacea* in May

HALICTIDAE

***SPHECODES* Latreille, 1805**

Sphecodes sp.

In the field study conducted in May, the example of the pollinator was not collected. Pollen grains of *Malva sp.* and *Plantago sp.* and spores of *Drechslera* were identified from the *Sphecodes sp.* specimens collected in October (Figure 6, Table 1 and 2).

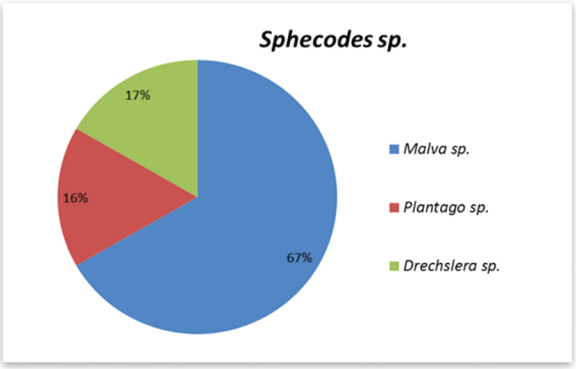


Figure 6. Pie graph of pollen observed on *Sphecodes sp.* in October.

ICHNEMONIDAE

PHYGADEUON Gravenhorst, 1829

Phygadeuon sp.

Pollen of Cupressaceae, Graminaceae, Liliaceae, *Salix* sp., *Senecio* sp. and *Taraxacum* sp. taxa and spores of *Alternaria* were identified and counted from the specimens of *Phygadeuon* sp. collected in May, (Figure 7, Table 1 and 2). In the field study conducted in October, the specimens of this pollinator were not collected.

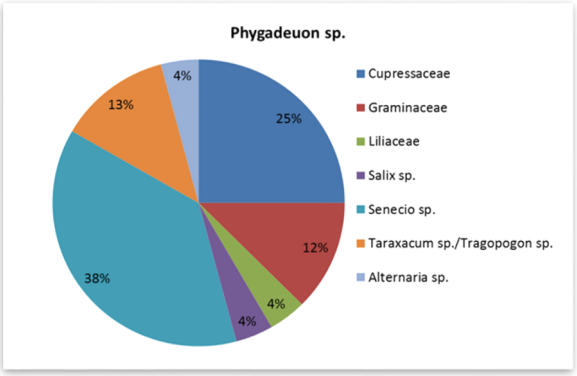


Figure 7. Pie graph of pollen and spores observed on *Phygadeuon sp.* in May

MEGACHILIDAE

MEGACHILE Latreille, 1802

***Megachile* sp.**

Pollen grains of *Echium* sp., Graminaceae, Lamiaceae, Anthemidae, Cupressaceae and fungi spores of *Pleospora* sp. and *Epicoccum* sp. were identified and counted from the specimen of *Megachile sp.*, collected in May, (Figure 8, Table 1 and 2). In the field study conducted in October, the specimen of this pollinator was not collected.

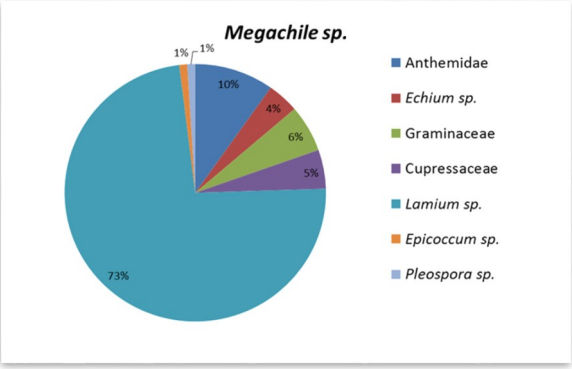


Figure 8. Pie graph of pollen observed on *Megachile sp.*in May.

VESPIDAE

***POLISTES* Latreille, 1802**

***Polistes dominula* (Christ, 1791)**

Pollen grains of *Rumex* sp., and *Pinus sp.* were observed from the specimen of *P. dominula* collected in May (Figure 9, Table 1 and 2). In the field study conducted in October, the specimen of this pollinator was not collected.

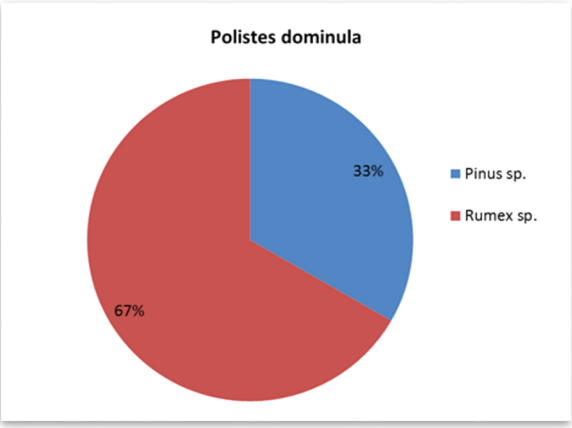


Figure 9. Pie graph of pollen observed on *Polistes dominula* in May.

***SYMMORPHUS* Wesmael, 1836**

Symmorphus sp.

In the field study conducted in May, the specimen of this pollinator was not collected. Pollen grains of *Brassica* sp., Chenopodiaceae, Fabaceae and spores of *Alternaria* sp. were observed from the specimen of *Symmorphus* sp. collected in October (Figure 10, Table 1 and 2).

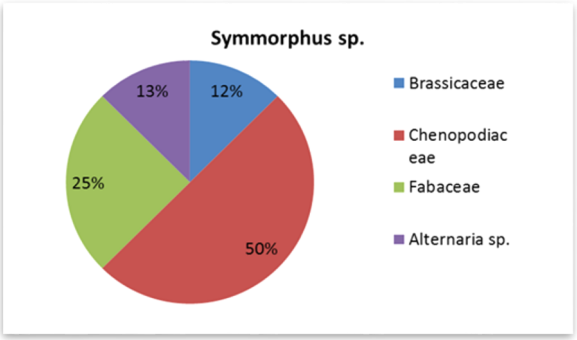


Figure 10. Pie graph of pollen observed on *Symmorphus* sp. in October.

Discussion

As a result of this study carried out to determine Hymenoptera species visited flowers of wild and cultivated plants in Kastamonu University campus, 9 taxa belonging to 5 families of the Hymenoptera group were identified. 40 different plant taxa and 6 different fungal spores were identified and counted as results of investigations of slides prepared by washing the bees specimens with 70 % alcohol.

Honey bee has a type of leg called collector leg. Special collecting organs are found in the legs and abdomens of pollen carriers and worker bees. Hairs present in the body of bees play an important role in pollination. It is stated that a honey bee can visit 50-1000 flowers at a distance of 1-3 km during a visit between 30 minutes and 4 hours. Meteorological factors such as rainfall, wind speed and temperature are said to be very effective in pollination activities of honey bees. It is stated that Honeybees rarely fly at temperatures below 13 °C or at wind speed over 24-32 km/h [4]. Honeybees are considered to be a very important primer pollinator not only because of the production of honey and honey wax, but also they pollinate more than 100 commercially important agricultural plants and numerous wild plants [22, 23, 24].

In our study, on *A. melifera anatolica* sample collected in May, fungal spores of 2 taxa and pollen of 22 taxa were identified among them 90% belonged to *Taraxacum* sp./ *Tragopogon* sp. On the samples collected in October, the amount of pollen and number of taxa were less as compared to May. From specimen collected in October pollen of *Brassica sp., Lapsana* sp., *Daucus* sp. and fungi spores *Puccini* sp. were observed. This difference, as mentioned in the above studies, suggests that the decrease in number of plants during the pollination period and the negative effect on the decreasing temperature value in October, when the number of plants in the pollina-

tion period is high and the weather conditions are favorable in May. It reveals that honey bears play a pollinating role in both seasons, although at different levels.

It is stated that, only about 15% of over 100 species of plants which produce the world's food sources are pollinated by domesticated bees and 80% are pollinated by wild bees and other pollinators [2, 25]. For this reason, wild bees that are outside honey bees are of great importance for pollination. One of the most important of these wild bee species is bumble bees. Bombus bees are very valuable pollinator insects that play a role in the pollination of many plants of great importance to humanity and are a wide spreading area in the world. These bees, which have a fairly flashy, attractive and colorful appearance, are generally more bulky and fuzzy, stronger and more spoiled than the honeybees (*A. mellifera*). Thanks to their long tongues, they can visit the deep-scented flowers and work in low temperatures, under bad weather conditions and in low light to pollinate the flowers. At the same time they can pollinate 6-8 times more flowers per minute than honey honeys [26].

In the study, pollen of 7 taxa were found on *B. terrestris* species in May. In October, pollen of 2 taxa were observed. In month of May, 56% of total pollen belongs to *Syringia* sp. and 93 % of total pollen observed in October belongs to *Trifolium* sp. The pollen found on *B. terrestris* mostly belongs to the tubular or papillionide type flowers.

Most *Ceratina* members are solitary while some are living socially. Species which belong to the genus *Ceratina* are generally small in size (2-3 mm), so they are thought to be very less effective in pollination [27]. Inouye [28] stated that small-sized bees are not important particularly for pollinating large flowers.

In this study, pollen of 2 taxa and fungal spore of 1 taxa were identified from the specimen of *Ceratina* sp. in May and pollen of 3 taxa were identified In October. The observed pollen was found to be 97% belong to *Lamium* sp. in the month of May While 91% belong to *Lapsana* sp. in the month of October. All of these Pollen belongs to herbaceous plants.

Xylocopa violaceae, known as violet carpenter bees, is a common species of Europe. They usually make their nests in the dead woods. The adults hibernate in the tunnels they open into the trees and usually wake up in April-May from their hibernation. Female bees place their eggs together pollen balls in separate sections of nests. Due to their large sizes, they are very effective in pollination. They fly quickly around the trees, especially around the upper branches, carrying out pollina-

tion in the flowers around them [27]. There are a number of studies emphasize these bees as an important pollinators of Fabaceae and Lamiaceae species with asymmetric flowers [29, 30]. In our study, pollen of 18 taxa and 3 fungal spores were identified on the *X. violacea* species in May. The identified pollen mostly belongs to the Lamiaceae. This taxa is followed by Rosaceae, *Quercus* sp., *Spirae* sp. taxa respectively.

Halictidae species are common in almost all over the world. Species are generally dark colored with metallic color differences. All species of the Halictidae family are fed with pollen and have an important role in pollination [15]. Pollen belonging to *Malva* sp. and *Plantago* sp. taxa and 1 fungi taxa were observed on the *Sphecodes* sp. specimens collected during the October. In the field study conducted in May, the specimen of this pollinator was not collected.

Species from Vespidae family, such as *Vespa orientalis* L., *Vespula vulgaris* L., *Vespula germanica* (Fabricius), *V. rufa* (L.) (Vespinae), *Polistes associus* Kohl, *P. bischoffi* (Weyrauch), *P. gallicus* L., *P. dominulus* Christ), *P. nimpha* (Christ) (Polistinae) and some Eumeninae species were found visiting the flowers of fruit trees. *P. dominula* species are fed with nectar and pollen of flowers to meet their energy needs. Their contribution to pollination is very limited because they do not have the ability to retain pollen grains of body hair [27]. In this study, pollens of *Rumex* sp. and *Pinus* sp. were detected. It is estimated that these pollen grains were infected from the *Pinus* pollen found in the atmosphere at the time of the bee harvest, or from the *Rumex* plant that they visited for hunting.

Phygadeuon sp. are the insects that live as parasites on other insects and hold an important place in the biological struggle. In this study, pollen of 6 taxa and 1 fungus spore were found on sample collected in May.

Megachilidae is the second largest bee family in the world with over 4000 species defined [31, 32]. These solitary bees are ecologically and economically important for pollination of both natural and cultivated plants. For example, *Megachile rotundata* plays a leading role in the pollination of alfalfa plants worldwide [31, 33, 34]. In our work, pollen of 5 taxa and 2 fungal spores were identified on the Megachile sp. species collected in May. Pollen of 3 taxa were observed on the species *Symmorphus sp.* collected in October.

As a result, the Apidae family appears to be the most effective family in Hymenoptera order in terms of pollination. Vespidae family members were found to be the least effective families in pollination according to pollen numbers they carried.

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Kastamonu Üniversitesi Kampüs Alanındaki Tozlaştırıcı Hymenoptera Takımına Ait Türlerin İncelenmesi

ÖZ

Tozlaşma, tohumlu bitkilerde polen olarak adlandırılan erkek gametofitin dişi organın stigması üzerine taşınması olarak tanımlanmaktadır. Tozlaşma meyve oluşumu ve verimli tohumların gelişmesi için hayati öneme sahiptir. Bazı bitkiler kendi kendini dölleme yeteneğine sahipken bitkilerin büyük çoğunluğu tozlaşma için taşıyıcı bir vektöre ihtiyaç duymaktadır. Bu çalışmada Kastamonu Üniversitesi kampüs alanında tozlaştırıcı çeşitliliğinin saptanması ve tozlaştırıcı vektörlerin üzerindeki polen çeşitliliğinin belirlenmesi amaçlanmıştır. Bu amaçla 2016 Mayıs ve Ekim aylarında kampüs alanında arazi çalışması gerçekleştirilmiş, toplanan örnekler teşhis edilmiş ve üzerinde taşıdıkları polenler belirlenmiştir. Çalışma sonucunda Mayıs ayında toplanan örnekler arasında Hymenoptera takımının 4 familyasına (Apidae, Ichnemonidae, Megachilidae, Vespidae) ait 7 tür/alttür (*Xylocopa violacea* (Linnaeus, 1758), *Apis mellifera anatolica* Maa, 1953, *Ceratina sp.*, *Bombus terrestris* (Linnaeus, 1758), *Megachile sp.*, *Polistes dominula*, *Phygadeuon sp.*) tespit edilmiştir. Ekim ayında toplanan örnekler arasında ise Hymenoptera takımının 3 familyasına (Apidae, Halictidae, Vespidae) ait 5 tür/alttür (*Ceratina sp.*, *Sphecodes sp.*, *Symmorphus sp.*, *Apis mellifera anatolica* Maa, 1953, *Bombus terrestris* (Linnaeus, 1758)) tespit edilmiştir. Toplanan bu taksonların alkolle yıkandıktan sonra yapılan incelemelerinde 40 taksona ait polene ve 6 taksona ait mantar sporuna rastlanmıştır.

Anahtar Kelimeler: Tozlaşma, Hymenoptera, polen, entemogami

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MELLIFERA RESEARCH ARTICLE

Anticytotoxic and Antimutagenic Effects of Propolis on Human Lymphocytes In Vitro

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ABSTRACT

Propolis is a natural product which is collected from different plants by honeybees to provide antiseptic environment for colonies. It has been used as a therapeutical agent in alternative medicine since ancient times. The aim of this study was to investigate antimutagenic and anticytotoxic effects of propolis extract from Turkey's Hakkari region against aflatoxin B1 (AFB1) on human lymphocytes *in vitro*. Chemical content of propolis extract which was determined by Gas Chromatography–Mass Spectrometry (GC-MS) and it was observed to contain high amounts of flavonoids. The mutagenicity test results showed that AFB_1 caused DNA damages and increased sister chromatid exchange (SCE) frequency. Propolis reduced SCE frequency and showed strong antimutagenic effect against AFB1 on human lymphocytes. In addition, Cytotoxic and anticytotoxic effect of propolis was examined by LDH (lactate dehydrogenase) leakage test. Consequently, our findings showed that propolis had strong anticytotoxic and antigenotoxic properties against aflatoxin B_1.

Keywords: Aflatoxin B1, Propolis; GC/MS analysis, Sister Chromatid Exchange (SCE) Test, LDH (lactate dehydrogenase) leakage assay

Introduction

Propolis, a resinous material collected by honeybees from plant exudates, has recently aroused the interest of scientists for the study of its constituents and biological activities [1]. It has been used since ancient times by people in alternative medicine for its antibacterial, antiviral, antifungal, cytotoxic, antioxidant and many other properties [2, 3, 4, 5, 6]. Propolis contains a variety of substances including phenolic compounds such as flavonoids, aromatic acids and their derivatives, esters, alcohols and trace elements [7,8]. To date, over 500 chemical components have been defined in the chemical structure of propolis. Its chemical content differs according to the season and the region in which it is collected [9].

Aspergillus species, producing aflatoxins,

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can cause contamination of many foods including oilseeds, groundnuts, maize, pistachios, hazelnuts, wheat, barley, soya, rice and dried fruits [10]. Aflatoxin contamination of the food and food products are mainly caused by improper storage and transportation conditions [11]. Aflatoxins are highly toxic, mutagenic, teratogenic and carcinogenic compounds and for this reason they are considered as threat for human health [12]. Aflatoxins can cause serious health problems such as chronic and acute aflatoxicosis, cirrohosis and hepatic cancer [13]. AFB1 is the most potent carcinogenic agent among aflatoxins. Previous studies indicated that AFB1 is mutagenic in many test systems like chromosomal aberrations, micronuclei, sister chromatid exchange , unscheduled DNA synthesis and chromosomal strand [14, 15, 16].

It has been known for a long time that some substances exhibit mutagenic effect on genomic material in living organisms. Several quantitative tests are used to show such genotoxic effects. They are used in studies conducted both in vitro and in vivo. One of the most frequently used system among these is sister chromatid exchange (SCE) test. Known to be the part exchange between sister chromatids during metaphase without altering the chromosome morphology, SCE test is a fast, easily-applicable, low-cost, sensitive and reliable test system [17, 18, 19, 20]. The test developed by Taylor et al. (1957) [21] is still confidentially used with some modifications. SCE test, which is utilized in the determination of the toxic dosages of mutagens and carcinogens that damage live genomic material, and the investigation of the mutagenic or antimutagenic traits of substances to be used for the prevention of cytogenetic damage, is a highly important test due to being used for the sensitive and quantitative analysis of genetic damage [22, 23].

In this study, we aimed to determine the genotoxic and cytotoxic effects of propolis on human lymphocyte *in vitro* using SCE assay and lactate dehydrogenase (LDH) leakage assay. Additionally, significant compounds that form propolis and their relative amounts were determined by using GC-MS.

Materials and Methods

Preparation of propolis sample

The propolis sample that used in this study was collected from Hakkari region of Turkey. The sample was hardened in a freezer and ground in a handy grinder. Then one hundred grams of sample was dissolved in 300 ml of 96% ethanol. This mixture was incubated for two weeks at 30 °C in a tightly closed dark colored bottle. After two weeks, the supernatant was filtered twice with Whatman No. 4 and No.1 filter paper, respectively. The final solution was diluted in 1:10 ratio (w/v) with etanol (96%). A portion of this final solution was evaporated to obtain completely dry sample. About 5 mg of dry substance was mixed with 75 µl of dry pyridine and 50 µl bis (trimethylsilyl) trifluoroacetamide (BSTFA) heated at 80 °C for 20 min and then the final supernatant was analyzed by GC-MS.

GC-MS analysis

GC-MS analysis of ethanol extract of propolis were performed using a GC 6890N from Agilent (Palo Alto, CA, USA) coupled with mass detector (MS5973, Agilent) fitted with a DB-5 MS capillary column (30 m x 0.25mm and 0.25 µm of film thickness). The column oven temperature was initially held at 50°C for 1 min, then programmed to rise to 150 °C at a rate of 10 °C/min and held for 2 min. Finally, temperature was increased to

280 with 20 °C/min. heating ramp and kept at 280 °C for 30 min. Helium was used as the carrier gas at a flow rate of 0.7 mL/min.

Sister Chromatid Exchange (SCE) Test

Heparin, at a ratio of 1/10, was added to the 1 ml peripheral blood samples of donors. The blood samples were added to the 5 ml chromosome medium B supplemented with 6 µg/ml 5′ -bromo-2′ -deoxyuridine in sterile conditions. The cultures were incubated at 37 °C for 72 hours. 0.06 µg/ml colchicine was added at 2 h before the harvesting of the culture. The SCE tests were performed as described by Perry and Evans (1975) [24]; Evans (1984) [25]; Perry and Thompson (1984) [26]; but with some modifications [27]. Then, slides were stained with %5 Giemsa (pH = 6.8) prepared in Sorensen buffer solution, for 20–25 min; washed in distilled water; dried at room temperature. The slides were stained with Giemsa according to the method of Perry and Wolff (1974) [28]; Speit and Haupter (1985) [29], with some modifications Yüzbaşıoğlu et al., (2006) [27].

In order to determine the genotoxic and mutagenic effects of heat shock stress, peripheral blood samples taken from the volunteer donors were transplanted as 13 drops (0.5 mL) into chromosome media that were heparinized at a 1/10 ratio in sterile cabins (Labormed). To determine SCEs, the cells were incubated for 72 h at 37 ± 0.5 °C by adding fresh 10 µg/mL 5-bromo-2-deoxyuridine solution (Sigma, CAS number: 59-14-3) into culture tubes at the beginning of the incubation. At the end of 72 h, the length of culture time employed, cells were precipitated by centrifugation for 10 min at 1200 rpm and then the supernatant was removed. The precipitate was homogenized, a warmed (37 °C) hypotonic solution (0.4% KCl) was added, and the cells were treated at 37 °C for 20 min. At the end of the period, the suspension was precipitated by centrifuging for 10 min at 1200 rpm and the supernatant was removed. After the addition of cold fixative (1:3 glacial acetic acid and methanol), the cells were held at room temperature for 15 min and centrifugation was repeated three times so that the cell pellet in the tube was homogenized. Cell pellet was dropped onto a cold slide from a height of 25 cm. After the slides dried under room temperature, they were stained with a 5% Giemsa stain prepared in a Sorensen buffer and covered with Entellan. To investigate SCEs, the fluorescence plus Giemsa method, developed by Speit and Haupter (1985) [29] was modified and used.

Cytotoxicity assay

Lymphocyte cells were seeded into 96-well plates at a density of $1x10^4$ cells/mL. After 24 h of seeding, cells were treated with different concentrations of propolis or media alone as a control. The cytotoxicity of propolis on cultured human lymphocytes was also assayed at 48 h by using following method.

LDH (lactate dehydrogenase) leakage assay

LDH leakage assay was carried out with a LDH-cytotoxicity assay kit (Cayman Chemical Company) according to the manufacturer's protocol. In brief, 10^4 to 10^5 cells/well were seeded in 96-well plates and exposed to different concentrations of propolis (0–20 µg/mL) for 24 h. At the end of exposure, the 96-well plate was centrifuged at 400 × g for 5 min to settle the propolis present in the solution. Next, 100 µL of supernatant was transferred to a well of a 96-well plate that already contained 100 µL of reaction mixture from a Bio-Vision kit and was incubated for 30 min at room temperature. After incubation, the absorbance of

the solution was measured at 490 nm using a microplate reader (Synergy-HT, BioTek, Winooski, VT, USA). LDH levels in the medium versus the cells were quantified and compared with the control values according to the instructions of the kit.

Statistical analysis

Statistical analysis was performed using SPSS® software (version 18.0, SPSS, Chicago, IL, USA). Statistical analysis was performed using one-way analysis of variance (ANOVA). The experimental values were expressed as the mean±standard error (SE). The statistical significances and comparisons between different groups were evaluated with Duncan's test. Dose-response relationships were determined from the correlation coefficients. P< 0.05 was considered as the level of significance.

Results and Discussion

Mutagenicity Tests

In the present study, different concentrations of propolis were performed with the sister chromatid exchange (SCE) and LDH tests which widely used as a short term test system. In SCEs test, positive control (AFB_1) used significantly increased the SCEs frequencies on peripheral lymphocytes when compared with the control as seen in Table 1. Such an increase was found to be statistically significant ($p < 0.05$). It was observed that treatment group with different concentrations (P-1: 5µg/ml; P-2: 10µg/ml; P-3: 20µg/ml) of propolis together with AFB_1 (5 µM) decreased the SCEs frequencies compared with intoxicated with AFB_1.

In smilar to our results, it was reported frequencies of SCEs in peripheral lymphocytes was significantly increased by the direct-acting mutagen AFB1 compared with controls [30]. Similarly, Türkez and Yousef (2009) [31] found that treatment with propolis provide antigenotoxic effects by AFB1 at different degree. Lima et al. (2005) [32] demonstrated the antimutagenic activity of propolis against DNA damage induced by 1,2-dimethylhydrazine in rat colon cells by using comet test. According to recent studies, the toxicity of AFB1 is mainly due to lipid peroxide and

Table 1. The frequencies of SCEs in human lymphocytes exposure to AFB_1 and propolis.

Groups	Metaphase	Range of SCEs	SCEs/Cell	SCEs/Cell ± S.E
Control	60	3-7	328	3,28±0,11[a]
AFB_1(5 µM)	60	3-10	519	5,19±0,54[d]
Propolis (10 µg/ml)	60	2-15	348	3,48±0,15[a]
AFB_1+P-1 (5 µg/ml)	60	3-11	469	4,70±0,07[c]
AFB_1+P-2 (10 µg/ml)	60	3-10	395	3,96±0,06[b]
AFB_1+P-3 (20 µg/ml)	60	2-12	340	3,40±0,15[a]

[a] $p < 0.05$ compared with control.

[b] $p < 0.05$ compared with control.

[c] $p < 0.05$ compared with control.

[d] $p < 0.05$ compared with AFB_1 (5 µM) group.

oxidative damage which causes different types of cellular damage, including DNA breaks [33, 34].

Cytotoxic Effect

Lactate dehydrogenase (LDH) leakage tests showed that propolis doesn't have cytotoxic effect but have strong anticytotoxic effect against AFB_1. Especially, the highest concentration (20 µg/ml) was determined as the effective concentration. LDH leakage test, which is a cell membrane damage test and the indicator of cytotoxic damage, showed that propolis did not damage the cell. In addition, propolis has prevented it against the cytotoxic effect of AFB_1, and reduced LDH enzymes activities.

Many studies and research groups have confirmed that propolis possesses anticancer activity [35]. Hasan et al. (2015) [36] showed that propolis (from Makassar region) exhibited anticytotoxic effect in Michigan Cancer Foundation-7 breast cancer cell line. Milosevic-Dordevic et al. (2015) [37] demonstrated that the tested ethanolic extracts of propolis exhibited antimutagenic effect on human peripheral blood lymphocytes and anticancer activity on breast cancer cell line (MDA-MB-23).

Determination of Chemical Composition

The chemical composition of propolis sample, which was collected from Turkey (Hakkari) was determined by GC–MS.

GC-MS analysis of propolis indicated that it contains different concentrations of compounds that belong to fatty acids and their esters, flavonoids, hydrocarbons, carboxylic acids and their esters, ketones and monoterpenes groups (Table 2). The following compounds were identified in high ratios in propolis sample; ethyl oleate (6.90%), tectochrysin (6.25%), pinocembrin(6.11%), pinostrobin chalcone (5.72%). The level of ethyl oleate (% 6.90) belong to fatty acids and their esters had the highest concentration. In addition, flavonoids had high level in propolis. These findings are in agreement with different researchers. It has been reported that propolis from Turkey-Artvin region, containıng flavonoids and ethyl oleate as domi-

Table 2: Chemical composition of the propolis from Turkey-Hakkari

Compound Groups	Compounds	% of Total Ion Current
Fatty acids and their esters	Palmitic acid	0.26
	Palmitic acid,ethyl ester	0.77
	Ethyl Oleate	6.90
	Total	**7.93**
Flavonoids	Pinostrobin chalcone	5.72
	Pinocembrin	6.11
	Tectochrysin	6.25
	Chrysin	1.98
	Total	**20.06**
Hydrocarbons	Eicosane	0.13
	Heneicosane	0.36
	9-Tricosene, (Z)-	0.33
	Docosane	1.80
	Nonadecane	1.00
	17-Pentatriacontene	2.44
	(Z)-14-Methyl-8-hexadecen-1-ol	0.24
	(Z)-13-Methyl-11-pentadecen-1-ol acetate	0.60
	Total	**6.90**
Carboxylic acids and their esters	Pentadecanoic acid, ethyl ester	0.29
	Total	**0.29**
Ketones	2-Heptadecanone	1.09
	2-Nonadecanone	2.79
	Total	**3.88**
Monoterpenes	Δ^3-Carene	0.04
	α-Pinene	0.03
	Total	**0.07**

nant, had antibacterial activity against Enterecoccus faecalis [38]. Likewise, many studies have shown that flavonoids were the main propolis components [39,40,41,42].

Flavonoids, one of the main groups of phenolic compounds, are the key compounds for determination of propolis quality. It is well known that the flavonoid concentration will affect the biological activity of propolis [3,43]. Most of the flavonoids, such as acacetin, chrysin, galangin, naringenin, and pinocembrin are important metabolites that activate the antioxidant system and use of in vitro experimental systems has shown that they also possess antioxidant, anti-inflammatory, antiallergic, antiviral, and anticarcinogenic, properties [44]. In addition, Liu et al. (2008) [45] showed that pinocembrin increased neuronal viability, decreased lactate dehydrogenase release, inhibited the production of NO and ROS, increased glutathione levels, and down-regulated the expression of neuronal NO synthase (nNOS) and iNOS in primary cortical neurons subjected to oxygen–glucose deprivation/reoxygenation (OGD/R). Besides, pinocembrin is also able to regulate mitochondrial function and apoptosis [46]. Previous investigations have demonstrated that pinostrobin chalcone has various pharmacological activities, antioxidant activity, and neuroprotective effects [47]. Tectochrysin has previously been reported to have inhibitory effect on cell growth of colon cancer cells (SW480, HCT116) [48]. That is why propolis, due to the high amount of flavonoids content, may have prevented the mutagenic effect of AFB1 causing DNA damage, therefore preventing the sister chromatid exchange in DNA. Also, not having a cytotoxic effect, propolis exhibited a strong anticytotoxic effect by protecting the cell against the toxic effect of AFB1. This anticytotoxic effect may be based on the compounds of flavonoids and other chemical group in its content.

It was also revealed in the studies that secondary metabolites in the propolis content such as ketone and terpene have a protective effect. Some ketones and terpenes have biological activities such as strong antimicrobial and low cytotoxic activities [49]. In addition, the enormous structural diversity presented by this class of natural products ensures a broad range of biological properties ranging from anti-cancer and anti-malarial activities to tumor promotion and ion-channel binding [50].

In more detailed studies to be conducted in future, important active substances in the propolis content can be purified, and it can be identified what substance(s) cause(s) the anticytotoxic and antimutagenic effects. Hence, Hakkari propolis having strong anticytotoxic and antimutagenic effects can be used as an alternative drug in the cancer researches. The anticytotoxic and antimutagenic effects of Hakkari propolis were revealed in this study. More detailed studies are planned in future to investigate the antioxidant and anticancer effects of propolis.

Propolisin Antisitotoksik ve Antimutajenik Etkilerinin İnsan Lenfositlerinde *İn Vitro* Olarak Belirlenmesi

ÖZ

Propolis, koloniler için antiseptik bir çevre oluşturmak amacıyla bal arıları tarafından farklı bitkilerden toplanan, reçinemsi doğal bir üründür. Antik çağlardan beri alternatif tıpta terapötik bir ajan olarak kullanılmaktadır. Bu çalışmanın amacı Hakkari, Türkiye bölgesinden toplanan propolis ekstraktının AFBı'e (aflatoxin Bı) karşı insan lenfosit hücrel-

erinde *in vitro* olarak antisitotoksik ve antigenotoksik etkilerinin araştırılmasıdır. Propolisin kimyasal içeriği GC-MS (Gaz kromatografi – Kütle spektrometre) ile belirlenmiş ve yüksek oranda flavonoid içerdiği belirlenmiştir. Mutajenite test sonuçları AFB_1'in kardeş kromatid değişimi (SCE) frekansını arttırdığını ve DNA hasarlarına neden olduğunu göstermiştir. Propolis ise *in vitro* ortamda AFB1'e karşı insan lenfositlerinde güçlü antimutajenik etki göstermiş ve SCE frekansını azaltmıştır. Buna ek olarak propolis'in sitotoksik ve antisitotoksik etkisi LDH (Laktat dehidrogenaz) salınım testi ile belirlenmiştir. Testler sonucunda propolisin aflatoksin B_1'e karşı antisitotoksik ve antigenotoksik etkiye sahip olduğu görülmüştür.

Anahtar Kelimeler: Aflatoksin B1, Propolis, GC/MS analizi, Kardeş Kromatid Değişimi (SCE) Testi, LDH (laktat dehidrogenaz) salınım testi

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MELLIFERA RESEARCH ARTICLE

Halictus Latreille (Halictidae: Apoidea: Hymenoptera) Fauna of Hacettepe University Beytepe Campus (Ankara)

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ABSTRACT

The genus *Halictus* Latreille, 1804 belongs to the family Halictidae. It contains over 300 species in the world and 35 of these species are distributed in Turkey. Faunistic studies on this genus are limited in Turkey and new local studies of the taxa are needed to be established. In the study, 205 individuals collected from Hacettepe University Beytepe Campus were identified as 12 species and among them *H. grossellus* Ebmer, 1978 was the new record for Ankara province. The diagnostic key for females of *Halictus* species which were distributed in the field is given in this study. Also, it was found that species of the family Asteracea were the most frequently visited plants by *Halictus* species in this area.

Keywords: Halictidae, pollinator, fauna, phenology, diagnostic key, Turkey

Introduction

The family Halictidae (Apoidea: Hymenoptera) is represented with 72 genera and nearly 3500 species in the world [1]. According to Michener [2] this family can be divided into four subfamilies which are Halictinae, Nomiodinae, Nomiinae, and Rophitinae. Among, Halictinae is one of the very large and nearly cosmopolitan subfamily [3].

Genus *Halictus* Latreille, 1804 is one of the most common genera of Halictinae and it was divided into 12 subgenera by Pesenko [4, 5]. The genus contains almost 90 species in the Palaearctic Region [1] and 35 of these species belonging to 8 subgenera are distributed in Turkey [6]. *Halictus* species are pollinators or at least visitors of economically important plant species such as *Helianthus annuus* L. [7], *Medicago sativa* L. [8], *Eriobotrya japonica* Lindl. [9], *Malus* sp. [10], *Capsicum annuum* L. [11], and *Prunus avium* L. [12]. They are also visitors and may be potential pollinators of natural vegetation such as the members of Asteraceae, Boraginaceae, Fabaceae, Lamiaceae, Ranunculaceae, and Salicaceae [8, 13, 14]. The unique features of *Halictus* species are coming from not because of their great help in this pollination service [15], but also from their social plas-

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ticity [16].

Although some recent faunistic studies [6, 14] on the genus *Halictus* of Turkey had been reported before, new local studies of the taxa are needed to be established. Since there were only few studies [17, 18] that had mainly focused on the halictid fauna of Ankara province, our study aimed to make contribution to this issue.

Materials and Methods

In this study, we used the collection material deposited in the Apoidea collection of Morphometry Laboratory of Hacettepe University's Department of Biology in Ankara, Turkey. From this collection, we only focused on the specimens that have been collected by various researchers with pan traps (yellow, blue and white) and sweep net between 2005 and 2013 from Beytepe campus of Hacettepe University, Ankara (39° 52' 16" N, 32° 44' 11" E; 1050 m). That area is nearly 100 km^2 and two sides of the campus were also delimited by small valley which sometimes poses weak stream. Area is surrounded by artificially planted *Pinus nigra* J.F. Arnold woods, and open areas between woods display typical step vegetation. The campus flora contains 510 taxa, which 65 of them endemic, belonging to 57 family and also 145 cultured plants [19].

Identification of the bee species was made by according to works of Pesenko *et al.* [8], Pesenko [20], Dikmen and Aytekin [14] and Dikmen *et al.* [6].

Results

After the examination of 205 specimens, 12 *Halictus* species were determined from Beytepe Campus. *H. resurgens* and *H. patellatus* are the most abundant species and the other species are the rarest ones, except *H. pentheri* and *H. maculatus*, in that area (Table 1). Females were found between April and September whereas male ones had been observed between July and September (Table 2).

Table 1. Numbers of female and male specimens for each species in the collection.

Species	Females	Males	Total
H. patellatus Morawitz, 1874	39	4	43
H. luganicus Blüthgen, 1936	4	1	5
H. brunnescens (Eversmann, 1852)	1	6	7
H. quadricinctus (Fabricius, 1776)	-	1	1
H. cochleariatarsis (Dours, 1872)	2	-	2
H. resurgens Nurse, 1903	90	24	112
H. grossellus Ebmer, 1978	-	1	1
H. pentheri Blüthgen, 1923	12	1	13
H. sajoi Blüthgen, 1923	3	-	3
H. tetrazonianellus Strand, 1909	4	-	4
H. asperulus Pérez, 1895	1	-	1
H. maculatus Smith, 1848	11	-	11
Total	167	38	205

Table 2. Female and male specimens were collected by months.

Species	April	May	June	July	August	September
H. patellatus	-	+ ♀	+ ♀	+ ♀,♂	+ ♀	-
H. luganicus	-	-	-	+ ♀,♂	-	-
H. brunnescens	-	-	-	+ ♀,♂	+ ♂	-
H. quadricinctus	-	-	-	-	+ ♂	-
H. cochlearitarsis	+ ♀	+ ♀	-	-	-	-
H. resurgens	+ ♀	+ ♀	+ ♀	+ ♀,♂	+ ♀,♂	-
H. grossellus	-	-	-	+ ♂	-	-
H. pentheri	-	+ ♀	+ ♀	+ ♀	-	+ ♀,♂
H. sajoi	-	+ ♀	+ ♀	+ ♀	-	-
H. tetrazonianellus	-	+ ♀	-	+ ♀	-	-
H. asperulus	-	-	+ ♀	-	-	-
H. maculatus	-	+ ♀	+ ♀	+ ♀	-	-

Figure 1. A. Mesoscutum of *H. sexcinctus* (female), B. Mesoscutum of *H. brunnescens* (female); Metapostnotum and metanotum of **C.** *H. tetrazonianellus* (female) **D.** *H. asperulus* (female) **E.** *H. pentheri* (female) (Photo: Burcu DAŞER ÖZGİŞİ)

Diagnostic Key for *Halictus* Species (Female)

1- Mesoscutum densely and in general regularly punctured (Fig. 1.A) 2

1'- Mesoscutum very sparsely and irregularly punctured (Fig. 1.B) ..... *H. brunnescens*

2- Apical parts of metatibia and metatarsus light brown to yellow ..... *H. patellatus*

2'- All legs completely dark 3

3- All posterior hair bands of terga complete (Fig. 2.B) 4

3'- T1-2 or all posterior hair bands of terga interrupted medially (Fig. 2.C-D) 5

4- T1 with brownish and less appressed furca-like hairs proximally (Fig. 2.A) .....
..... *H. cochleariatarsis*

4'- T1 without furca-like hairs proximally ..... *H. resurgens*

5- All posterior hair bands of terga broadly interrupted (Fig. 2.D) ..... *H. maculatus*

5'- T4-5 posterior hair bands complete and T3 sometimes interrupted or narrowed medially 6

6- Posterior marginal fields of terga hyaline (yellowish); posterior hair bands of terga coarse.....
..... *H. luganicus*

6'- Posterior marginal fields of terga dark; posterior hair bands of terga relatively fine in structure 7

7- Metapostnotum triangular (Fig. 1.C) ..... *H. tetrazonianellus*

7'- Metapostnotum crescent-shaped (Fig. 1.D-E) 8

8- Metapostnotum as long as or a little longer than metanotum (Fig. 1.D) ..... *H. asperulus*

8'- Metapostnotum barely 1,5 times longer than metanotum (Fig. 1.E) 9

9- Lateral surface of propodeum sparsely punctured, slightly shiny ..... *H. sajoi*

9'- Lateral surface of propodeum densely punctured, dull ..... *H. pentheri*

Discussion

Even though current faunistic study on the genus *Halictus* of Turkey were reported [6, 14], local faunistic studies on this genus are limited in Turkey. Because of the data deficiency in this topic, it is important to make contribution on the distribution of the local bee populations in understanding the potential pollinator bee diversity of Turkey. One of the first faunistic studies on this genus was reported 28 taxa and 12 of them recorded in Ankara province [21] whereas the latest faunistic study on this genus was reported 35 taxa and Ankara province showed the highest species diversity with, 18 records (Table 3) [6, 14]. In this

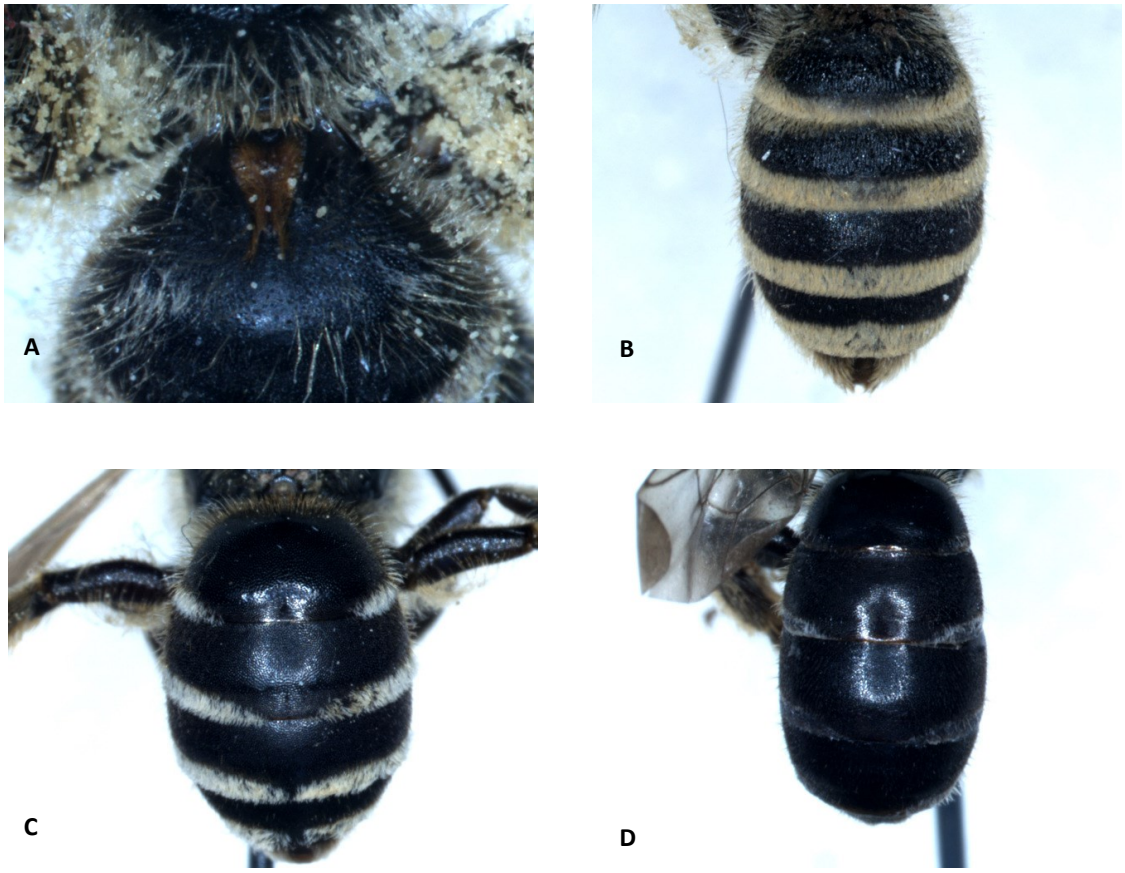

Figure 2. A. Furca-like hairs, *H. cochleariatarsis* (female). Abdominal terga of **B.** *H. cochleariatarsis* (female) **C.** *H. tetrazonianellus* (female) **D.** *H. maculatus* (female) (Photo: Burcu DAŞER ÖZGİŞİ)

study, we determined 12 *Halictus* species from Beytepe, Ankara and *H. grossellus* reported for the first time from Ankara. According to studies which were done so far indicate that *H. asperulus*, *H. brunnescens*, *H. cochleariatarsis*, *H. quadricinctus*, *H. maculatus*, *H. patellatus*, *H. pentheri*, *H. sajoi*, *H. tetrazonianellus*, and *H. resurgens* can be found in every region of Turkey and *H. luganicus* is distributed in Western part of Turkey [14]. *H. grossellus* was recorded just from Southeastern Turkey before [14]. Because of that our study suggested its distribution area to be broader than previously known.

According to our data from collected materials, although density is low, earliest female specimens of *Halictus* can be seen in April whereas earliest males can be seen in June. Female specimens between May and July are much denser than August and September. On the other hand, male's density is highest in August. Our observations confirm the data reported by the authors [8, 22, 23]. Also, our observation for the flower-visits of *Halictus* species suggested that species of the family Asteraceae were the most frequently visited plants.

Table 3. *Halictus* species are found in Turkey. "+" demonstrates the presence and "-" demonstrates the absence of relevant species. All data mentioned here according to Dikmen *et al.* [6].

	Ankara	Beytepe
H. adjikenticus	-	-
H. aegypticola	-	-
H. alfkenellus	+	-
H. asperulus	+	+
H. berlandi	-	-
H. beytueschebapensis	-	-
H. brunnescens	+	+
H. cochlearitarsis	+	+
H. compressus	+	-
H. dschulfensis	-	-
H. falcinellus	-	-
H. fatsensis	-	-
H. georgicus	-	-
H. gordius	-	-
H. graecus	-	-
H. grossellus	-	+
H. luganicus	+	+
H. maculatus	+	+
H. patellatus	+	+
H. pentheri	+	+
H. ponticus	-	-
H. quadricinctoides	-	-
H. quadricinctus	+	+
H. resurgens	+	+
H. rubicundus	-	-
H. sajoi	+	+
H. scabiosae	+	-
H. senilis	-	-
H. sexcinctus	+	-
H. simplex	+	-
H. squamosus	+	-
H. submodernus	-	-
H. tetrazonianellus	+	+
H. tetrazonius	+	-
H. xanthoprymnus	-	-
Total number of species	18	12

Conclusion

Halictus species are pollinators or at least visitors of economically important species and natural vegetation. In this study, 205 *Halictus* specimens were collected and identified as 12 species from Beytepe and *H. grossellus* was the new record for Ankara province. Also, it was found that species of the family Asteraceae were the most frequently visited plants by *Halictus* species in this area. Since the landscape of Beytepe do not contain many different habitats and it is relatively small area, the proportion of the species richness might be found lower than expected. In spite of the fact that the study reports few species numbers, the proportional comparison would be more informative. Beytepe with 12 *Halictus* species reflects nearly 67% of this genus species in Ankara and almost 35% of this genus species in Turkey (Table 3).

Acknowledgements

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Hacettepe Üniversitesi Beytepe Kampüsü (Ankara) *Halictus* Latreille (Halictidae: Apoidea: Hymenoptera) Cinsi Faunası

ÖZ

Halictus Latreille, 1804 cinsi Halictidae familyasında bulunmaktadır. Dünyada 300'den fazla türe sahiptir ve bunlardan 35'i Türkiye'de yayılış göstermektedir. Türkiye'de bu cins üzerine faunistik çalışmaları sınırlıdır ve bu cins ile ilgili yeni yerel çalışmalara ihtiyaç duyulmaktadır. Çalışmada Beytepe'den toplanan 205 bireyin 12 türe ait olduğu tespit edilmiş olup, *H. grossellus* Ebmer, 1978 Ankara için yeni kayıt duru-

mundadır. Bu çalışmada alanda yayılış gösteren *Halictus* türlerinin dişileri için teşhis anahtarı verilmiştir. Aynı zamanda, alanda bulunan *Halictus* türlerinin en çok Asteraceae familyası türleri bitkileri ziyaret ettiği de tespit edilmiştir.

Anahtar kelimeler: Halictidae, polinatör, fauna, fenoloji, teşhis anahtarı, Türkiye

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MELLIFERA RESEARCH ARTICLE

Pollen Morphology of Opium Poppy (*Papaver somniferum* L.) Pollen Collected by Honeybees and Honeybees Tendency to Opium Poppy Flowers

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ABSTRACT

The purpose of this study is to investigate the relationship between opium poppy plants with honeybees. Honey and pollen samples were collected from apiaries near the poppy fields in Afyonkarahisar. Mellissopalynologic analysis, pollen morphology and Kjeldahl protein analysis were done. Also, honey samples were examined before and after the blooming of poppy flowers, and honeybees (*Apis mellifera* L.) tendency to poppy flowers was identified. Pollen measurements were made separately on white and purple poppy flowers pollen. Dimension of 50 pollens were measured from each pollen type. The pollen grains from white and purple poppy flowers are similar in morphology; tricolpate in type, oblate spheroid in shape and microechinate in ornamentation. According to the protein results, it was found average 40.86% in white flowered and average 36.82% in purple flowered opium poppy pollen. Also honeybees' tendency to opium poppy flowers were determined before and after their blooming, and 84% trend was observed.

Keywords: Opium poppy (*Papaver somniferum* L.), pollen morphology, melissopalynology, honeybee (*Apis mellifera* L.), pollen, honey

Introduction

Opium Poppy (*Papaver somniferum* L.) belonging to Papaveraceae family has white or purple flowers. Their sizes are between 30 and 120 cm in length [1]. The plant is cultivated in Afyonkarahisar, Isparta and Burdur in Turkey. Their capsules which are known as "opium" are used in medicine and pharmacy for their various alkaloids (morphine, codeine and papaverine)[1].

Honeybee products are important for beekeepers and consumers as a food and medicinal drug [2]. Thus, their contents and authenticity are significant for people health. For example, pollen has a high protein content, which varies from 7 to 40% [3]. Also bee pollen is a natural source of car-

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bohydrates, crude fibres, proteins and lipids as well as minor components such as amino acids, minerals, trace elements, vitamins, carotenoids, phenolic compounds, flavonoids, sterol, terpenes and etc. [4]. Besides its nutritional value, bee pollen shows various human health-promoting effects such as antitumor, chemopreventive/ chemoprotective, antimicrobial, antifungal, antioxidant, anti-radiation and anti-inflammatory activities [5-9].

In this study, honey and pollen samples were collected from apiaries near the poppy fields in Afyonkarahisar. Mellissopalynologic analysis and pollen morphological studies were done. Also, the field observations were made in the region. Honey samples were examined before and after the blooming of poppy flowers, and honeybees (*Apis mellifera* L.) tendency to poppy flowers were identified.

Materials and Methods

Collection of materials

Pollen samples used in the research were collected from Afyonkarahisar region, where the opium poppy fields are intensively widespread. We collected pollen samples separately near the white and purple opium poppy fields. Also pollen samples were collected from opium poppy flowers for pollen morphological studies. Pollen samples was put into glass jars right after collection from the traps and the jars were brought in a refrigerated container to the laboratory.

Preparation of preperats from material

The investigation followed the Wodehouse method [10] for preparing pollen slides.

Microscopic studies of pollen samples

Pollen slides were researched with a Nikon Eclipse E400 microscope, and immersion objective (x100) was used in the description of pollens. In the research all the area of 18x18mm² was checked. The relevant sources consulted in the diagnosis of the pollens were from Sorkun (2008) [11] and palDat (2016) [12] as well as prepared reference preparats.

Measurements of pollen samples

One interval of micrometric ruler used in pollen measurements was calculated as a 1 mm. Polar, equatorial axes and AMB diameters was measured on 50 pollen garins from each sample until the Gausse curve obtained. Also, exine (sexine and nexine) thickness, intine thickness, longitude of colpus (Clg), latitude of colpus (Clt), longitude of porus (Plg), latitude of porus (Plt), height of spines (dh), base width of spines (dt) and distance of colpus peaks (t) were measured on 50 pollen grains. Plg, Plt etc. could not be measured in some pollen samples because they were not very clear.

Means of pollen measurements (M) and standard deviation (Std) were calculated according to Sokal and Rohlf (1969) [13]. The formulas used are shown below.

Mean; $M = m+a\ 1/n \sum xy$

Standard deviation; $Std = \pm a\sqrt{1/n \sum x^2y - u^2}$

$(u = 1/n \sum xy)$

Microscopic analysis of honeys

For microscopic analysis Wodehouse (1935) [10] and Sorkun (2008) [11] methods were followed, and honey preparations were examined by a Nikon Eclipse E400 microscope.

Preparates from honey samples

Preparates to identify in 10 grams of honey are

obtained as follows:

500 grams of stock honey was well stirred with a sterile glass stick and 10 grams of it was separated for obtain preparats. The sample and 20 ml distilled water were mixed in a tube and were left in a water bath of 45°C for 30-45 minutes. Then this melted honey mixture was centrifuged in 3500 rpm for 45 minutes. Water in centrifuged tubes was removed and tubes were left upside down on a drying mat for full drainage. The material was taken from the bottom of the tube and plated on a lam with basic fucsin-glycerin gelatin mixture.

Basic fucsin-glycerin-gelatine mixture and honey were taken with the edge of a sterile needle was transferred to a microscope slide and put on a hotplate set at 40°C. When the gelatine was melted, 18 × 18 mm^2 cover slips were placed on the samples. Pollen slides were researched with a Nikon Eclipse E400 microscope. Immersion objective (x100) was used for identification of pollens. During microscopic studies all the area of 18x18mm^2 was checked. 200 pollens were counted for every sample and pollen types were determined according to their botanical origin.

Identification of honey sample preparates

The relevant sources used in the identification of the pollen were from Persano Oddo and Piro (2004) [14], Özkök Tüylü and Sorkun (2007) [15], Sorkun (2008) [11], palDat (2016) [12] as well as reference slides.

The determination of the botanical origin was based on the relative frequencies of nectariferous species' pollen types. The frequency classes of pollen grains were given as predominant (>45%), secondary pollen (15–45%), important minor pollen (3–15%) and minor pollen (1–3%) [16].

Generally a honey can be defined as unifloral if the "characteristic" pollen (e.g. *Brassica* in rape honey) exceeds 45%. It is considered honeydew if the ratio "HE/PG" exceeds 3. These are general guidelines but many pollen types are underrepresented (*Robinia pseudoacacia*, *Citrus* spp., *Tilia* spp.) or overrepresented (*Castanea sativa*, *Eucaliptus* spp.). For instance, to characterize acacia honey as unifloral, *R.pseudoacacia* pollen must be over 15%, citrus must have at least 10% of *Citrus* spp. pollen while, for chestnut honey, a content of 90% of *Castanea* pollen is required to classify honey as unifloral [16].

Kjeldahl protein analysis in pollen samples

The Kjeldahl method is an analytical method for the determination of nitrogen in the trinegative state in certain organic compounds, that was developed in 1883 by Johan Kjeldahl, a Danish chemist. The method is used extensively in the determination of the amount of protein in food, in which the percentage of nitrogen measured is converted to the equivalent protein content by use of an appropriate numerical factor [17].

In pollen samples total nitrogen was determined by using the Kjeldahl method adapted for the Kjeltec System digestion and distillation units (Leco FP- 528).

Protein amount was measured both for pollen samples dried in a pollen drying machine at 45 °C for 6 hours and for wet pollen samples. Total protein was calculated by multiplying the pollen nitrogen content by 5.6 [18].

Results and Discussion

As a result of the analysis this results were identified (Table 1-2, Figure 1-3).

Morphologic analysis of both *Papaver somniferum* L. white and purple flower's pollen showed that the plants have microechinate ornemantation and tricolpate pollen type. Also pollen measurements have been carried out. According to this; *P. somniferum* L. white flower pollen (P=27.78mm; E=29.04mm; L=29.02mm; Clg=22.78mm; Clt=8.34mm; Exine=1mm; Intine=0.5mm), *P. somniferum* L. purple flower pollen (P=28.76 mm; E=30.28 mm; L=29.98 mm; Clg=23.78mm; Clt=9.9mm; Exine=1mm; Intine=0.41mm). Sorkun (2008) [11] also found for *P. somniferum* tricolpate pollen type and microechinate ornemantation. Besides these results, pollen aperture membrane peculiarity is important in *Papaver* species and it is ornamented like other ones (*Papaver dubium, Papaver alpinum, Papaver rhoeas*) [12].

Table 1. Pollen morphology and % protein amount results of opium poppy pollens

Definition	White flowered opium poppy pollen	Purple flowered opium poppy pollen
Ornamentation type	Microechinate	Microechinate
Pollen type	Tricolpate	Tricolpate
Pollen shape	Oblat Sferoid	Oblat Sferoid
P/E	0.95	0.95
Polar axis (P)(mm)	27.78±1.0448	28.76±1.0307
Equatorial axis (E) (mm)	29.04±0.8236	30.28±0.9389
AMB diameter (L) (mm)	29.02±0.7345	29.98±1.288
Colpus lenght (Clg) (mm)	22.78	23.78
Colpus latitude (Clt) (mm)	8.34	9.9
Exine (mm) thickness	1	1
Intine(mm) thickness	0.5	0.41
% Protein	40.86	36.82

In this study Kjeldahl protein analysis regarding Nx5.6 show that *P. somniferum* L. has the high protein content between 36.82% to 40.86%. Also Özkök Tüylü and Sorkun (2006) [15] determined *P. rhoeas* L. has the highest protein content of 24.13%, while *T. pratense* L. and *T. repens* L. from Fabaceae family are next with 23.77%. However Bonvehi and Jorda, (1997) [19] reported regarding Nx5.6 in 52 different types of dry pollen was between 12.6-18.2g/100g, and average content was 15.3g/100g. Rabie *et al.,* (1983) [18] reported the amount of average protein as 18% in his studies regarding Nx5.6 with 21 species of dry pollen samples. So, consumption of *P. somniferum* L. becomes important because of their rich protein contents.

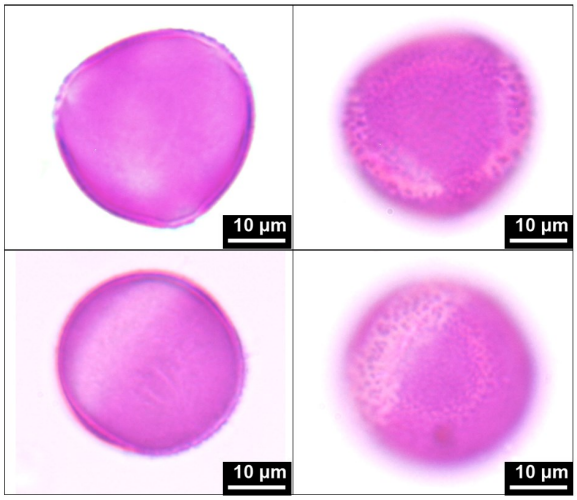

Figure 1. Pollen images of *Papaver somniferum* L. (10x100). (Photo: Aslı Özkök)

Besides, pollen morphology and chemistry we investigated honey samples which found in the opium poppy fields. Honeybees' tendency to opium poppy flowers were determined before and after their blooming and 84% trend was observed. Before opium poppy flowers predominant type of pollen in honey samples was Ranunculaceae (61%) but after opium poppy flowers predominant type of pollen in honey samples was Papaveraceae (84%). Also Srivastava and Singh (2006) [20] found that opium poppy flowers attracted honey-

Table 2. Honey samples before and after *Papaver somniferum* flowers blooming

Honey type	Total Pollen Number (TPN)	Predominant type of pollen (>45%)	Secondary pollen (15-45%)	Important Minor Pollen (3-15%)	Minor Pollen (<3%)	Province
Before *Papaver somniferum* flowers blooming	51 147	Ranunculaceae (61%)	Salicaceae (19.7%)	Rosaceae (10.8%) Papaveraceae (4%) Fabaceae (3.8%)	Asteraceae (0.5%) Ericaceae (0.2%)	Afyonkarahisar
After *Papaver somniferum* flowers blooming	120 831	Papaveraceae (84%)	-	Fabaceae (10.2%) Apiaceae (3.6%) Rosaceae (1.7%) Gramineae (0.5%)	-	Afyonkarahisar

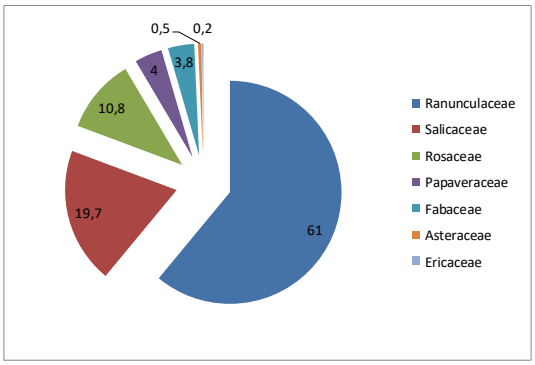

Figure 2. % Pollen tendency of honeybees before *Papaver somniferum* flowers blooming

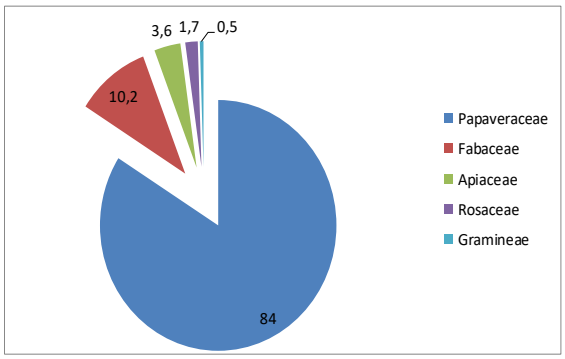

Figure 3. % Pollen tendency of honeybees after *Papaver somniferum* flowers blooming

bees because of theirs physico-chemical properties and alkoloid contents.

Conclusion

As a conclusion, all results showed us that high protein content of opium poppy pollen may have attracted honeybees. Beside these studies, further studies are essential for evaluating relationship between *Papaver somniferum* pollens and honeybees.

Acknowledgements

We are grateful to the beekeepers in Afyonkarahisar region, who helped us during the field study.

Bal Arıları Tarafından Toplanan Haşhaş (*Papaver somniferum* L.) Polenlerinin Morfolojisi ve Bal Arılarının Haşhaş Çiçeklerine Eğilimi

ÖZ

Bu çalışmanın amacı haşhaş bitkisi ile balarısı arasındaki ilişkiyi araştırmaktır. Afyonkarahisar'daki haşhaş ekili alanlara yakın bölgelerdeki arılıklardan bal ve polen örnekleri toplanmış ve bu örneklerin melissopalinoloji, polen morfolojisi ve Kjeldahl protein analizleri yapılmıştır. Aynı zamanda bölgede haşhaş çiçekleri açmadan önce ve açtıktan sonra alan gözlemleri yapılmış, arılıklardan toplanan bal örnekleri incelenmiş ve bal arılarının (*Apis mellifera* L.) haşhaş çiçeklerine eğilimi araştırılmıştır. Buna göre beyaz ve mor haşhaş çiçeklerinin polenlerinin ayrı ayrı polen morfolojisi çalışmaları yapılmış ve polen ölçümleri belirlenmiştir. Her çiçek poleninden 50 polen ölçülmüştür. Polen tipinin her iki grupta da trikolpat tipte ve oblat sferoid şekilli olduğu bulunmuş ve mikroekhinat orne-

mantasyon saptanmıştır. Kjeldahl protein analizi sonuçlarına göre ise beyaz çiçekli haşhaş poleninde protein miktarı ortalama %40.86, mor çiçekli haşhaş poleninde ise protein miktarı ortalama %36.82 olarak bulunmuştur. Bununla birlikte haşhaş çiçekleri çiçeklenmeden ve çiçeklendikten sonraki ballardaki melissopalinolojik analizlere bakılmış ve %84 oranında haşhaş çiçeğine eğilim olduğu saptanmıştır.

Anahtar Kelimeler: Haşhaş (*Papaver somniferum* L.), polen morfolojisi, melissopalinoloji, bal arısı (*Apis mellifera* L.), polen, bal

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CONTENTS / İÇİNDEKİLER