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RESEARCH PAPER

Surveying, identification and characterization for the potential honeybee (*Apis mellifera* L.) pollen sources in the arid region of Riyadh-Saudi Arabia

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Botanical sources

Abstract

This study was conducted targeting identification and documentation of major honeybee plants as pollen sources and their phenology in the central arid region of Saudi Arabia (Riyadh). Pollen loads were collected using pollen traps and were classified according to their color then traced back to plant species level. Throughout the year, sixteen plant species belonging to 10 plant families has been recorded and investigated by Light Microscopy (LM) and Scanning Electron Microscopy (SEM). Asian mustard (*Brassica tournefortii* Gouan.) (15.91%), willow wattle (*Acacia salicina* Lindl.) (15.17%), mesquite (*Prosopis juliflora*) (Sw.) DC. (21.81%) and cat's head (*Tribulus terresetris* L. var. *terrestris*) (15.52%) were the dominant sources of pollen. The period from May to July was found to be a significant blooming period and the scarcity period was from December to March in the study area. Color, size and shape of the potential pollen sources were variable between different species. Beekeepers could trap pollen loads that were collected from these sources during February, April and July, respectively. In conclusion, wise use and rehabilitation of these potentially identified bee plant species shall be considered in attempting beekeeping development.

Introduction

Pollen grains play a vital key role for honey bee nutrition. Honey bees gather pollen grains from plants to get the protein they need to survive (Al-Ghamdi, 2007a; Arien et al., 2020), colony strength (Belay et al., 2015), and productivity (Shawer et al., 2021). The type and quantity of the accessible flora, which is considered the primary source of pollen, have a significant impact on the potential for various colony products and success in beekeeping development. (Lau et al., 2019). Natural vegetation botanical makeup changes according to topography, climate, and soil type (Asefa et al., 2020). Consequently, the diversity of pollen depends on plant habitat, environmental conditions, distributions and flowering season (Amro, 2021; Begum et al., 2021).

Many researchers focused on palynological studies to help beekeepers in the abundance, distribution and flower blooming calendars of the main pollen sources in a various study area (Abou-Shaara, 2015; Adgaba et al., 2017; Taha et al., 2019). In order to satisfy their nutritional needs, bees should be given an array of different floral resources. This will promote healthier populations (Brodschneider & Crailsheim, 2010). For optimal honey production, beekeepers need to know when the major and minor nectar- and pollen-producing plants in the area of their apiaries are in bloom. This will allow them to decide when to introduce various management methods to their colonies (Agashe, 2021). Moreover, highlighting the periods in which beekeepers can collect bee-pollen from the major pollen sources (Taha, 2015) and determine the suitable period to introduce pollen substitutes and supplements (Amro et al., 2020) in order to economize the cost of feeding.

Little is known about the indigenous melliferous flora of Riyadh region wish described as the harshest environmental conditions of Saudi Arabia (Abou-Shaara et al., 2013). The density of blooming plants has decreased as a result of these circumstances, resulting in a shortage of pollen available and nectar sources (Al-Ghamdi, 2007a). Thus, a high percentage of imported honey bees and a significant number of local honey bee colonies are dying annually (Al-Ghamdi, 2009).

This study aims to survey, identify and provide an account of pollen morphological features of the plants in the central region of Saudi Arabia using light and scanning electron microscopy. As well as, further research on the ecology and floral preferences of honey bees in developed landscapes will be built on the quantity and suitability of each plant species as a pollen supply for honey bee nutritional demands.

Material and Methods

The experiments were carried out in the laboratory and apiary (24° 34′ 27″ N 46° 41′ 18 ″ E). Five colonies, equal in strength of the native honey bee race (*Apis mellifera jeminitica*) and imported Carniolan hybrid (*Apis mellifera carnica*) headed by sister queens, were located in Derab farm (56° 39' 36.21" E) in Riyadh during 2016 from January to December.

Sample Collection

To collect pollen samples from available pollen forage plants in the study region, five colonies from native honey bee race (*A. m. jeminitica*) and another from imported honey bee (*A. m. carnica*) were used. A pollen trap was fixed on the hive entrance of each colony. Every fifteen days, pollen traps were run for 3 days, giving the colonies 12 days of free entering pollen. Each sampling's pollen pellets were weighed and kept at -10°C until pollen classification and separation.

Description of Used Pollen Trap

The used pollen trap composed of wooden box with a slope roof and two vertical metal strips each of 32 cm length x 17 cm width. Each strip has 16 holes sq./inch (5 mm in diameter/hole). Pollen loads fallen through a horizontal wire gauze screen into a collecting tray which emptied as required (Abd El Salam et al., 2022). For increasing trap efficiency of native colonies, the board of trap entrance was changed with another one (3.75 mm in diameter/hole) to become suitable for the small size of native bee workers (*A. m. jeminitica*). 100 returning pollen foragers were observed as they entered the hive with pollen loads through an empty trap to gauge the effectiveness of the trap. The number of pellets that fell onto the tray was counted, and the efficiency was computed using the formula provided by Abd El Salam et al. (2022), as the following:

$$\textbf{Trap efficiency} = \frac{Number\ of\ pollen\ pellets\ in\ the\ trap\ box}{200} x100$$

Separation of Pollen Pellets

The fresh pollen pellets for every trap was cleaned and weighed. Pollen types were separated by color of pellets using small drawing brush. Every group contains the same color more than 70% of the total was considered as the major pollen source, while those with 50-70% and from 30-50% of the total were considered as moderate and minor pollen sources, respectively (Kirk, 1994).

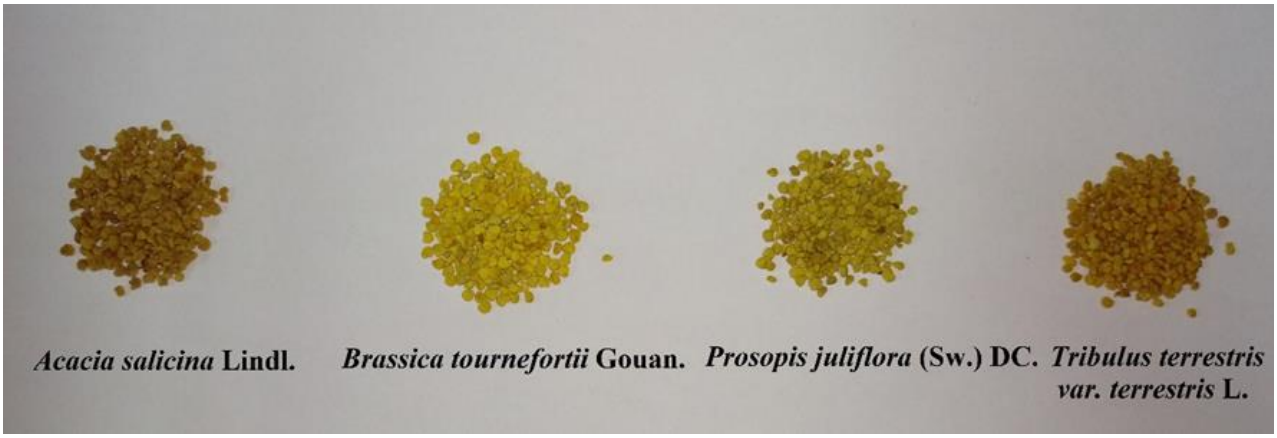

Figure 1: Pollen pellets colors varied from brown in willow wattles (*Acacia salicina* Lindl.), lemon in asian mustard (*Brassica tournefortii* Gouan.), olive in mesquite (*Prosopis juliflora* (Sw.) DC.) and in cat's head (*Tribulus terrestris* L. var. *terrestris*) pollen pellets were appeared in dark beige coloration.

Identification of Pollen Sources

Once pellets were split into groups based on colour, using the structure of the pollen grains as a guide, groups were further separated into various pollen types. (Dimou & Thrasyvoulou, 2007). During the same flowering period, in order to prepare pollen grains reference slides, pollen grains were directly collected from the adjacent opening flowers of the congruent blooming plants around the apiary. The morphology of each species of pollen grains was then compared with that of pollen pellets collected from pollen traps.

Identification of collected plants was carried out at King Saud University Herbarium in College of Sciences, where they were deposited. Family names were according to angiosperm phylogeny website. Method of Collenette (1999), was used for nomenclature of genera and species, the latest taxonomic changes as well as author update.

Preparing Standard Pollen Grain Slide

The specimens' fully grown anthers were removed, and they were then processed using the standard acetolysis procedure (Erdtman, 1960). Only newly opened flowers were used to prevent contamination from other pollen sources. Only newly opened flowers were used, so that contamination from other pollens is avoided. A few drops of isopropyl alcohol (IPA) were added and leaved for 10 minutes to detach the pollen grains from the anthers and remove the waxy covering from them. A drop of molten glycerin jelly was placed on a warm coverslip and slowly upturned slide lowered so that the pollen grains contact the jelly. The slide was left on the hotplate for 5 mins to allow the stain to penetrate the pollen grains. The slide was labeled with the name of the plant and the date of pollen collection.

Measurement of Pollen Grain Dimensions

The diameter of pollen grains was measured using an ocular micrometer (n=10) for each pollen type. According to Erdtman (1954), the pollen grain's polar (P) and equatorial (E) axes' lengths and the P/E ratio (index shape) were measured.

Light Microscope Examinations

Under a light microscope (Olympus –CH20i BIMU) provided with Camera Axicocam 512 color, under E40, 0.65 and Oil immersion (E100, 1.25), using a 10x eye piece, measurements and morphological observations were conducted. This procedure was conducted to get a clearer vision for the structure of the tested pollen grains obtained from the main pollen sources in the study area.

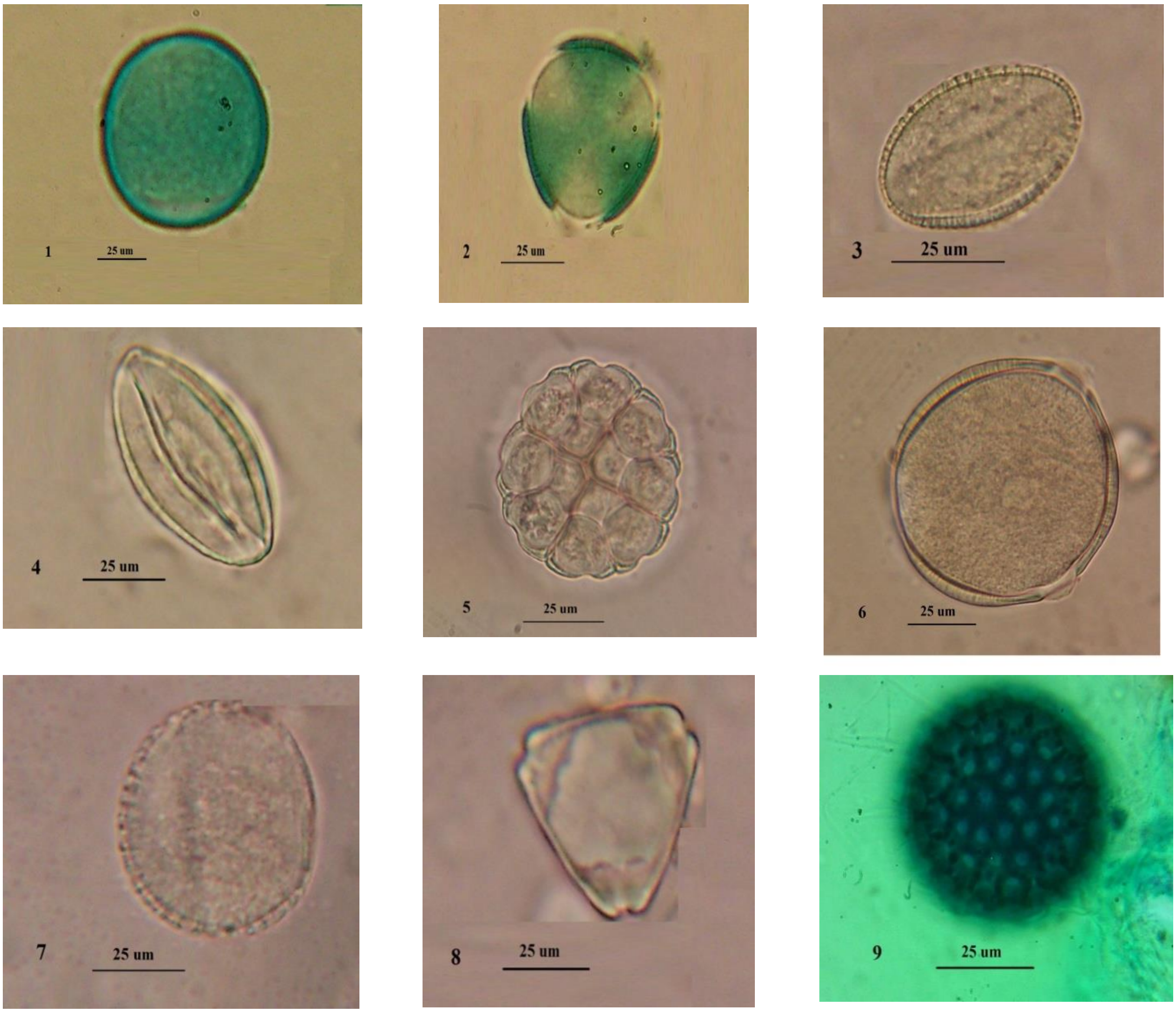

Figure 2: Light micrographs (LM) showing shaped and dimensions of pollen grain collected from Riyadh region.

1- *Phoenix dactylifera* L. 2- *Eruca sativa* (Miller) Thell. 3- *Brassica tournefortii* Gouan. 4- *Prosopis juliflora* (Sw.) DC. 5- *Acacia salicina* Lindl. 6- *Leuceana leucocephala* (Lam.) De Wit. 7- *Parkinsonia aculeate* L. 8- *Eucalyptus camaldulensis* Dehnh. 9- *Tribulus terrestris* L. var*. terrestris magnification* (1000 x).

Scanning Electron Microscopic Examinations

The scanning electron microscope was used for observing pollen wall surface. After dehydration on a silica gel drier, small dry quantities of pollen grain were mounted on scanning electron microscopy (SEM) stubs using double slided adhesive. The samples were then coated with gold in a JOEL JFC 1100E ion-sputtering device and examined in JOEL JSM 5400 LV scanning electron microscope, operated at accelerated voltage of 15 KV at the Scanning Electron Microscope Unit. The terminology of Boesewinkel and Bouman (1984), was used to describe the achene coat characteristics.

Statistical Analysis

The experiment was designed using a fully randomized design. For the aforementioned parameters, Analysis of variance (ANOVA) was used to statistically examine the given data. The Duncan's multiple range test (Duncan, 1955) was used to compare the means at a significance level of 5% with the SAS 9.1.3 programme (SAS Institute, 2004).

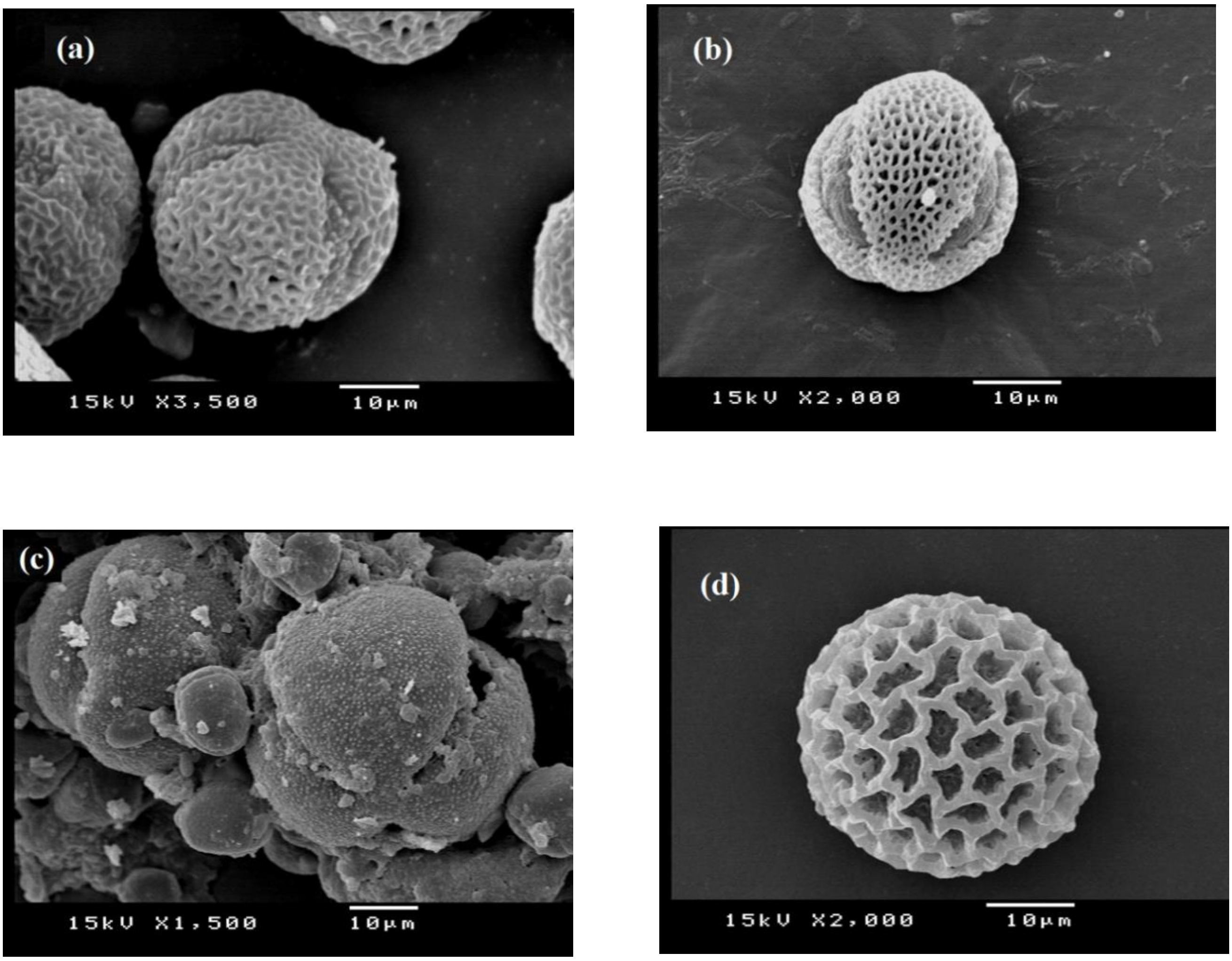

Figure 3. Scanning electron microscope (SEM) micrographs showing surface structure of selected pollen grains from Riyadh region.

a- *Prosopis juliflora* (Sw.) DC. b- *Brassica tournefortii* Gouan. c- *Acacia salicina* Lindl. d- *Tribulus terrestris* L. var*. terrestris* (Scale bars represent 10 µm for photomicrograph)

Results

Botanical Sources of Pollen and their Flowering Periods

The flowering periods of pollen sources in study area are shown in Table 1. Sixteen pollen sources belonging to ten plant families were identified. The most frequent pollen plant sources were from families Brassicaceae and Fabaceae. Honey bee plant species *Brassica tournefortii* Gouan, *Acacia salicina* Lindl., *Prosopis juliflora* (Sw.) DC., and *Tribulus terrestris* var. *terrestris* were ranked as the first four pollen plants and formed more than 70% in most of collected samples. These plants were considered the major pollen sources. On the other hand, *Phoenix dactylifera* L., *Eruca sativa* (Miller) Thell., *Leuceana leucocephala* (Lam.) De Wit., *Parkinsonia aculeata* L. and *Eucalyptus camaldulensis* Dehnh which formed 50-70% of the samples considered as moderate pollen sources. However, *Taraxacum officinale* F.H. Wigg, *Borago officinalis* L., *Citrullus colocynthis* (L.) Schrad., *Helianthus annuus* L., *Portulaca oleraceas* L. and *Nigella arvensis* var. *arabica* L., which formed 30-50% considered as minor pollen sources.

The asian mustard (*B. tournefortii*) was recorded during the periods from the first January till late March. However, flowering of willow wattle (*A. salicina*) was recorded three times, during mid-February till mid-April, mid-July till mid-August and from mid-October till mid-December. While, mesquite pollen was collected in the study area from mid-April till mid-August. The rest of pollen sources were recorded through 2016 season in differential periods. In general, results showed that the period from mid-April till late November recorded the highest presence of the pollen sources.

Table 1: Flowering periods of pollen botanical sources in Riyadh during 2016.

Common name	Family	Scientific name	Abundance	Months											
				Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Date palm	Arecaceae	*Phoenix dactylifera* L.	**xx**		▬	▬									
Dandelion	Asteraceae	*Taraxacum officinale* F.H. Wigg	**x**				▬	▬	▬	▬					
Sunflower	Asteraceae	*Helianthus annuus* L.	**x**			▬					▬				
Borage	Boraginaceae	*Borago officinalis* L.	**x**										▬	▬	▬
Salad rocket	Brassicaceae	*Eruca sativa* (Miller) Thell.	**xx**	▬	▬								▬	▬	▬
Asian mustard	Brassicaceae	*Brassica tournefortii* Gouan.	**xxx**	▬	▬	▬									
Colocynth	Cucurbitaceae	*Citrullus colocynthis* (L.) Schrad.	**x**					▬	▬	▬	▬	▬	▬		
Willow wattle	Fabaceae	*Acacia salicina* Lindl.	**xxx**		▬	▬	▬			▬	▬		▬	▬	▬
White lead tree	Fabaceae	*Leuceana leucocephala* (Lam.) De Wit	**xx**				▬	▬	▬	▬	▬	▬	▬	▬	
Parkinsonia	Fabaceae	*Parkinsonia leculeate* L.	**xx**						▬	▬	▬	▬	▬	▬	▬
Blue palo verde	Fabaceae	*Cercidium floridum* AZ.	**x**				▬	▬	▬						
Mesquite	Fabaceae	*Prosopis juliflora* (Sw.) DC.	**xxx**				▬	▬	▬	▬	▬				
Camphor	Myrtaceae	*Eucalyptus camaldulensis* Dehnh.	**xx**					▬	▬	▬	▬	▬			
Verdolaga	Portulacaceae	*Portulaca oleraceas* L.	**x**			▬							▬		
Nigella	Ranunculaceae	*Nigella arvensis* var. *arabica* L.	**x**					▬				▬			
Cat's head	Zygophyllaceae	*Tribulus terrestris* L. var. *terrestris*	**xxx**				▬	▬	▬	▬	▬				

x = Minor Pollen source (30% – 50%)**, xx=** Moderate Pollen source (50% - 70%) and **xxx**= Major Pollen source (more than 70%)

Abundance of the pollen sources

Availability and quantities of trapped pollen from the main pollen sources collected by Yemeni race and Carniolan hybrid honey bees was clarified in Table 2. The dominant plant families in the area were Fabaceae (22.64%) and Brassieaceae (15.53%), followed by Zygophyllaceae (14.58%). Pollen types varied in time, frequency, and species richness. The obtained data showed that the used plant sources were continuously presented in the area of study. In general, honey bee foragers collect most pollen amount from mesquite, asian mustard, cat's head, and willow wattle, respectively. Quantities of pollen loads collected by Yemeni race from all plant sources were higher than those collected by Carniolan hybrid. Highly significant variations ($P < 0.05$) were recorded between the quantity of pollen loads collected by Yemeni and Carniolan hybrid bees. The higher quantities of pollen loads collected by Yemeni race can be arranged descending as follows: 2833.57 > 2067.50 > 2017.19 > 1970.84 gm/colony for mesquite, asian mustard, cat's head, and willow wattle, respectively. Asian mustard and mesquite plants were presented during 3 months each. Except December, the rest of plant sources appeared during two months each. Mesquite plants lasted in the study area for the longest period and their pollen were constantly present in the pollen traps and recorded in 11 samples during the entire study period. It followed by asian mustard, willow wattle and cat's head. However, salad rocket exhibited the lowest period of presence, where it occurred in 3 samples only. Although, the Yemeni race collected pollen loads more than Carniolan hybrid bees, the percentage from total collected pollen showed semi equal values between the two races.

Table 2. Presence and quantity of trapped pollen load (gm/colony/flowering period) in Yemeni race and Carniolan hybrid honeybee colonies from the main pollen botanical sources in Riyadh during 2016.

Common name	Family name	Scientific name	Presence in traps (Catch No.)	Quantity of pollen loads (gm/colony/flowering period)			
				Total (±S.D.**)		% from total collected pollen	
				Yemeni race	Carniolan hybrid	Yemeni race	Carniolan hybrid
Asian mustard	Brassicaceae	*Brassica tournefortii* Gouan.	Jan. 17 to Mar 6 (9)	2067.50 ± 31.60 b*	1110.09 ± 15.23 e	15.91 ± 0.09 c	15.15 ± 0.25 d
Willow wattle	Fabaceae	*Acacia salicina* Lindl.	Mar. 12 to Apr. 26 (9)	1970.84 ± 56.72 c	933.12 ± 30.04 g	15.17 ± 0.24 d	12.74 ± 0.49 f
Cat's head	Zygophyllaceae	*Tribulus terrestris* L. var. *terrestris*	May 2 to Jun. 19 (9)	2017.19 ± 69.92 c	999.53 ± 30.71 f	15.52 ± 0.29 cd	13.64 ± 0.29 e
Camphor	Myrtaceae	*Eucalyptus camaldulensis* Dehnh.	Jun. 25 to Jul. 13 (4)	1038.50 ± 38.43 f	630.97 ± 55.99 j	8.00 ± 0.35 i	8.60 ± 0.68 h
Mesquite	Fabaceae	*Prosopis juliflora* (Sw.) DC.	Jul. 19 to Sep. 19 (11)	2833.57 ± 71.43 a	1719.61 ± 31.89 d	21.81 ± 0.32 b	23.47 ± 0.45 a
White lead tree	Fabaceae	*Leuceana leucocephala* (Lam.) De Wit	Sep. 23 to Oct. 11 (4)	838.88 ± 40.50 h	495.40 ± 17.22 k	6.45 ± 0.30 j	6.76 ± 0.24 j
Parkinsonia	Fabaceae	*Parkinsonia aculeata* L.	Oct. 17 to Nov. 29 (8)	1128.31 ± 26.94 e	742.79 ± 13.51 i	8.68 ± 0.17 h	10.13 ± 0.12 c
Salad rocket	Brassicaceae	*Eruca sativa* (Miller) Thell.	Dec. 5 to Dec. 18 (3)	470.82 ± 38.43 k	315.59 ± 16.26 m	3.62 ± 0.35 m	4.38 ± 0.23 l
Date palm	Arecaceae	*Phoenix dactylifera* L.	Jan. 24 to Mar. 11 (4)	622.99 ± 23.26 j	378.82 ± 3.49 l	4.79 ± 0.20 k	5.17 ± 0.03 k

* Means followed by the same letter are not significantly different at 0.05 level of probability, by Duncan's multiple range test.
**S.D.= Standard deviation

Pollen Characterization

Pollen grain dimensions and coloration

Data presented in Table 3 show the shapes, colors and dimensions of the pollen grains collected from study region which represented the major and moderate pollen botanical sources. Pollen grains which contained the highest length and width belonged to 9 plant species and 5 families. It is clear that family Fabaceae was represented by 4 plant genera, followed by family Brassicacea by 2 genera and families Arecaceae, Myrtaceae and Zygophyllaceae by one genus for each.

Pollen grains of the white lead tree (*L. leucocephala*) showed the highest length of polar and equatorial of the collected grains with an average of 50.4 µm polar and 47.9 µm equatorial lengths followed by cat's head (*T. terrestris*) grains by 47.4 polar and 47.6 µm equatorial lengths. Pollen grains of willow wattle (*A. salicina*) ranked the third one by 42.7 polar and 36.4 µm equatorial lengths. The remaining pollen grains exhibited the least values of polar and equatorial lengths, and camphor (*E. camaldulensis*) ranked the last with an average of 17.4 polar and 17.0 µ equatorial lengths. It is important to note that pollen grains length

Table 3. Pollen grain dimensions and pellets color of main pollen botanical sources collected by honeybee colonies from Riyadh during 2016.

Common name	Family	Scientific name	Length axis (µm)		Shape index P/E	Pollen pellets color
			Polar (P) ± S.D*	Equatorial (E) ±S.D		
Date palm	Arecaceae	*Phoenix dactylifera* L.	19.3±0.08	19.3±0.08	1.0	Creamy
Salad rocket	Brassicaceae	*Eruca sativa* (Miller) Thell.	19.9±0.11	20.1±0.08	1.0	Dark yellow
Asian mustard	Brassicaceae	*Brassica tournefortii* Gouan.	32.6±0.13	26.9±0.13	1.2	Lemon
Willow wattle	Fabaceae	*Acacia salicina* Lindl.	42.7±0.28	36.4±0.50	1.2	Brown
White lead tree	Fabaceae	*Leuceana leucocephala* (Lam.) De Wit	50.4±0.22	47.9±0.36	1.1	Light yellow
Parkinsonia	Fabaceae	*Parkinsonia leculeate* L.	22.4±0.31	21.9±0.21	1.0	Dark orange
Mesquite	Fabaceae	*Prosopis juliflora* (Sw.) DC.	28.1±0.23	27.3±0.11	1.0	Olive
Camphor	Myrtaceae	*Eucalyptus camaldulensis* Dehnh.	17.4±0.09	17.0±0.08	1.0	Bright brown
Cat's head	Zygophyllaceae	*Tribulus terrestris* L. var. *terrestris*	47.4±0.36	47.6±0.27	1.0	Dark beige

***S.D.=** Standard deviation

of *L. leucocephala* is equal 2.89 and 2.82 fold of polar and equatorial lengths of *E. camaldulensis* pollen grain.

Morphological Characterization of Pollen Grains

By using the light microscope (LM), the shape and dimensions of pollen grains collected from botanical origin in the study area showed wide differences (Fig. 1). The pollen grains of *P. dactylifera*, *E. sativa*, *P. aculeate*, *P. juliflora*, *E. camaldulensis* and *T. terrestris*, were spheroid (shape index 1.0), whereas, *B. tournefortii* and *A. salicina* were sub-prolate (shape index 1.2). Only *L. leucocephala* pollen grains were prolate-spheroid (shape index 1.1).

Description of the main pollen sources using scanning electron microscope (SEM) is illustrated in Fig. 2. The results showed that pollen grains of *P. juliflora* (Fig. 2a) are largely homogeneous. The shape index is prolate-spherodial. The mature pollen grains are tricellular and tricolporate with a regulate exine. On the other side, *B. tournefortii* pollen grains are tricoplate (Fig. 2b) whereas, they have a sub-prolate shape, and the exine is a finely perforated reticulate sculpture with lumina that are smaller than the muri or equal in size to them. However, *A. salicina* pollen grains are spherical ovoidal with openings at the proximal surface and colpus at the distal surface, scattered as an interconnected-polyad (Fig. 2c). In regards to *T. terrestris,* pollen grains were found to be spheroidal (Fig. 2d). The exine decoration is reticulate with straight to slightly expressed wavy boundaries (muri) and a straightforward columnar structure.

Discussion

Pollen Botanical Sources and Flowering Periods

The current results showed that rape, date palm and the Acacia trees are the main pollen sources in the harshest environmental region of Saudi Arabia (Riyadh). In the same area Alqarni (2020), recorded notable foraging behaviour for the *A. m. jemenitica* (indigenous) and *A. m. carnica* (exotic) on the Acacia trees (*Acacia gerrardii* Benth.). In eastern province of Saudi Arabia in Al-hasa region (semi-arid), Al-Jabr and Nour (2001), stated that rape, date palm and the Acacia trees considered main pollen sources for honey bees. In addition, Taha (2015), recorded family Brassicaceae among the most abundant pollen sources in the same region.

In the present study, acacia trees were notable along the study period, in addition the long presence period of *Acacia salicina* pollen flow in the field confirmed by Khan et al. (2019). He came to the conclusion that because Acacias have the majority of the characteristics needed to resist harsh climatic circumstances, they are the most effective "survivors" in desert environments. Also, Bilisik et al. (2008), stated that the honey bee foragers focused on a few plant species at a particular time despite the richness and diverse flora in the study area. According to their findings, the pollen varieties exhibiting high quantities were prevalent around the hives. They also, reported that the Brassicaceae (*Papaver* spp.), is the main pollen taxa collected by honey bees in the area. Among the plant families which studied by Da luz et al. (2010), and Mayda et al. (2020), Asteraceae, Fabaceae, Arecaceae, and Myrtaceae were recorded as the major richness plant families of pollen sources types. In addition, the latter researcher confirmed that the elemental analysis showed that all samples were rich in essential minerals.

In reference to a particular plant, prosopis was recognized as a significant source of nectar for honey bees (Zaitoun et al., 2009). Also, Suryanarayana et al. (1992), found that numerous insects, notably honey bees, willfully visit Asteraceae inflorescences of the capitulum type. This information insures that the plant sources identifying during this study have special importance as pollen sources for honey bee in Riyadh region. In Saudi Arabia, Al-Ghamdi (2007b), confirmed our results by recorded *E. sativa*, *Eucalyptus rostrata, L. leucocephala, Brassica juncea, Acacia farmesiana* and *A. salicina* as the most promising bee plants during their flowering periods in relation to various honey bee activities. Also, he noted that most of the species were controllable and that weedy vegetation might be seen as a significant source of food for bees because it commonly appeared in the pollen spectrum even in well farmed areas.

Concerning the data obtained from Table1*,* it was observed that *H. annuus*, *E. sativa*, *A. salicina*, *P. oleraceas* and *N. arvensis* bloomed 2-3 times a year. The present results supported by the observation of Al-Ghamdi (2007b) and Amro et al. (2020), in which they stated that availability of secondary sources of honey bee forage plants is seen as a very critical issue and helps bees to live in times of scarcity in the Riyadh region. Date palm (*P. dactylifera* L.) (Arecaceae) was identified as another significant resource utilized by both Yemeni and Carniolan races in the study area, and in several samples, it was the dominant source of pollen. The importance of Arecaceae pollen as a resource for *A. mellifera* was described by Lau et al. (2019). In addition, Asteraceae ornamental species are thought to be particularly significant insect attractor plants (Denisow et al., 2014). Also, Importance of mesquite trees (*P. juliflora*) as a pollen source for honey bee colonies was confirmed (Zaitoun et al. 2009). They concluded that the mesquite is an important plant species which represents practically the only source of nectar and pollen to bees in April and May in several of the arid and semi-arid zones of Durango, Mexico. It is a crucial plant because it has a great capacity to fix nitrogen in arid environments and during droughts, and it offers shelter and food to a variety of species who feed its nectar, pollen, leaves, and fruits.

Abundance of the pollen sources

Our results showed highest collected pollen loads initiated by Yemeni race and Carniolan hybrid honey bee have been recorded during summer months when honey bees foraged on *L. Leucocephala*, *P. Juliflora* and *T. terrestris*, followed by spring season during the flowering periods of *P. dactylifera*, *B. tournefortii* and *A. salicina*. In the same line, Alqarni (2020) recorded that *A. m. jeminitica* recruited significantly ($P < 0.01$) more active pollen-gathering bees than the *A. m. carnica* especially on Acacia trees. Plants families Aracaceae, Brassicaceae, Fabaceae, Myrtaceae and Zygophyllacae were the most flowering plants visited by honey bee colonies as a pollen sources in the present investigation. The importance of these families as a pollen sources for honey bees were confirmed (Alves & Dos Santos, 2016; Alqarni, 2020). They recorded that Aracaceae and Fabaceae were the most important families supplying honey bee colonies with high amount of pollen. In a comparative study from Turkey and Romania bee pollen, Mărgăoan et al. (2021), suggested that the fatty acids contents were closely correlated with the above-mentioned parameters especially with the botanical origin and antibacterial activity. Our findings suggest that BP is a rich source of unsaturated fatty acids and bioactive compounds, which can be considered a value-added product and concluded that geographical location is a determining factor for plant pollen viability.

Data presented in Table 2, highlighted that native bees were significantly ($P < 0.05$) more active foragers than the exotic one. In the same line, Al-Ghamdi et al. (2020), stated that the examined nursery stage under Acacia species flow performed well and within acceptable ranges, potentially qualifying them as suitable biological tools for introducing genotypes into apicultural landscape restoration projects. Also, Taha (2015), point out to families Arecaceae, Brassicaceae and Fabaceae as the most plentiful sources of pollen for honey bee colonies in Alhasa region, Saudi Arabia. Highly significant differences between quantities of pollen collected from different families, as well as, different plant species and honey races were recorded. Our field experiments indicated statistically significant differences among tested honey bee strains for all quantitative pollen yield parameters. The percentages of collected pollen loads were statistically significant higher ($P < 0.05$) in indigenous bees (*A. m. jeminitica*) than the imported one especially for the plant species *A. salicina*, and *T. terrestris*. On the other hand, the statistical analyses showed the vigor of imported bees (*A. m. carnica*) to collect more pollen from *P. dactylifera, E. sativa, P. aculeate* and *P. juliflora*. Hence, the amount of pollen produced by different species can vary greatly, and the reward of a given species might vary depending on the area and growth season. In the same line Amro et al. (2020), conclude that it is indispensable to use both races (Carniolan and Yemeni bees) in extremely dry desert areas, because each of them has specifications and capabilities that allow them to forage and collect pollen in a way that distinguishes each one from the other.

The present results showed that mesquite (*P. juliflora*) pollen pellets achieve superior vs the other tested plants followed by asian mustard (*B. tourneforii*), cat's head (*T. terrestris*) and willow wattle (*A. salicina*) plants. These results were matched with Taha (2015), who considered that *Prosopis* spp., *Brassica* spp., *Tribulus* spp. were the most important pollen sources for honey bee colonies. He observed that bee foragers focused only on four flowering plants to collect major quantities of pollen in Alhasa region. This phenomenon was previously illustrated by Klein et al. (2019). They verified that *A. mellifera* can concentrate foraging efforts on a single type of flowers. Therefore, the fidelity in the studied areas could be attributed to the scarcity of other flowering species, due to seasonal inundation of the floodplain. This fact could force honey bees to visit flowers of a limited number of plants. Also, floral fidelity (Goodwin, 2012) could be related to the distance of the hives to floral sources. In addition, color, shape and flowers odour, energy requirement and caloric rewards offered by flowers also determine whether honey bee can be a dependable flower visitor (Dobson & Bergström, 2000).

Continuous presence of *T. terrestris* in the present study reinforced by Yankova- Tsvetkova et al. (2011). They claimed that the high pollen and embryo viability established as well as sexual reproduction are important factors in determining if *T. terrestris* has a suitable amount of reproductive potential and whether its successful realization ensures the stability of its populations. Naghiloo and Siahkolaee (2019), also, referred to *T. terrestris* as being extensively dispersed due to its facile seed transmission in desert, warm, and moderately temperate parts of the globe. This species' ability to successfully use its reproductive potential is also a result of its trait as an entomophilous plant that generates a significant amount of pollen grains.

Pollen Characterization

The obtained results showed considerable variation in pollen morphology has been detected between the plant sources. Except of cat's head and asian mustard the largest polar length and equatorial length of the collected pollen grains in this work belonged to family Fabaceae. However, cat's head pollen shape was in agreement with Rouhakhsh et al. (2014), who documented that *T. terrestris* pollen grains are oblate-spheroidal, radially symmetric, pantoporate. Also, Semerdjieva et al. (2011), reported that exine ornamentation for *T. terrestris* is reticulate and the sizes of the pollen grains ranged between 35 and 47.5 µm with an average 43.5 µm.

In this investigation, pollen morphology of *E. sativa* and *B. tournefortii* showed similar phenomena with 39 species belonging to family Brassicaceae, studied in Saudi Arabia by Umber et al. (2021). The dominant feature is tricolpate for the pollen grains which ranged in form from prolate spheroidal to subprolate to prolate. The same author recorded that *Schimpera arabica* (Brassicaceae), which has the smallest grains in this family, has a polar axis of 16 µm and an equatorial diameter of 15 µm. However, *Malcolmia pygmaea*, has the largest polar axis of 37 µm and an equatorial diameter of 21.5 µm. Additionally, the finding of Gosling et al. (2013) supported ours by classifying family Brassicaceae as a stenopalynous, whereas the pollen grains are usually reticulate and tricolpate.

Conclusion

The large amounts of pollen obtained from asian mustard and mesquite reflect its significance as important pollen forage plant for honey bees in this area. Plants families Aracaceae, Brassicae, Fabaceae, Myrtaceae and Zygophyllacae were the most flowering plants visited by honey bee colonies as pollen sources in Riyadh region. The beekeepers interested in collecting pollen can be advised to hold their pollen traps from mid-January to the first of march and mid-July to mid-September as the most suitable periods for collecting pollen in the Riyadh region. Further studies on honey bee colonies operating in the harsh environmental conditions of central Arabia are needed.

Ethical Statement

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Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

All authors of this manuscript contributed equally to the design and/or execution of the experiments described in the manuscript. A.A prepared and edited the final version of this manuscript. All authors approved the final manuscript.

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RESEARCH PAPER

Determination of Consumers' Consciousness Levels of Consumption of Bee Products

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Abstract

In this study, it was aimed to determine the honey consumption consciousness levels of consumers residing in Ordu province. For this purpose, a face-to-face survey was conducted with 387 people determined by simple random sampling. Determination of the honey consumption consciousness levels of consumers residing in Ordu province was carried out using a face-to-face questionnaire. 38% of the consumers were men and 62% were women, whereas 15% of the consumers were farmers, 15.2% were self-employed, 13.4% were workers, 39.3% were civil servants, 4.1% were retirees and 12.9% were tradesmen. In the study, 65.6% of the consumers stated that they had sufficient knowledge about honey, 26.1% did not, and 8.3% stated that they did not have any idea. Again, it has been determined that 59.2% of the consumers have sufficient knowledge about pollen, 32% did not and 8.8% have no idea about it. Similarly, 50.4% of the consumers stated that they knew about beeswax, 39.5% stated that they did not, and 10.1% stated that they did not have any idea. Moreover, 47.8% of the consumers stated that they knew about royal jelly, 39.8% did not, and 12.4% stated that they did not have any idea about it. Again, it was determined that 46% of the consumers did not know about bee venom, 34.9% knowledge and 19.1% had no idea about it. At the same time, 53% of the consumers stated that they knew about propolis, 33.6% did not, and 13.4% stated that they did not have any idea.

Introduction

The beekeeping activity can be called the most dependent livestock activity due to the habits of honey bees and their collecting raw materials from nature. Although honey is the most well-known product of the beekeeping activity, there are also several bee products such as beeswax, pollen, royal jelly, and propolis. Although the name of honey products, which are very beneficial regarding human health, are well known, the benefits of honey products are not known well by consumers. It is determined that pollen strengthens the immune system, shows as an antibiotic effect against microorganisms causing severe diseases and has antibacterial and antiviral properties. Beeswax is mostly used to make honeycomb. It is also used in the cosmetic and pharmaceutical industry, dye and varnish production. Honey products, especially honey, are used in various areas from food to cosmetics industry, and in recent years they have been widely used in apitherapy (Baki et al., 2017). Though there are many studies concerning honey consumption in Turkey (Bölüktepe & Yılmaz, 2006; Bölüktepe & Yılmaz, 2008; Tunca et al., 2015; Niyaz & Demirbaş, 2017), and in other countries (Arvanitoniyannis & Krystallis, 2006; Pocol, 2011; Schifani et al., 2016), the number of studies that examine the honey consumption behaviours is limited. Honey is becoming an increasingly popular product among consumers. Honey is a product with very rich symbolism and it is present in all cultures, but its consumption is a variable category. It is, therefore, necessary to identify the wishes and expectations of consumers, which must correspond to the perception they have about this product (Haderbache & Mohammed, 2015).

Ordu province, located in the Eastern Black Sea Region, has recently attracted more and more people's attention with its natural beauties, vegetation, sea, mountains and beautiful plateaus. Beekeeping is widely practiced in Ordu, which is also the first in hazelnut production in Turkey. Bees provide people with products such as honey, beeswax, royal jelly, bee venom, pollen, and propolis, which are very valuable. At the same time, bees play an important role in maintaining the natural balance and in agricultural production with their contribution to pollination in

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plants. For this reason, it has a very important role in maintaining the biological diversity of Turkey (Pirim et al., 2011; Sıralı, 2015; Sıralı, 2017).

Ordu province has a great economic potential in terms of beekeeping, and a significant part of the people, especially in the districts located at high altitudes, make their living from beekeeping (Sıralı, 2017). Beekeeping has become more profitable with the transition from traditional production methods to modern production methods in Ordu province and the transfer of bees to other regions. Beekeepers from Ordu, who make use of the flora in different regions of Turkey, make a significant contribution to the economy of the province with the honey and bee products they produce. For this reason, beekeeping has become the most important agricultural sector after hazelnut cultivation in Ordu (Sıralı, 2015). In Table 1, the number of enterprises, the number of bee hives and honey production are given according to the districts in Ordu province (Anonymous, 2020). The objective of this research is to determine the consumption consciousness levels of consumers residing in Ordu province regarding honey bee products (beeswax, pollen, royal jelly and propolis). At the same time, these results will enable people working in this field to better understand the level of consciousness of consumers in terms of honey and its products, and will contribute to the development of some solution proposals for honey consumption.

Table 1. Number of enterprises, total number of hives and honey production by districts in Ordu province

District Name	Number of enterprises	Number of hives	Honey production (Ton)
Altınordu	395	93060	2525
Ulubey	316	76147	2284
Gölköy	290	72600	2170
Gürgentepe	305	75000	2200
Perşembe	302	67200	1950
Ünye	210	44500	1424
Kabataş	143	40000	1250
Çatalpınar	190	33500	1300
Fatsa	152	32200	805
Çamaş	77	11992	358
Kumru	56	8870	265
Kabadüz	25	4750	140
Aybastı	30	4100	121
Gülyalı	26	2540	78
İkizce	35	2660	76
Mesudiye	32	1100	37
Korgan	14	1150	31
Akkuş	17	860	25
Çaybaşı	21	1129	18
Total	**2636**	**573358**	**17057**

Materials and Methods

Materials

In this study, face-to-face interviews were used as data collection methods and questionnaire forms were used as data collection tool. After the consumers were informed about the survey, it was ensured that the consumers answered the questions correctly.

Method

The population of the study consisted of citizens residing in Altınordu district, which is the central district of Ordu province. The number of surveys was determined by using simple random sampling method. The equation given below was used to determine the number of surveys and a survey was conducted with a total of 387 people (Akbay & Yıldız Tiryaki, 2007). Due to possible problems that may arise in the surveys, 10% more of the minimum sample size was surveyed. Thus, a total of 426 questionnaires were made. However, 39 surveys were excluded from the evaluation due to various lack of information and inconsistency issues, and 387 surveys were evaluated in the study.

$n= (t^2 * p * q) / d^2$

n: sample volume,

t^2 = Confidence coefficient (for 95% confidence, the coefficient was taken as 1.96),

p= Ratio value of the population (0.50),

q=1-P=0.50,

d^2 = Accepted sampling is the margin of error.

Statistical Evaluation

First of all, frequency analysis of the answers given by the respondents to all the questions in the questionnaires was performed, and the frequency values were calculated as numerical (n) and percentage

(%) all calculations were made using SPSS (2008) statistical package program.

Results and Discussion

Demographic Characteristics of Consumers

In the research, numerical (n) and percent (%) frequency values were obtained from the answers given to the questions asked to determine the personal characteristics of the consumer participating in the survey study and the social-demographic and economic characteristics of the families. The frequency values obtained are given in Table 2.

In this study, 38% of the consumers participating in the survey were men and 62% were women. Also, of the

Table 2. Demographic Characteristics of Consumers

No	Demographic Characteristics	Options	Frequency	
			n	%
1	Profession of consumers	Farmer	58	15
		Self-employment	59	15.2
		Worker	52	13.4
		Civil servant	152	39.3
		Retired	16	4.1
		Tradesmen	50	12.9
2	Gender of consumers	Male	147	38
		Female	240	62
3	Monthly income of consumers (TL)	Below 2500	114	29.5
		2500-4000	71	18.3
		4001-6000	110	28.4
		6001-8000	79	20.4
		8001-10000	12	3.1
		10000 and above	1	0.3
4	Age of consumers (years)	30 years and less	91	23.5
		31-40 years	146	37.7
		41-50 years	93	24
		51 years and older	57	14.7
5	Education level of consumers	Illiterate	3	0.8
		Primary education	98	25.3
		High school	112	28.9
		University	174	45
6	Number of individuals in the household of consumers	Less than 4	187	48.3
		Between 4-6	167	43.2
		7 and above	33	8.5
7	Social security of consumers	None	47	12.1
		Green card	18	4.7
		Social Security Organization for Artisans and the Self-Employed	65	16.8
		Social security agency	257	66.4

participants, 15% are farmers, 15.2% are self-employees, 13.4% are workers, 39.3% are civil servants, 4.1% are retired and 12.9% are tradesmen were determined. The monthly income level of the participants is below 2500 Turkish Liras (TL) in 29.5%, between 2500-4000 TL in 18.3%, between 4001-6000 TL in 28.4%, between 6001-8000 TL in 20.4%, between 8001-10000 TL in 3.1%. On the other hand, 0.3% of them were found to be above 10000 TL. It was determined that 23.5% of the participants were under the age of 30, 37.7% were 31-40 years old, 24% were 41-50 years old, and 14.7% were 51 years old and over. When the educational status of the participants is examined; it was determined that 0.8% were illiterate, 25.3% were primary school graduates, 28.9% were high school graduates and 45% were university graduates. While the rate of consumers in the households of the survey participants is four or less is 48.3%, the rate of those between four and six is 43.2% and the rate of those with seven or more is 8.5. In terms of social security, it was determined that 4.7% of the participants had a green card, 16.8% had Social Security Organization for Artisans and the Self-Employed, 66.4% had Social Security Agency and 12.1% had no social security.

In a previous study conducted by Bölüktepe and Yılmaz (2006), 38.2% of the participants were high school graduates, 24.1% were university and college graduates, 35.1% were primary school graduates and 2.7% were literate. When the occupational distributions are analyzed, 34% of the participants are housewives, 19.3% are private sector employees, 15.1% are public employees, 14.1% are tradesmen, 3.7% are employers and 3.3% of them are farmers. Niyaz and Demirbaş (2017) stated in their research that 52% of the consumers are female and 48% are male. In a similar study, 63.5% of the students participating in the study by Saral and Yavuz (2020) were women and 36.5% were men. Akdemir (2019) found that 44.25% of the participants were men, 55.75% were women, and Bölüktepe and Yılmaz (2008) determined that 54% of the participants were women and 46% were men. Kumova and Korkmaz (2000) stated that 38.88% of the surveyed the consumers' education levels were high school, 36.72% were high school, 16.67% were primary school, 7.48% were secondary school and 0.25% were only literate. Akdemir (2019) determined that 2.5% of the consumers do not have social security and 1% of the consumers who have social security have green card. Similarly, in the study conducted by Baki et al., (2017), it was determined that 97.5% of the consumers have social security. Again, in the study conducted by Karahan and Özbakır (2019), it was determined that 80.7% of the participants were male, 19.3% were female, and 36.1% of them were undergraduate considering their educational status. Gyau et al. (2014) determined that education and age are significant factors affecting consumer decisions when purchasing honey in terms of consumer characteristics.

Main Findings Regarding the Consumption Consciousness of Bee Products of Consumers

The frequency values obtained from the answers given to the questions about the consumption consciousness level of consumers and their families regarding bee products are given in Table 3.

Table 3. Statistical Values Regarding the Consumption Consciousness of Bee Products of Consumers

No	Questions	Options	Frequency	
			n	%
1	Do you know enough about honey?	No	101	26.1
		No idea	32	8.3
		Yes	254	65.6
2	Do you know enough about pollen?	No	124	32
		No idea	34	8.8
		Yes	229	59.2
3	Do you know enough about beeswax?	No	153	39.5
		No idea	39	10.1
		Yes	195	50.4
4	Do you know enough about royal jelly?	No	154	39.8
		No idea	48	12.4
		Yes	185	47.8
5	Do you know enough about bee venom?	No	178	46
		No idea	74	19.1
		Yes	135	34.9
6	Do you know enough about propolis?	No	130	33.6
		No idea	52	13.4
		Yes	205	53

It was determined that, 65.6% of the consumers stated that they knew about honey, 26.1% did not, and 8.3% of the consumers stated that they did not have any idea about honey. According to these findings, it can be said that the level of consciousness of the consumers is high. In the study conducted by Dağdemir and Akdemir (2021), it was found that 47.25% of the consumers had wrong information about unsweetened honey, 41.25% had no idea about it, and only 11.5% had correct information about unsweetened honey.

While 59.2% of the consumers stated that they knew about pollen, 32% did not, and 8.8% stated that they did not have any idea about it. Also, 50.4% of the consumers answered that they knew about beeswax, 39.5% answered that they did not, and 10.1% stated that they did not have any idea about it. Moreover, 47.8% of the consumers stated that they knew about royal jelly, 39.8% did not, and 12.4% had no idea about it. Again, while 34.9% stated that they had knowledge and 19.1% had no idea about it, 46% of the consumers stated that they did not know about bee venom. In the study, 53% of the consumers who participated in the survey stated that they knew about propolis, 33.6% knew and 13.4% had no idea. In the study conducted by Tunca (2015), 22% of the consumers stated that they had one way or another heard of propolis from bee products, while 78% stated that they had never heard of it. In a study by Dağdemir and Akdemir (2021), it was determined that the consumers did not know pollen, royal jelly, beeswax, bee venom and propolis at a rate of 46.75%, 67.50%, 69.75%, 87.00% and 81.30%, respectively. In the study conducted by Bölüktepe and Yılmaz (2008), the recognition levels of pollen, royal jelly, beeswax, bee venom and propolis were determined as 61.10%, 52.80%, 46.40%, 16.30% and 8.90%, respectively. In the study conducted by Niyaz and Demirtaş (2017), it was determined that consumers knew pollen, royal jelly, beeswax, propolis and bee venom at a rate of 69%, 50%, 58%, 21.70% and 27.40%, respectively. Consumers' levels of knowledge about propolis, pollen, bee venom and royal jelly were 28.2%, 22.9%, 56.8% and 23.3%, respectively (Tunca et al., 2015).

Main Findings Regarding Honey Consumption Consciousness of Consumers

The frequency values (n and %) obtained from the answers given to the questions asked to determine the honey consumption consciousness of the consumers and their families participating in the research are given in Table 4.

According to our findings, 97.4% of the consumers participating in the survey stated that they consume honey, while 2.6% do not. It was determined that 0.8% of the consumers who do not consume honey, do not trust the produced bee products, 0.5% find honey expensive and 0.3% have diabetes. On the other hand, 50.9% of the consumers who consume honey stated that they consumed honey for health, and 41.9% stated that they consumed honey as a food source.

When consumers were asked which type of honey they prefer, 68.2% of the consumers stated that they preferred liquid honey while 31.8% preferred comb honey. In this study, 16% of the consumers stated that they consume honey comb because it is more nutritious, 17.8% of them prefer to consume honey comb because it is more difficult to cheat and 21.4% of them like to consume honey comb. In addition, 44.7% of them stated that they did not have any idea about honey comb. In the study conducted by Dağdemir and Akdemir (2021), it was determined that 54.75% of the consumers prefer comb honey, and the most important reason for this is that it is more beneficial and healthy (37.90%).

Likewise, 60.5% of the consumers stated that they prefer liquid honey because it is easier to consume, 14.2% of the consumers stated as it looks cleaner and 12.7% of the consumers prefer it because it is easier to store. On the other hand, 12.7% of the the consumers stated that they did not have any idea about filtered honey. Akdemir and Dağdemir (2021) stated in their study that consumers prefer filtered honey at a rate of 84.50%, and the most important reason for this is that it is easy to consume (43.79%). In the study conducted by Niyaz and Demirbaş (2017), it was determined that filtered honey is more preferred than honey comb honey. Similarly in another study, Sayılı (2013) stated that 86.76% of the consumers in the province of Tokat preferred to consume filtered honey.

When the consumers were asked about their honey preferences according to the source, it was determined that 62.8% of the consumers preferred flower honey. In a previous study, Coşkun (2019) found that the consumers mostly preferred flower honey (67.9%), while Baki et al. (2017) stated that 31% of the consumers consumed flower honey. To the question of how much honey is consumed per week in your household, 66.4% of the consumers stated that they consume 0.5 kg of honey on average. In a similar study, Coşkun (2019) found that 34% of the consumers consume an average of 0-2 kg of honey per year. Tunca et al. (2015) carried out research in 11 provinces in Turkey. They found that the percentage of consumers consuming 0-500 grams of honey per month is around 40%. In addition, 51.2% of consumers buy honey from beekeepers whom they generally know, and 41% of consumers buy honey from market and bazaar. Klickovic et al. (2017) found that 47% of consumers consume honey several times a week. On the other hand, the percentage of respondents who consume honey very rarely is 12%. In another study, the frequency of honey consumption was asked to young people, the percentage of respondents who consume honey every day is around 11%, while the percentage of responders who consume honey occasionally is about 55% (Zak, 2017).

Moreover, 15.8% of the consumers participating in the research stated that they do beekeeping. It was determined that 9.6% of beekeeping consumers attended courses related to beekeeping.

Table 4. Statistical Values on Honey Consumption Consciousness of Consumers

No	Questions	Options	Frequency	
			n	%
1	Do you consume honey?	No	10	2.6
		Yes	377	97.4
2	If your answer is no, why?	Expensive	2	0.5
		I don't trust	3	0.8
		I am diabetic	1	0.3
		Others	4	1
		Non-responders	377	97.4
3	If your answer is yes, for what purpose do you consume honey?	As a source of nutrients	162	41.9
		For my health	197	50.9
		Others	18	4.7
		Non-responders	10	2.6
4	What kind of honey do you prefer?	Comb honey	123	31.8
		Liquid honey	264	68.2
5	What are the factors affecting your choice of comb honey?	Because it is more nutritious	62	16
		Because cheating is more difficult to confuse	69	17.8
		I like to consume comb honey	83	21.4
		I have no idea	173	44.7
6	What are the factors affecting your choice of liquid honey?	It looks cleaner	55	14.2
		Easier to consume	234	60.5
		Easier to store	49	12.7
		No idea	49	12.7
7	Which honey do you prefer according to its source?	Flower honey	243	62.8
		Glandular honey	5	1.3
		Chestnut honey	139	35.9
8	How much honey is consumed per week in your household (kg)?	0.5	257	66.4
		0.5-1.0	90	23.3
		1.0- 2.0	28	7.2
		2 and above	12	3.1
9	Do you do beekeeping?	No	328	84.8
		Yes	59	15.2
10	If yes, have you attended a training/course on beekeeping?	I did not participate	22	5.7
		I did participate	37	9.6
		Non-responders	328	84.8
11	If your answer is yes, for what purpose do you do beekeeping?	As a hobby	17	4.4
		Commercially	20	5.2
		For family need	22	5.7
		Non-responders	328	84.8
12	What is your opinion about crystallized honey?	Due to the natural structure of honey	182	47
		Honey is not pure	70	18.1
		Honey is spoiled	6	1.6
		I have no idea	129	33.3

In the research, 5.7% of beekeeping consumers stated that they do beekeeping for the needs of the family, 5.2% are beekeeping for commercial purposes and 4.4% of them are beekeeping as a hobby. At the same time, when consumers were asked about their opinions about crystallized honey, 47% of the consumers stated that it is due to honeys natural structure, 18.1% of the consumers stated that honey is not pure and 1.6% of the consumers stated that honey is spoiled. On the other hand, 33.3% of the consumers answered that they do not have any idea about crystallized honey. According to the result obtained from the study, it can be said that consumers are generally conscious about this issue. In the study conducted by Coşkun (2019), when consumers were asked about their opinions about crystallized honey; 52.52% of the consumers stated that honey originates from its natural structure, 19.45% of the consumers stated that honey is not pure and 8.96% of the consumers stated that honey is spoiled. However, 19.07% of them stated that they had no idea about frozen honey. In the study conducted by Aytop et al., (2019), it was determined that 84.4% of the consumers consume honey. In a similar study, Onuç (2020) determined that honey is mostly consumed for nutritional purposes at breakfast. Kos Skubic et al. (2018) found that the price of products is the most important factor affecting a consumer's willingness to buy honey. Schifani et al. (2016) and Nabwire (2016) stated that consumers prefer buying local honey and are willing to pay more to local honey products. In addition, according to Batt and Liu (2012), brand reputation, the origin and the price of honey products are the most important factors affecting consumers' attitudes towards buying honey.

Conclusion

It is known that approximately 40% of the protein that needs to be consumed in order to a person to have a sufficient and balanced diet should be met by proteins of animal origin. Proteins of animal origin contain essential amino acids required for human health in a sufficient and balanced manner. Malnutrition has negative effects on people's health all over the world. For this reason, safe food production and balanced nutrition will be the most important health factor in the future as in the past and today. Honey is one of the most important protein sources of animal origin.

As in the whole world, the demand for organic agricultural products in Turkey is increasing gradually depending on the income level. One of these products is honey. In this study, it was tried to determine what the consumers thought about organic honey and it was determined that 86.6% of the consumers paid attention to the organic honey. These findings show that the consciousness level of consumers is quite high on this issue. For this reason, it is necessary to increase the production of organic honey in order to meet these demands of consumers. In the current study, 88.6% of the consumers stated that they do not trust the honey purchased from the internet. Again, 71.6% of the consumers stated that they obtain honey directly from the honey producer and this is safer. It has also been determined that 84% of the consumers pay attention to the expiration date of honey when purchasing honey from the markets, and it has been understood that the level of consciousness of the consumers is high. The publications containing confusing, incorrect and incomplete information about bee products in the visual and written media affect people negatively about honey consumption. For this reason, the information to be given about bee products in the written or visual media should be given by people and institutions who are experts in their field, and the consciousness level of the consumers should be increased.

Ethical Statement

There are no ethical issues with the publication of this article.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Author Contributions

Author 1: Investigation, Writing
Author 2: Investigation, Writing – Review & Editing, Supervision

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RESEARCH PAPER

The Importance of Plants Belonging to Asteraceae Family as a Pollen Source in Beekeeping

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Abstract

In beekeeping, flowering plants grown in flora are important in terms of nectar and pollen. These plants are the only basic food sources for honey bees. This research was carried out in an area of approximately 2000 ha in Ordu, Altınordu District, and Dedeli Locality to identify the existing taxa and pollen sources in the flora. Hazelnut (*Corylus avellana* L.) is grown intensively in the study area. Along with hazelnut gardens, there are also forest areas consisting of several decares. Plant taxa were identified during the flowering period in March, April, and May the growing period and flight activity time of honeybees. Additionally, 200 samples were collected from the pollen pellets of three bee colonies placed in the area between 07.00 am and 03.00 pm, one day a week for three months. As a result of a two-year study, 156 taxa belonging to the Asteraceae family were identified. Considering the number of taxa (with five taxa) and pollen pellet density (16.53%), it was observed that the taxa belonging to Asteraceae family were preferred more than other taxa. *Taraxacum officinale* W., *Bellis perennis* L., *Doronicum* spp. B., *Sonchus asper* L., and *Calendula arvensis* L. have been found to be important by honey bees as a source of pollen for the Asteraceae family. Thus, it has been determined that honey bees do not prefer every taxa equally in the flora as a source of pollen.

Introduction

Beekeeping is an agricultural activity that involves the use of honey bee (*Apis mellifera* L.) colonies to produce honey, pollen, and royal jelly, and facilitate pollination during periods of high nectar flow in the region (Güler, 2017). Bees and flowering plants are natural partners, with each performing certain functions for the other's survival and reproduction, creating a bond of coexistence (Sorkun et al., 2012).

Many flowering plants are pollinated by insects, with bees being the most important. Of the approximately 250000 flowering plant taxa in the world, it is known that 20000 are visited by bees (Kaufman, 1989).

The honey bee (*Apis mellifera* L.) collects pollen from flowers' stamens of seed plants and combines this pollen with their oral secretions into pellets (Çobanoğlu et al., 2021). Pollen is the only natural protein source for honey bees. The need for pollen also varies with the seasonal changes in the reproduction activities and production of larval food in the bee colony. The times when they have the greatest need for pollen are the beginning of spring and summer, when the colonies show reproductive, larval rearing, comb building, and multiplication behaviors (Güler, 2017).

In the flora of Türkiye, plants such as milkvetch, ivy, henbit, thyme, lavender, mint, mustard, clover, white clover, red clover, crimson clover, sweet clover, and bird's-foot trefoil are also very important nectar and pollen sources for bees (Genç & Dodoloğlu, 2011).

The Asteraceae family has a worldwide distribution, with special relevance in the Mediterranean, Eastern Europe and Asia Minor, being acknowledged about 25000 species integrated in approximately 1000 genera (Bessada et al., 2015).

In beekeeping, for high productivity to be achieved, the region where beekeeping is conducted should be rich in pollen and nectar sources. Additionally, the beekeeper should have knowledge about the flora of the area where beekeeping is practiced and should be familiar with the nectar and pollen sources that the bees utilize. The identification of local nectar and pollen sources is important in this regard.

In this study, it is aimed to determine the flower plants that are belonging to Asteraceae family and also important for local pollen sources for honeybees in the spring season in Dedeli Locacity in Ordu.

Material and Methods

The study was conducted between 2013 and 2015. In 2014, data could not be collected due to unfavorable weather conditions. The research was conducted in Altınordu District, Ordu Province, using three Langstroth type wooden (Anonymous, 1979) beehives with pollen traps (with a diameter of 5 mm and made of plastic material) during the flowering periods of March, April, and May, when the honey bees start their flight activities and development phases. Pollen samples were collected from each of the three hives every week for three months, between 7.00 am - 03.00 pm and stored in -18°C deep freezer. On the days when the traps were active, 200 pollen pellets were randomly selected from each of the three hives according to Sawyer (1988), and those with a density of more than 45% were evaluated in the dominant (D) group, those between 16% and 45% in the secondary (S) group, those between 3% and 15% in the minor (M) group, and those less than 3% in the trace (T) group. To prepare sample pollen preparations, the pollen pellets collected from the traps were mixed with 15 mL of 0.7% physiological saline solution in 50 mL Falcon tubes using a shaker. From this mixture, a drop was placed on a slide using a 3 mL Pasteur pipette (made of polyethylene) and covered with a coverslip. Then, the slide was examined under a camera-equipped light microscope with a 40x/0.65 objective to identify the pollen grains and determine their sizes in horizontal and vertical directions (in micrometers, µm). The prepared slides, where the measurement and photography processes were performed, were secured with balsam on the slide-slide mount to create sample preparations. Ten measurements were made for each plant species' pollen (Anonymous, 2005).

The pollen preparations were prepared according to the Wodehouse (1935) method. In this method, the pollen collected from the anthers of flowers is placed on a clean slide and 2-3 drops of 96% ethanol are added to remove resin, oil, and air bubbles by heating in an oven (30-40°C). The pollen detected on the slide is then dropped with 1-2 mm^3 of basic fuchsin-glycerin gelatin, heated to melt. In this way, the pollen is evenly distributed on the slide and sealed with a lamella without any air bubbles (Aytuğ et al., 1971). In order to facilitate the freezing of preparations through labeling, they kept upside down at room temperature for 1-2 days. These preparations were examined under a light microscope (Zeiss Axio Scope A1) with a camera (40x/0.65) to determine the pollen. In addition, during this period, pollen from the anthers of flowers of plants in the region was collected to determine the plant taxa in the field, and photographs were taken.

Results and Discussion

In this study, 10 plant taxa belonging to the Asteraceae family were identified. It was observed that the number of plant taxa (with five taxa) and pollen pellet density (16.53%) from the Asteraceae family were preferred more than other flowers. *Taraxacum officinale* W. (dandelion), *Bellis perennis* L. (daisy), *Doronicum* spp. B. (leopard's bane), *Sonchus asper* L. (prickly sow thistle) and *Calendula arvensis* L. (marigold) from the Asteraceae family gained importance as pollen sources for honey bees (*Apis mellifera* L.). Images belonging to some defined pollen are given in Figure 1 and Figure 2.

Although various plants belonging to the Asteraceae family were found in the flora, such as *Cirsium arvense* (field thistle), *Matricaria chamomilla* (chamomile), *Tanacetum parthenium* (feverfew), *Tussilago farfara* (coltsfoot), and *Senecio vulgaris* (common groundsel), none of these specific plant species were observed in the pollen gathered by the bees. It was once again confirmed that honey bees do not prefer every plant in the flora as a pollen source. The pollen density of plants belonging to the Asteraceae taxa between 2013 and 2015 is shown in Table 1.

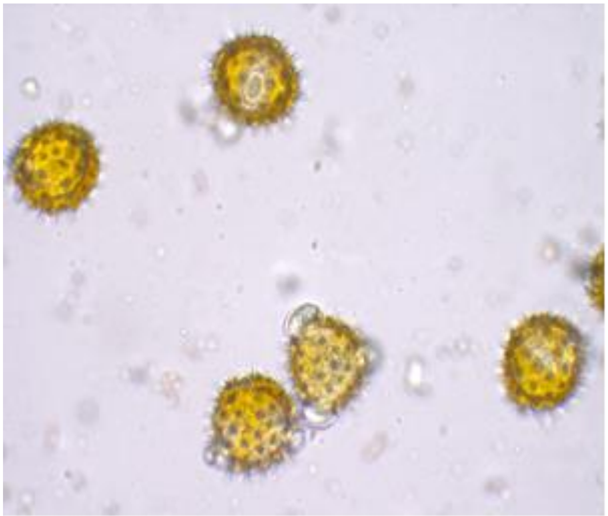

Figure 1. *Calendula arvensis* L. (marigold)

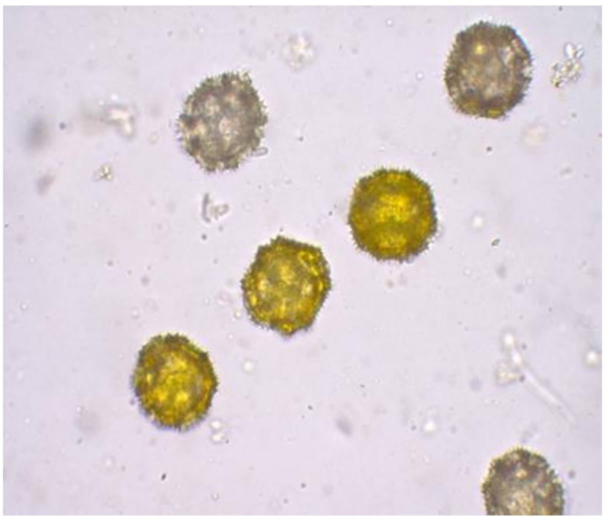

Figure 2. *Sonchus asper L. (prickly sow thistle)*

It has been observed that pollen from other plant species is not preferred by honey bees as a dominant pollen source (>45%) during these periods.

As can be seen from the table, there is a difference in the pollen density between years and months of the same year. The reason for the difference between months may be due to differences in the flowering periods of the species. The difference between years, on the other hand, can be explained by changes in climatic conditions. In a similar manner, it has been reported in a study conducted by Yaşar et al. (2006) in the Anzer Plateau of Rize province that climate factors have an impact on the foraging activity of bees in collecting nectar and pollen.

Baydar and Gürel (1998) reported that, in a study conducted by placing pollen traps on four colonies throughout the year in the natural flora of Antalya, it was determined that honey bees meet their pollen needs from 40 plant taxa belonging to 16 families. The most preferred taxa were those belonging to the Asteraceae and Fabaceae families. *Euphorbia characias*, *Taraxacum* spp., *Daphne sericea*, *Asphodelus fistolosus, Sinapsis arvensis, Raphanus raphanistrum, Calicotome villosa, Cistus creticus, Cistus salviifolius, Crepis* spp., *Acacia cyanophylla, Papaver rhoeas, Rubus sanctus, Myrtus communi, Vitex agnus-castus, Inula viscosa, Urginea maritima, Cerotonia siliqua* and *Eucalyptus* spp. were identified as the most important pollen sources.

Table 1. The pollen density of plants belonging to the Asteraceae taxa between 2013 and 2015

Pollen Sources		Months					
Family	**Taxa**	**March**		**April**		**May**	
		2013	**2015**	**2013**	**2015**	**2013**	**2015**
Asteraceae	*Taraxacum officinale* W.	S	M	T	T	T	-
	Bellis perennis L.	T	-	S	M	M	M
	Doronicum spp.	T	-	T	-	-	-
	Sonchus asper L.	-	-	-	-	M	-
	Calendula arvensis L.	-	-	-	-	T	-

** Dominant (D) >45%, Secondary (S) 16-45%, Minor (M) 3-15%, Trace (T) <3%*

In a study of 45 honey samples collected from various areas of the Rize-Anzer Plateau, microscopic analysis found 19 families (with the most common families being Asteraceae at 16%, Fabaceae at 14%, Lamiaceae at 14%, and Rosaceae at 8%) with 42 pollen taxa (Sorkun, 2003).

In studies conducted in various regions of New Zealand to determine pollen preferences of bees in the spring, it was found that *Cordyline australis, Taraxacum* spp.*, Trifolium* spp.*, Ulex europaeus, Pseudopanax crassifolius, Salix* spp. plants were highly preferred in the Kaitaia region, *Ranunculus* spp.*, Taraxacum* spp. plants were preferred in the Raetihi region, taxa from the Asteraceae family were preferred in the North Canterbury region, the *Ulex europaeus* taxa was preferred in the Wainuimata region, and the *Pennantia corymbosa* taxa were preferred in the Dunedin region (Webby, 2004).

In a study conducted by Dimou and Thrasyvoulou (2007), pollen pellets were collected daily from 4 colonies and the flowering plants around the apiary were recorded. Based on field records, it was found that 204 taxa belonging to the Asteraceae, Fabaceae and Rosaceae families were the most important taxa.

Karaca (2008) identified 595 plant taxa belonging to 73 families that honey bees (*Apis mellifera* L.) could use in the Aydın region. Based on previous studies, the plant taxa in the top three were; 129 taxa from the Fabaceae family (22%), 57 taxa from the Asteraceae family (10%), and 49 taxa from the Labiateae family (8%), while other families followed.

Sorkun and Süer (2013) identified 54 taxa belonging to 31 families from the pollen collected from three regions in Bursa. Of these taxa, the most was found in the Asteraceae family (16%).

Baydar and Gürel (1998) have stated that *Taraxacum* spp.*, Sinapsis arvensis, Cistus salviifolius, Calendula arvensis, Trifolium* spp. *and Salvia* spp. are preferred by bees as pollen sources, and Webby (2004) has indicated that *Taraxacum* spp., *Trifolium* spp., and *Salix* spp. are also preferred. At the family level, Sorkun (2003) found that Asteraceae was the most important family, comprising 16% of the pollen collected, Dimou and Thrasyvoulou (2007) found that Asteraceae, Fabaceae, and Rosaceae were important, Karaca (2008) found that Asteraceae was the most important family, comprising 57 taxa (10% of the total) and Sorkun and Süer (2013) found that Asteraceae was an important pollen source for bees. These results show similar trends in the pollen preferences of bees.

Conclusion

In this study, ten plants taxa belonging to the Asteraceae family were identified. Although several plants from the Asteraceae family were found in the flora, some of them were not observed in the pollen collected by bees. This confirms that honeybees do not choose every plant in the flora as a pollen source. The variation in pollen density throughout the years and months may be due to differences in flowering periods and climatic conditions.

Ethical Statement

Not applicable.

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Conflict of Interest

The authors declare there are no conflict of interest.

Author Contributions

Author 1: Investigation, Writing – review & editing; Supervision, Formal Analysis

Author 2: Investigation, Writing – review & editing

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RESEARCH PAPER

Comparative Morphological Analysis of Honey Bees (*Apis mellifera* L.) from the Western and Central Black Sea Region, with Emphasis on Natural Diversity

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Abstract

Due to migratory beekeeping and nationwide sales of queens and colonies, genetic mixes are a major concern. Nonetheless, providing beekeepers with quality breeding material will boost production. Two hundred colonies were chosen from the Western and Central Black Sea Region, specifically from Düzce, Kastamonu, Sinop, and Ordu. These colonies were selected from locations where migratory beekeeping is not practiced, and the beekeeping firms involved have abstained from using commercial queen bees for three generations, between 2014 and 2022. Each sample has 10 worker bees with 41 morphometric measures. The local bee genotype was compared to Caucasian, Anatolian, and Yığılca genotypes, whose comprehensive morphological traits were known. Four local bee genotypes differed significantly (P<0.05) in 39 morphological features. These four bee genotypes clustered into three function value groups. With 98.9% accuracy, the 271 worker bee samples from the four genotypes were categorized in respective fields. Anticipated were strong morphological similarities between worker bee samples from the nearby regions of Yığılca and WCBS. The Caucasian bee subspecies, along with the other three genotypes, had a significant level of morphological likeness and overlap. Selection may affect qualitative characters (color, etc.) like quantitative characters across generations. Protect, generate, and provide breeding material with better productivity, wintering capacity, and morphology.

Introduction

Honeybee (*Apis mellifera* L.) populations can be found in almost every region of the world, despite the fact that the African Continent is the species' original home. These honey bee populations have adapted to very different ecological conditions and have different structures in terms of morphological, physiological, behavioral, and molecular structure as a result of these adaptations (Alpatov, 1929; Bilash et al., 1976; Ruttner, 1988; Rinderer et al., 2010). Honey bee populations that have settled in different isolated regions have developed distinct morphological characteristics as a result of natural selection, genetic drift, and mutation (Ruttner et al., 1978; Settar, 1983; Bodur et al., 2007; Güler, 2010). These morphological differences have been attributed to natural selection, genetic drift, and mutation. Variations in populations that were established in distinct isolating zones propagated independently of one another, which resulted to the emergence of new genotypes (Alpatov, 1929). When the climate had reached a stable state, they were redistributed to their previous habitats, where they met and shared genetic material (Alpatov, 1929; Ruttner, 1988; Smith, 1991). The subspecies of the honeybee were categorized into three major lineages: A (Africa), C (Carnica), and M (Mellifera). This classification was developed by taking into consideration the physical characteristics of the honey bee (Ruttner et al., 1978; Rinderer et al., 1993; Smith et al., 1997; Palmer et al., 2000; Ruttner et al., 2000; Kandemir et al., 2000). The abbreviation of Region Honey Bees is WCBS.

Breeders don't recommend some types of bees because they don't make enough honey, swarm a lot, or are very mean. Native bee breeds were threatened with extinction as a result of the preference for alien kinds. In terms of consistency in local settings, however, European breeds fall short of breeders' goals. Since pure European-breed colonies cannot produce more honey than breeding stock under these circumstances, and because their hybrids grow more aggressive, pure European-breed colonies cannot produce more honey than breeding stock. The Western Black Sea is commonly recognized as one of the most important bee gene pools in Anatolia. Although the area lies outside of the bees' migration route, it is favorable for the preservation of genetic and breeding material. On the population's behavior, performance, and morphology, however, there is inadequate study and data. In this regard, it is unknown whether the population in the region is homogeneous, whether it is exposed to the mixture, whether it is a distinct taxonomic unit, and whether it is related to the taxonomy of races in Anatolia, such as *Apis mellifera anatoliaca, Apis mellifera caucasica, Apis mellifera carnica, Apis mellifera syriaca, Apis mellifera* (Kandemir et al., 2000; Güler, 2010)

According to Ruttner (1988), *A. m. caucasica* is found from Samsun to the north-east of Turkey, *A. m. meda* is found at Southeastern Anatolia, *A. m. syriaca* is found along the Turkish-Syrian border and in the province of Hatay *A.m. syriaca*, and *A. m. anatoliaca* Mugla honey bee genotype were found in the rest of Turkey reported in 2022.

The objective of this study is to analyze the morphological structure of the bee population in the Central and Western Black Sea Region. This population displays clear distinctions from the existing bee races in Turkey and has been the focus of a relatively little amount of research up to this point. This population is being preserved in its native territory so that it can continue to contribute to the genetic diversity of Anatolian bees and ensure the continued viability of beekeeping in the country as a whole. The research entails both the characterization of material that has been subjected to three generations of selection and the comparison of its morphological structure with bee races and genotypes that have adapted to geographical areas that are nearby.

Material and Methods

Material

The material has been utilized to produce 200 colonies, all of which were collected in 2014 from stationary apiaries located at some regions of the Western and Central Black Sea Regions. In these regions there is no practice migratory beekeeping and do not make use of commercial queens. Western and Central Black Sea Region Honey Bees is abbreviating WCBS.

Method

These colonies were chosen because the index values of the 50 colonies that formed in the 25% slice showed that they did better in terms of honey production, how well they raised their young, and how well they survived the winter. Each chosen colony was represented by four queens from the same family in each generation. In this direction, queen bees were raised every two years in May and June. Ten microliters of homogenized semen from colonies other than their own was used to artificially inseminate these queens. The colonies took the queen bees that had been fertilized, and the herd size was back up to 200. The study, which began in 2014, is now in its third generation, with a performance test every other year and a selection every other year. At the end of the third generation, worker bee samples were taken from 50 colonies during the swarming period. Morphometric measurements were taken of 10 worker bees and 41 characters in each sample (Alpatov, 1929; Ruttner et al., 1978; Güler & Kaftanoglu, 1999a; Güven, 2003).

The morphological data of 98 Anatolian and 98 Caucasian race samples, as well as 25 samples of Düzce Yığılca ecotypes, previously examined by the Faculty of Agriculture at 19 Mayıs University, were compared with 50 samples of WBCS in our study. Totally 271 samples were used. The utilization of the Anatolian race, Caucasian race, and Yığılca ecotype in the comparison is attributed to their original distribution areas of the WBCS bee.

Morphological Evaluation

The samples were collected in June, when the colonies had the young worker bees. Ten worker bees from each sample were used to collect morphometric measurements of 41 common morphological features. The morphological characters measured were the following in each worker bee: fourth tergite length of hairs (LH, mm), fourth tergite hair band width (WTa, mm), fourth tergite hair shiny surface width (WTb, mm), tomentum index (TI, ratio), length of proboscis (LPr, mm), length of femur (LF, mm), length of tibia (LT, mm), length of metatarsus (LM, mm), width of metatarsus (WM, mm), metatarsal index (MI, ratio) , hind leg length (LHL, mm), third tergite width (WT_3, mm), third sternite width (WS_3, mm), wax gland surface length (MSU, mm), wax gland surface width (WWM, mm), distance between wax surfaces (MAM, mm), sixth sternite length (LS_6, mm), sixth sternite width (WS_6, mm), sternum index (S_6I, ratio), wing length (LH, mm), wing width (KG, mm), cubital vein a length (LCa, mm), cubital vein b length (b, mm), cubital index (CI, ratio), second tergum (CT_2), third tergum (CT_3), and scutellum (CSc) colors, as well as the morphometric measurements of wing vein angles A_4, B_4, D_7, E_9, G_{18}, J_{10}, J_{16}, K_{19}, N_{23}, and O_{26} (Alpatov, 1929; Dupraw, 1965; Ruttner et al., 1978; Moritz, 1991; Kauhausenkeller ve ark., 1997; Akyol, 1998; Güler & Kaftanoğlu, 1999a; Güler, 2001; Güler & Bek, 2002; Güler et al., 2010). The measurements were measured by stereomicroscope in the morphometric measurement package.

Statistical Evaluation

In this investigation, the morphological characteristics of worker bees from four distinct genotypes were thoroughly analyzed. Single-factor analysis of variance (ANOVA) was applied to the data for 41 morphological traits to assess the variations within the categories. Subsequently, Multivariate Discriminant Analysis was utilized to ascertain the level of variation within the region. To evaluate the differences between groups, ANOVA was used to determine the mean values and standard deviations, and the DUNCAN multiple comparison test was used to compare the means. Analysing the relationships between traits, distinguishing morphological characteristics, and non-distinguishing ones was also part of the analysis. It was determined the functions representing these characteristics and their discriminate power, as well as Fisher's linear, standard, and non-standard Discriminant Function Coefficients representing the genotypes, and the Constant Discrimination Coefficients. The study also utilized Discriminant Analysis to determine if there were significant differences among the groups, analyzing variance and providing means with standard deviation. The tables displayed averages with standard deviations. Manova was also used in conjunction with Multivariate Discriminant Analysis to assess the degree of variation within characteristics.

Results

In terms of morphological measurements, comparison of the WCBS and Yığılca genotypes alone would not be adequate or correct. These two bee genotypes are thus mostly Anatolian and Caucasian based. It would be more appropriate to compare the morphological structure of the breeds. The standard morphological characteristics of 271 worker bee samples from Anatolian and Caucasian bee varieties, as well as Yığılca and WCBS bee genotypes, were investigated in this study. The data were sourced from the beekeeping unit at 19 Mayıs University Faculty of Agriculture. In Tables 1, 2, 3, and 4, the mean and

Table 1. Morphological traits (mean±sem) for honeybee genotype groups

Character	Provinces				Average
	WCBS	Anatolia	Caucasian	Yığılca	
LH	0.221±0.004^{c}	0.252±0.003^{b}	0.267±0.003^{a}	0.223±0.002^{c}	0.238±0.001
Wta	0.871±0.005^{b}	0.858±0.003^{b}	0.931±0.008^{a}	0.826±0.005^{c}	0.858±0.003
Wtb	0.329±0.005^{c}	0.536±0.003^{a}	0.440±0.012^{b}	0.523±0.003^{a}	0.483±0.005
LPr	6.545±0.018^{b}	6.436±0.011^{c}	6.528±0.018^{b}	6.671±0.014^{a}	6.549±0.009
LF	2.638±0.009^{b}	2.627±0.006^{b}	2.798±0.051^{a}	2,653±0.006^{b}	2.660±0.007
LT	3.273±0.010^{b}	3.098±0.006^{c}	3.429±0.013^{a}	3.299±0.008^{b}	3.241±0.008
LM	2.022±0.006^{c}	1.971±0.004^{d}	2.145±0.035^{a}	2.068±0.005^{b}	2.036±0.006
WM	1.157±0.004^{c}	1.164±0.004^{c}	1.281±0.006^{a}	1.180±0.004^{b}	1.183±0.003
W T_3	2.210±0.006^{b}	2.016±0.004^{d}	2.256±0.011^{a}	2.132±0.004^{c}	2.121±0.005
W T_4	2.154±0.007^{b}	1.982±0.004^{d}	2.199±0.014^{a}	2.086±0.005^{c}	2.076±0.005
WS_3	2.743±0.005^{c}	2.777±0.005^{b}	2.883±0.018^{a}	2.769±0.005^{b}	2.781±0 004
LWM	1.423±0.004	1.451±0.004	1.423±0.043	1.446±0.003	1.441±0.005
WWM	2.351±0.005^{b}	2.325±0.005^{b}	2.257±0.027^{c}	2.384±0.004^{a}	2.342±0.004
DWM	0.287±0.001^{c}	0.304±0.002^{a}	0.295±0.004^{b}	0.291±0.001^{bc}	0.296±0.001
LFW	9.107±0.024^{b}	8.376±0.009^{c}	9.562±0.054^{a}	9.056±0.018^{b}	8.891±0.026
WFW	3.105±0.009^{b}	2.821±0.004^{c}	3.320±0.043^{a}	3,134±0.007^{b}	3.043±0.012
LCa	0.528±0.004^{b}	0.479±0.002^{c}	0.483±0.004^{c}	0.539±0.002^{a}	0.509±0.002
LCb	0.241±0.002^{b}	0.250±0.001^{a}	0.219±0.003^{c}	0.241±0.001^{b}	0.241±0.001
LS_6	2.531±0.005^{c}	2.548±0.004^{bc}	2.671±0.012^{a}	2.557±0.005^{b}	2.564±0.004
WS_6	3.101±0.009^{b}	3.119±0.006^{b}	3.336±0.021^{a}	3.123±0.006^{b}	3.144±0.006

LH= length of hair, WTa=width tomentum a WTb= width tomentum b, LPr= Length of proboscis, LF= Length of femur, LT= Length of tibia LM = Length of metatarsus WM = Width of metatarsus, WT_3 = Width of tergite WS_3= Width of sternite LWM= Length of wax mirror, WWM= Widht of wax mirror, DWM= D between mirrörs, LFW= length of forewing, WFW=wing of forewing LCa= length of cubital a LCb= length of cubital b, LS_6= length of sternum 6 , WS_6= width of sternum 6 , WT_3+WT_4= BS body size standard error values for each group of bees studied are presented.

In a one-way variance analysis, it was determined that four regions of bees were significantly different from each other in terms of 39 morphological characters, except for the wing O_{26} vein angle and the length of the wax mirror (LWM) ($P < 0.05$). Especially when these four regional bees were characterized quantitatively or descriptively in terms of morphology, it was observed that they exhibited different structures.

In terms of WBCS, it was determined that the average largest wing B_4, E_9, and K_{19} vein angles, tomentum index (TI), cubital index (CI), sternum under index (S_6I), and second yellowest (CT_2), third (CT_3), fourth (CT_4) tergum and scutellum (CSC) color were found in WCBS genotype. On the other hand, this bee had the smallest average wing A_4, D_7, L_{13} vein angles, shortest length of hairs (LH), fourth tergum felt glossy ground width (WTb), metatarsal length (LM), metatarsal width (WM), sternum under width (WS6), wax mirror distance (DWM), sternum under length (LS_6), hind leg length (LHL) and metatarsal index (MI).

The Anatolian bee had the largest average fourth tergum felt glossy ground width (WTb), wax mirror distance (DWM), cubital b vein length (LCb), wing E_9 and L_{13} vein angles, scutellum (CSC) color and sternum under index (S_6I) value. On the other hand, it showed the smallest average length of proboscis (LPr), length of tibia (LT), metatarsal width (WM), shortest wing length (LFW), cubital a vein length (LCa), wing J_{10} vein angle, and third tergite color (CT_3).

Table 2. The mean and standard error values of the vein angles A4, B4, D7, E9, J10, J16, N23, L13, K19, G12, and O26 observed in worker bee samples from four regions

Character	Provinces				Average
	WCBS	Anatolia	Caucasian	Yığılca	
A_4	32.087±0.177 ^{c*}	32.935± 0.134^{b}	34.981±0.229 ^{a}	32.529±0.104 ^{ab}	32.898±0.088
B_4	104.646±0.422 ^{a}	102.152± 0.276^{b}	100.528±0.566 ^{c}	102.458± 0.301^{b}	102.501±0.188
D_7	100.597±0.276 ^{c}	101.655± 0.216^{b}	102.974±0.240 ^{a}	100.880±0.175 ^{c}	101.361±0.120
E_9	20.468±0.111 ^{a}	20.416±0.111 ^{a}	20.688±0.132 ^{a}	19.341± 0.074^{b}	20.084±0.062
J_{10}	52.238± 0.312^{b}	48.499±0.205 ^{c}	56.089±0.396 ^{a}	51.927± 0.203^{b}	51.305±0.191
J_{16}	91.435± 0.292^{b}	92.128±0.222 ^{ab}	87.661±0.348 ^{c}	92.297±0.229 ^{a}	91.507±0.157
L_{13}	14.430±0.095 ^{c}	17.774±0.097 ^{a}	14.344±0.204 ^{c}	15.304± 0.099^{b}	15.890±0.102
N_{23}	89.415± 0.289^{b}	88.520± 0.281^{b}	84.243±0.634 ^{c}	93.581±0.228 ^{a}	89.911±0.241
K_{19}	76.965±0.224 ^{a}	75.680± 0.195^{b}	76.288±0.307 ^{ab}	76.351±0.177 ^{ab}	76.218±0.110
G_{12}	92.010± 0.277^{b}	88.477±0.223 ^{c}	95.921± 0.394^{a}	91.968± 0.173^{b}	91.250±0.186
O_{26}	34.952±0.302	36.052±0.191	37.497±0.334	35.871±0.234	35.973±0.131

*=different letters indicate different averages

Among the genotypes, the Caucasian bees displayed the greatest diversity in measured morphological characteristics compared to other bee breeds, with the highest average values. This bee showed the longest hair (LH), the largest fourth tergum velvet ground width (WTb), length of femur (LF), length of tibia (LT), metatarsal length (LM), metatarsal width (WM), the widest width of tergite fourth tergum width (WT_3 and WT_4), third sternum width (WS_3), wing length (LFW), sternum lower length (LS_6), sternum lower width (WS6), the largest wing vein angles A_4, D_7, E_9, J_{10}, G_{12}, O_{26} , the largest hind leg length (ABU), the largest body size (WT_3+WT_4=BS), cubital index (CI) and metatarsal index (MI) (Tables 1, 2, 3, and 4). Additionally, it exhibited the lowest average values for wax gland surface width (WWM), cubital vein lengths a and b, the smallest wing vein angles B_4, J_{16}, L_{13}, N_{23}, fourth tergum color (CT_4), and the most yellow scutellum color (CSC). As seen, the Caucasian bee breed exhibited the longest hair (LH), the largest wing vein angles, the darkest color, the largest body, the longest hind leg, and the largest cubital (CI)

Table 3. Mean and standard error values of the second, third tergite and scutellum colors (scale) of worker bee samples from four regions

Character	Provinces				Average
	WCBS	Anatolia	Caucasian	Yığılca	
CT_2	7.330±0.125 [a]	6.539± 0.104[b]	5.380±0.177 [d]	5.793±0.085 [c]	6.275±0.067
CT_3	7.125±0.066 [a]	5.765±0.065 [c]	5.165± 0.199[d]	6.430± 0.074[b]	6.164±0.056
CT_4	4.438±0.059 [a]	2.859± 0.050[b]	2.609± 0.196[c]	4.257±0.069 [a]	3.596±0.060
CSc	2.337±0.096 [a]	2.207±0.094 [a]	0.376±0.066 [c]	1.226± 0.066[b]	1.660±0.060

CT_2 = color of second tergite, CT_3= color of third tergite, CT_4 = color of fourth tergite, CSc = color of scutellum, [*]= different letters indicate different averages

and metatarsal indices among the morphological structures.

The Yığılca bee genotype exhibits the highest values for average length of proboscis (LPr), fourth tergite wax patch width (WTb), wax gland surface area (WWM), cubital vein A1 length, wing vein angles J_{16} and N_{23}, fourth tergite color (CT_4), cubital index (CI), and sixth sternum index (S_6I). It has the shortest length of hairs on average (LH), the smallest fourth tergite wax band width (WTa), third (WT_3) and fourth (WT_4) tergite widths, the yellow second tergite color (CT_2), tomentum index (TI), the small body size (BS), and the small metatarsal index (MI).

Table 4. Mean and standard error values of tomentum, cubital and metatarsal index (ratio) and hind leg length (mm) determined by calculating worker bee samples from four regions

Character	Provinces				Average
	WCBS	Anatolia	Caucasian	Yığılca	
TI	2.819±0.068 [a]	1.654±0.018 [c]	2.253± 0.048[b]	1.612±0.015 [c]	1.922±0.031
LHL	7.934± 0.024[c]	7.698±0.015 [d]	8.373±0.077 [a]	8.022± 0.019[b]	7.937±0.018
BS	4.365± 0.012[b]	3.999±0.007 [d]	4.456±0.024 [a]	4.219±0.009 [c]	4.198±0.011
CI	2.227±0.033 [a]	1.958± 0.016[b]	2.214±0.028 [a]	2.266± 0.021[a]	2.145±0.014
MI	57.251± 0.177[c]	59.155± 0.220[b]	60.740±1,815 [a]	57.087± 0.144[c]	58.293±0.255
S_6 I	81.709±0.232 [a]	81.773±0.132 [a]	80.126± 0.243[b]	81.929±0.136 [a]	81.611±0.090

TI = tomentum index, LHL = hind leg length, BS($T_3 + T_4$)= body size, CI = cubital index, MI = metatarsal index, S_6 I = sixth sternum index, [*]= different letters indicate different averages.

In general, the WCBS breeding material showed similarity with the Anatolian bee race in 10 (WTa, LT, WM, WWM, WS_6, E_9, J_{16}, N_{23}, CSC, and S_6I) morphological characters, with the Yığılca genotype in 12 (LH, LT, LFW, a, D_7, J_{10}, G_{12}, CT_4, CI, MI, and S_6I) characters, and with the Caucasian bee race in 4 (LPr, E_9, L_{13}, and CI) characters.

In addition, multivariate discriminant analysis was carried out on 41 morphological characteristics of 271 worker bee samples that were representative of the four different bee genotypes. In the process of grouping, all of the other 40 characters, with the exception of the O26 character, were determined to be significant ($P<0.001$). In addition, the body size measure, also known as the BS character, was not taken into account in the discriminant analysis that was performed on the sample grouping, which was dependent on the tolerance test.

The application of Linear Discriminant Analysis to morphological characteristics led to the determination of the number of functions responsible for the grouping, as well as their appropriate values, variance levels, Wilks' lambda, and significance levels. Table 5 contains the values related to these factors, and the table itself can be found here.

In addition, the discriminant function represented by each morphological character or the characters that

Table 5. The values for the fitness values, variance, total variance levels, WilksLambda values and significance levels of the grouping functions

Function	Fitness Values	Variance (%)	Total Variance (%)	Wilks lambda	*P*
1	117,637 [a]	79.00	79.00	0.000	0.000
2	24,120 [a]	16.20	95.30	0.005	0.000
3	7.064 [a]	4.70	100.00	0.124	0.000

a : The first three discriminant functions were found to be successful in grouping.

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Table 6. Morphological characters describing grouping functions and their related structure matrix

Morphological character	Function		
	1	2	3
LT	.656 *	.264	.188
LPr	-.066 *	.058	-.007
E_9	.056 *	.019	.028
LH	-.073	.403 *	.303
WT3	-.026	.363 *	.139
WT3+ WT4(BS)	-.024	.337 *	.133
WTb	-.055	-.321 *	.226
L_{13}	.049	-.282 *	-.040
TI	-.218	.281 *	-.199
WT_4	-.019	.270 *	.111
WFW	-.064	.268 *	.231
CI	-.044	.104 *	.004
DWM	.017	-.063 *	.034
K_{16}	-.006	.047 *	-.033
MSU	-.003	-.024 *	-.003
O_{26}	.004	-.014 *	.000
WS6	.016	.076	.324 *
WM	.002	.078	.316 *
CSC	.041	-.061	-.275 *
LS_6	.006	.055	.275 *
CT_4	-.069	.102	-.269 *
CT_3	-.026	.067	-.265 *
A_4	.020	.018	.245 *
WS3	.013	.027	.242 *
G_{12}	-.030	.199	.220 *
ABU	-.029	.140	.212 *
J_{10}	-.026	.200	.210 *
CT_2	.035	.010	-.210 *
N_{23}	-.093	-.044	-.207 *
LFW	.043	-.041	.193 *
J_{16}	-.022	-.082	-.187 *
LCa	-.080	.092	-.169 *
LM	-.028	.088	.154 *
LCb	.003	-.086	-.146 *
B_4	.000	.027	-.140 *
LT	.002	.047	.139 *
WTa	.043	.064	.137 *
WWM	-.038	-.015	-.134 *
D_7	.017	-.005	.129 *
S_6I	-.014	-.040	-.120 *
MI	.020	-.010	.087 *

are effective in determining the function were determined and presented in Table 6.

Length of tibia (LT), length of proboscis (LPr), and wing E_9 vein angle characters were represented by the first function (F1), while length of hairs (LH), third tergite width (WT_3), body size (BS), fourth tergite shiny ground width (WTb), wing L_{13}, K_{16} vein angles, tomentum index (TI), fourth tergite width (WT4), wing width (WFW), cubital index (CI), and distance between wax glands (DWM) were represented by the second function (F2), and the other 25 characters were represented by the third function (F3). These three functions fully grouped the 271 worker bee samples representing the four regions. However, the first discriminant function alone could explain 79.00% of the total variance. The discrimination power of the first function can also be seen in the Wilks Lambda (0.000) value it received. As the WilksLambda value, which describes the power of a function in grouping, approaches 0, the discrimination power increases, which is easily seen in this study (Coley & Lohnes, 1971; Le, 2001). Moreover, this function achieved this discrimination power with only three morphological characters (LT, LPr, and E9) (Table 6). On the other hand, the WilksLambda value of the third function was low (0.124) and showed a grouping power of only 4.7% of the total variance. Therefore, it can be said that three functions are sufficient to group the samples. Indeed, the first and second functions explained 95.3% of the total variance. This result also shows that there is significant variation among genotypes in terms of their morphological structure (Güler & Kaftanoğlu, 1999b; Güler, 2010).

When the one-way analysis of variance (ANOVA) results from these three characters representing the discriminant function are examined, it is seen that the Caucasian bee has the longest length of tibia (LT) and the largest wing E_9 vein angle, while the Anatolian bee has the shortest length of proboscis and smallest tibia length.

The Anatolian, Caucasian bee races, WCBS and Yığılca genotypes were illustrated with 98, 35, 50 and 98 samples, respectively, in the group of 271 worker bee samples belong to four genotypes and evaluated using the Discriminant analysis method. The results of demonstrating the real or closest related groups and overlapping levels of the original samples are given in Table 7.

Table 7. The original and second most reliable predicted grouping numbers and ratios of 271 worker bee samples representing Anatolian, Caucasian bee breeds and WCBS and Yığılca genotypes

Original Regions	Estimated group membership			
	WCBS	**Anatolia**	**Yığılca**	**Caucasian**
WCBS	50 (100%)	0	0	0
Anatolia	0	98 (100%)	0	0
Yığılca	0	0	98 (100%)	0
Caucasian	2 (5.7 %)	1 (2.9 %)	1 (2.9 %)	31 (88.6 %)

The correct grouping levels of the original samples are 98.9%.

According to the discriminant analysis results of the samples representing the groups and the evaluation of their representation, discrimination, and similarities to their original groups, all 50 samples representing the WCBS genotype, 98 samples representing the Anatolian bee race, and 98 samples representing the Yığılca bee genotype have been grouped with 100% accuracy in their respective original groups. On the other hand, out of the total 35 samples representing the Caucasian bee race, two overlapped with the WCBS genotype, one with the Anatolian genotype, and one with the Yığılca genotype. Thirty-one samples represented the original Caucasian population. In this study, the Caucasian bee race showed morphological similarities of 5.7%, 2.9%, and 2.9% with the WCBS, Anatolian, and Yığılca bee genotypes, respectively.

The relationships, kinships, sharing areas, and which genotype formed the center group of the 271 worker bee samples representing these genotypes were determined in terms of their morphological structures, and the findings are presented in Figure 1.

Morphological difference according to discriminant functions (F1 and F2) is significant between

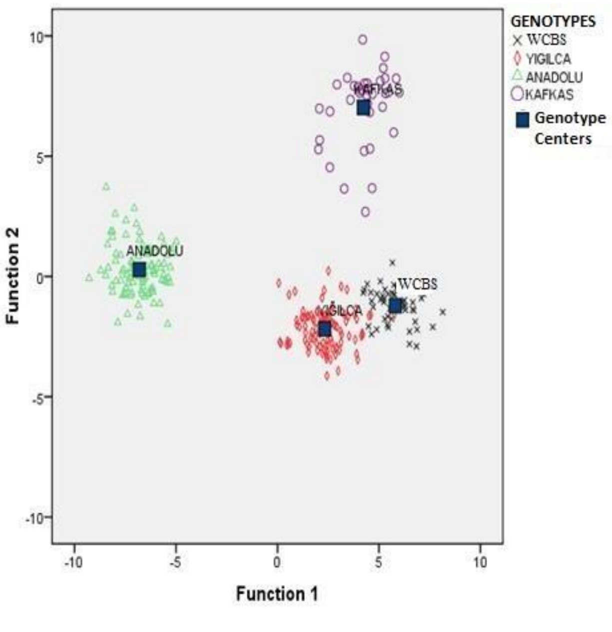

Figure 1. Distribution areas of 271 worker bee samples representing Anatolian, Caucasian bee breeds and WCBS and Yığılca genotypes in dimensionless measurement environment

all four bee genotypes (Table 7). In general, three different clustering areas depending on the size were formed in the coordinate system according to the function values. The first area of sharing was the Anatolian race, the second area was Yığılca and WCBS, and the third area was the Caucasian bee race. As can be seen in the dimensionless measurement ratio, Anatolian bee breed samples shared almost the same and narrow area in the coordinate system.

Discussion and Conclusion

Material obtained from different areas of the Central and Western Black Sea Region and subjected to three generations of selection was compared with Caucasian, Anatolian, and Yığılca bee races and genotypes in terms of 41 morphological characters. Statistical evaluation was performed using both one-way (randomized complete block design) and multivariate Discriminant Analysis, and it was observed that races and genotypes exhibited significantly different morphological structures in terms of all 39 characters except for LWM and wing O26 vein angle. On the other hand, three canonical discriminant functions correctly classified 271 samples belonging to 4 bee genotypes with a 98.9% accuracy rate. This level of discrimination power indicates that there is a significant genetic variation in terms of morphology. Indeed, it is seen that almost all of these races and genotypes are clearly separated from each other, and very low levels of overlapping sample matches occur in their distribution areas (Table 7).

The WCBS genotype is featured as the most yellow bee in terms of all color values (CT_2, CT_3, CT_4, and CSc) among the genotypes. The most yellow-colored bees distributed in Anatolia are the Syrian bee *(A. m. syriaca)* distributed in Southeast Anatolia and Anatolian bee races distributed in Central Anatolia (Ruttner et al., 1978; Akyol, 1998; Güler & Kaftanoğlu, 1999a; Gencer & Fıratli, 1999; Güler et al., 2012). Here, the WCBS exhibited a more yellow color than the Anatolian bee race except for Scutellum color. There is only a Scutellum color similarity between the Caucasian bee and the WCBS. However, in previous studies (Güler & Kaftanoğlu, 1999b; Güler & Toy, 2008; Güler et al., 2012), it was reported that Caucasian bees and Black Sea Region bees generally showed a dark color. In honey bees, the normal color of chitin is black, and this color is dominant over the yellow chitin color, which is recessive in effect. This dominant gene suppresses the effect of the recessive gene (Collins, 1986; Rinderer, 1986; Güler, 2017). Therefore, we estimate that the reason for the yellow color of the WCBS breeding material is due to the application of 3 generations of selection. It is known that the selection of behavior and performance traits of the breeding material was carried out using the index method. Therefore, the presence of yellow-colored colonies in the population and their selection as parents due to their high average index values increased the frequency of the recessive yellow color in the population over generations. It is thought that the color will become stabilized with one or two more generations of selection.

The WCBS genotype, after three generations of selection found to be similar to the Caucasian race in terms of wing vein angles (LPr), E_9 and L_{13}, and cubital index (CI), to the Anatolian race in terms of fourth tergum felt surface width (WTa), length of femur (LF), metatarsal width (WM), wax gland surface width (WWM), sixth sternum width (WS6), E_9 and N_{23} wing vein angles, scutellum color (CSc), and sternum bottom index (S_6I), and to the Yığılca genotype in terms of length of hairs (LH), length of femur (LF), length of tibia (LT), wing length (LFW), wing width (WFW), cubital B vein length, sternum bottom width (WS_6), D_7, J_{10}, G_{12} wing vein angles, fourth tergum color (CT4), cubital index (CI), metatarsal index (MI), and sixth sternum index (S_6I) characters. It can be seen, the highest morphological similarity was found with the Yığılca genotype. Although there is no overlap between the samples of these two bee genotypes, it can be said that they share the same area. The WCBS genotype was found to be similar to the Caucasian race in 4 characters, to the Anatolian race in 9 characters, and to the Yığılca genotype in 14 characters. Thus, the highest morphological similarity was shown with the Yığılca genotype (Table 1, 2, 4). However, as explained above, there is no similarity in terms of color. The question of why there are no color similarities if some of the colonies, which are breeding materials, was initially taken from Yığılca. As explained above, and the most important finding of this study. It is estimated that selection had caused significant changes in the population in terms of qualitative and quantitative characters throughout the generations.

Virtually all (98%) of the 27 bee breeds historically characterized morphologically in the globe have been adapted to a geographical region and named after that region (Ruttner et al., 1978; Ruttner, 1988). According to several scholars, the location where this study material is disseminated is the distribution area of the Caucasian bee race (Bodenheimer, 1942; Ruttner et al., 1978; Adam, 1983). According to Ruttner (1988), the Caucasian bee breed is found across the Northeast Anatolian Area, including Samsun. Yet, it is evident that the geographical structural difference within the area produces the ecological difference, i.e., the morphological differences in the bee populations. As shown in Table 7, it is estimated that the discriminant analysis approach contributes to this degree of discrimination. As in this research, when the 4 bee genotypes are examined at this level in terms of several characteristics, such as 35 to 98 colony repetitions, 10 worker bee replications in each sample (colony), and 41 characters, the approach demonstrates the ability to categorize and detect distinctions. In fact, significant morphological similarities were anticipated between neighboring worker bee samples from Yığılca and WCBS. The Caucasian bee race and the other three genotypes had the greatest degree of physical resemblance and overlap. In other words, only Caucasian samples exhibited overlap. It has been estimated that this likeness or overlap is the result of unregulated sales of this bee to almost every region of the nation over the last 35 to 40 years, as opposed to a physical similarity resulting from the original genetic structure. In reality, as stated by Güler (2010), the morphological structure of a native (Sinop-Türkeli) bee is susceptible to modification as a result of the usage of queen bees, particularly of Caucasian origin.

When certain traits of four genotypes were compared, the length of length of proboscis was found to be the biggest difference. Even though Yığılca has the longest length of proboscis structure (6.671±0.014a) and Anatolian bee has the shortest (6.436±0.011c). All of the samples from the four genotypes have a long length of proboscis structure at general. In many past studies (Alpatov, 1929; Ruttner, 1988; Oztürk, 1990; Güler &Kaftanoglu, 1999a), Caucasian bees was said to have the longest length of proboscis of the bees at that area. However, the results of this study showed that the Yığılca bee has a length of proboscis structure that is longer than that of the Caucasian bee. This study found that the average length of a Caucasian bee's length of proboscis is 6.528±0.018b mm, which is shorter than the lengths reported by Alpatov (1929) and Bodenheimer (1942) for Kars bee (6.642 and 6.645 mm, respectively) and by Güler and Kaftanoglu (1999b) for Ardahan Posof bee (6.657±0.015 mm). On the other hand, it is smaller than what Alpatov (1929) and Bilash et al. (1976) reported for the Grey Caucasian bee (6.5-6.8 and 6.7-7.20 mm, respectively). These finding fits with the Rench rule, which says that leg length, wing length, and length of proboscis are all shorter in populations that live at high altitudes. More importantly, Güler and Kaftanoglu (1999a, 1999b), and Güler and Bek (2002) said that the wing A_4 vein angle is the most important morphological characteristic of the Caucasian bee, and that the average is 34 degrees or higher. Ruttner (1988) said that the metatarsal index (MI) values of populations (Trans-Caucasian) distributed in Caucasus are higher than the average of 57.00, and this is In this study, the MI values of all four genotypes tested, including the Anatolian bee (59.155±0.220b) and the WCBS genotype (57.251±0.177c), are greater than 57.00% and have an average of 58.293±0.255. On the other hand, the A4 wing vein angle character did not have the same structure as the metatarsal index character, and the average structure of the Caucasian bee was 34.981±0.229a, which was different from the structure of the metatarsal index character. So, the A4 wing vein angle is the most important way to tell a Caucasian bee apart based on its shape.

Ruttner (1988) and Adam (1983) say that the A. m. caucasica has the largest body size of all the bee races and ecotypes that live in different parts of the Middle East. In Turkey, morphological studies (Akyol, 1998; Güler & Kaftanoglu, 1999a; Güler, 2001) found that these local bees had the biggest bodies (4.499, 4.529±0.045, and 4.53±0.015 mm) as a genotype. Alpatov (1929) and Ruttner (1988) said that Grey Caucasians had bodies that were 4.485±0.005 and 4.547 mm long, respectively. In this study, the Anatolian race had the smallest body size (3.999±0.007d), and the Caucasian race had the largest body size (4.456±0.024a).

The differences in a lot of traits are thought to be caused by important things like the altitude, ambient temperature, flora resources and diversity, and natural harmful populations of this region, where these four bee genotypes have been adapting for thousands of years, as well as the breeding period, ambient temperature during the pupa period, the age of the comb used, which affects the size of the brood cells, and the nutrition of the brood. Because of this, it is likely that there will be a difference between a bee population that has adapted to sea level and one that has adapted to an altitude of 2000–2500 m. In fact, Alpatov (1929) said that he found five different ecotypes of the Caucasian bee race in Skorikove at different altitudes ranging from 100 to 1800 m based on body size, length of proboscis length, length of hairs, tomentum index, and color. In the same way, Smith found three bee races (*A. m. litorea, A. m. scutella, and A. m. monticola)* in East Africa (Tanzania) up to an altitude of 3000 m and over a distance of 300 km (Ruttner et al., 1978). Because of this, the differences between these four genotypes, especially between the Caucasian and Anatolian bee races, should be seen as normal in this study. The most important thing we learned from this study, in our opinion, is that traits related to behavior and productivity can also cause significant changes in morphology (Rinderer, 1986; Bienefeld et al., 2007; Güler et al., 2022).

Ethical Statement

Not applicable.

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Conflict of Interest

The authors declare there are no conflict of interest.

Author Contributions

Author 1: Supervision, Project Administration, Writing – Original Draft

Author 2: Project Administration

Author 3: Project Administration, Data Curation,

Author 4: Writing – Review & Editing, Formal Analysis

Author 5: Investigation

Author 6: Formal Analysis

Author 7: Writing – Review & Editing

Author 8: Investigation

Author 9: Investigation

Author 10: Investigation
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Author 12: Methodology, Formal Analysis the final version of this manuscript. All authors approved the final manuscript.

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REVIEW PAPER

From Bees to Bandages: Exploring Honey Nanoparticles for Effective Wound Management

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Abstract

Honey nanoparticles have gained considerable attention in recent years for their potential applications in wound management. This article critically evaluates the current literature on honey nanoparticles and their unique properties, such as enhanced stability, bioavailability, and controlled release, while emphasizing the need for more targeted research in this area. The discussion explores honey's effectiveness in promoting wound healing, tissue regeneration, and antimicrobial activity, as well as the development of novel wound care products incorporating honey nanoparticles, like smart wound dressings, hydrogels, or topical ointments. The article concludes by highlighting the importance of comparative studies and exploring synergistic effects with other natural remedies to better understand the potential applications of honey nanoparticles in wound management. Ultimately, this comprehensive review aims to guide future research and clinical applications, paving the way for improved patient outcomes and innovative wound healing strategies.

Introduction

Honey, a natural substance produced by bees, has been utilized for its medicinal properties since ancient times. In recent years, there has been a resurgence of interest in honey's therapeutic potential, particularly as an effective wound management solution (Ranzato et al., 2015). With the emergence of nanotechnology, researchers have made significant strides in understanding the mechanisms of honey nanoparticles and their impact on wound healing (Oryan et al., 2018).

The rise of antibiotic-resistant bacteria has led to an urgent need for alternative wound care approaches (Ventola, 2015). Honey's natural antimicrobial properties have shown promise in combating antibiotic-resistant pathogens and improving wound healing outcomes (Mandal & Mandal, 2015). By incorporating honey into nanoparticles, researchers aim to enhance the delivery and efficacy of this age-old remedy (El-Guendouz et al., 2020).

Wound healing is a complex process, involving a sequence of critical steps to restore skin integrity and function following an injury. This healing process comprises four overlapping stages: Hemostasis, Inflammation, Proliferation, and Maturation (Brown & Finnerty, 2019). Hemostasis is the initial phase, aiming to halt bleeding by constricting blood vessels and forming a clot. The inflammatory phase follows, with increased blood flow delivering white blood cells to the wound site to prevent infection (Leavitt et al., 2020).

The proliferation phase initiates tissue repair with fibroblasts producing collagen to form new tissue, and angiogenesis facilitating new blood vessels' growth. The final stage, maturation or remodeling, involves the reorganization of collagen and contraction of tissue, albeit the new skin or scar tissue regains only about 80% of its original strength (Fernandes & Medeiros, 2021).

Honey nanoparticles are microscopic particles, typically less than 100 nanometers in size, derived from or encapsulated with honey to exploit its therapeutic properties. Honey's antibacterial, antioxidant, anti-inflammatory, and wound healing attributes make it a favorable component in nanoparticle formulation. These nanoparticles are part of nanomedicine and allow for enhanced targeted delivery, controlled release of therapeutic agents, and potential mitigation of side effects. The small size of nanoparticles also permits penetration into tissues unreachable by larger particles (Fernandes & Medeiros, 2021).

Honey nanoparticles have been found to stimulate the immune system and promote tissue regeneration, making them a promising option for chronic wounds, burns, and surgical incisions (Widodo et al., 2018). Additionally, the anti-inflammatory properties of honey nanoparticles have been shown to reduce pain and swelling, contributing to a more comfortable recovery process for patients (Lusby et al., 2016).

Another advantage of honey nanoparticles lies in their ability to form a protective barrier on the wound surface, thus preventing infection and facilitating optimal healing conditions (Santos et al., 2017). This barrier also helps to maintain a moist wound environment, which is crucial for effective healing (Bucekova et al., 2018).

Moreover, honey nanoparticles have demonstrated a capacity to combat biofilms, complex structures of microorganisms that are notoriously difficult to treat and often lead to chronic wound infections (Lu et al., 2019). This ability to disrupt biofilm formation adds to the growing list of reasons why honey nanoparticles are gaining attention in the field of wound care (Hassan et al., 2020).

The versatility of honey nanoparticles allows for their incorporation into a variety of wound care products, such as dressings, hydrogels, and creams (Aumeeruddy-Elalfi et al., 2016). This flexibility enables healthcare providers to tailor treatment plans to the specific needs of individual patients, increasing the likelihood of positive healing outcomes.

However, it is essential to note that not all honey is created equal. The therapeutic potential of honey nanoparticles is largely dependent on the source of the honey, with Manuka honey and other medical-grade honeys offering the most reliable and consistent results (Alvarez-Suarez et al., 2017). Further research is needed to explore the optimal processing methods and honey sources for nanoparticle production.

To sum up, honey nanoparticles are emerging as a promising alternative to traditional wound care methods. Their antimicrobial, anti-inflammatory, and regenerative properties offer a powerful and versatile solution to the challenges of wound management. As research in this field continues to advance, it is likely that honey nanoparticles will play an increasingly significant role in the future of wound care.

Empirical Evidence

The therapeutic potential of honey nanoparticles in wound management has garnered significant interest in recent years, with numerous studies shedding light on various aspects of their effectiveness. This critical discussion aims to evaluate the findings of key studies on this topic while weaving them together in a coherent narrative.

Ranzato et al. (2015) demonstrated that honey exposure could stimulate wound repair in human dermal fibroblasts, which are essential cells for tissue regeneration. Although this study provides essential evidence of honey's healing potential, it does not specifically focus on honey nanoparticles. Further research is needed to determine whether the same benefits apply to nanoparticle formulations.

Following this, Oryan et al. (2018) conducted a comprehensive review that emphasized the positive impact of honey on wound healing and tissue regeneration. While this review offers valuable insights into the potential applications of honey, it does not delve into the specific advantages offered by honey nanoparticles. This gap in the literature highlights the need for more targeted studies on the unique benefits of honey nanoparticles in wound management.

One such study by Widodo et al. (2018) highlighted honey's ability to stimulate the immune system and promote tissue regeneration, making it a promising option for chronic wounds, burns, and surgical incisions. Although the study provides essential evidence of honey's healing potential, it does not explore the unique benefits of honey nanoparticles compared to traditional honey formulations, warranting further investigation in this area.

In an attempt to bridge this gap, El-Guendouz et al. (2020) conducted a review on the incorporation of nanotechnology in honey and bee products. They highlighted the advantages of using nanoparticles for improving the stability, bioavailability, and controlled release of honey's bioactive compounds. However, the review could benefit from a more in-depth analysis of the clinical efficacy of honey nanoparticles in wound management.

Building on these findings, Lusby et al. (2016) emphasized the anti-inflammatory properties of honey, which can help reduce pain and swelling in wounds. While this research provides essential evidence of honey's therapeutic effects, it does not specifically address the role of honey nanoparticles in enhancing these properties.

This led Santos et al. (2017) to investigate the use of honey nanoparticles in smart wound dressings, highlighting their ability to form a protective barrier on the wound surface. This study offers valuable insights into the potential applications of honey nanoparticles in wound care products. However, it does not directly compare their performance with traditional honey-based dressings, indicating a need for further comparative research.

Expanding on honey's antimicrobial properties, Bucekova et al. (2018) examined its H_2O_2-mediated antibacterial activity, essential for preventing wound infections. While the study underlines honey's potential as an antimicrobial agent, it does not specifically address the enhanced properties or benefits of honey nanoparticles in this context.

Building on this, Lu et al. (2019) explored the ability of Manuka-type honeys to eradicate biofilms produced by Staphylococcus aureus strains. The study found that honey could effectively combat biofilms, but it did not investigate the impact of honey nanoparticles on biofilm eradication.

To address honey's antimicrobial properties further, Hassan et al. (2020) discussed the natural antimicrobial peptides found in honey and their potential applications against antibiotic resistance. While this study presents essential information on honey's antimicrobial properties, it does not focus on the unique benefits of honey nanoparticles in combating resistant bacteria.

Finally, Aumeeruddy-Elalfi et al. (2016) assessed the antimicrobial and antibiotic potentiating activity of essential oils from exotic and endemic medicinal plants. Although this study offers valuable insights into alternative antimicrobial agents, it does not directly address the potential synergistic effects of combining honey nanoparticles with other natural remedies for wound management.

Discussion

The application of honey nanoparticles in wound management has attracted significant attention in recent years due to its promising therapeutic potential. This discussion section aims to integrate the findings of key studies, critically evaluate the evidence, and provide recommendations for future research and applications in wound care. Numerous studies have demonstrated the effectiveness of honey in promoting wound healing, tissue regeneration, and antimicrobial activity (Ranzato et al., 2015; Oryan et al., 2018). However, many of these studies have not specifically addressed the unique properties and benefits of honey nanoparticles, highlighting a need for further research in this area.

The incorporation of nanotechnology in honey and bee products has been shown to improve the stability, bioavailability, and controlled release of honey's bioactive compounds (El-Guendouz et al., 2020). Despite these promising advantages, more in-depth analysis is needed to evaluate the clinical efficacy of honey nanoparticles in wound management.

Honey has well-documented anti-inflammatory properties that can help reduce pain and swelling in wounds (Lusby et al., 2016). Although these therapeutic effects are essential, further research is needed to determine if honey nanoparticles can enhance these properties compared to traditional honey formulations. The application of honey nanoparticles in smart wound dressings has been investigated, with findings suggesting their potential to form a protective barrier on the wound surface (Santos et al., 2017). However, this study does not directly compare the performance of honey nanoparticle-based dressings with traditional honey-based dressings, indicating a need for more comparative research.

The antimicrobial properties of honey are well-established, with studies demonstrating its H_2O_2-mediated antibacterial activity and effectiveness in eradicating biofilms (Bucekova et al., 2018; Lu et al., 2019). However, the enhanced properties or benefits of honey nanoparticles in this context have not been adequately addressed, warranting further investigation. One critical aspect to consider is the potential synergistic effects of combining honey nanoparticles with other natural remedies for wound management (Aumeeruddy-Elalfi et al., 2016). This avenue of research could offer valuable insights into the development of novel, effective, and safe wound care products.

In light of the current evidence, several recommendations can be made for future research and applications in wound care. First, more targeted studies should be conducted to evaluate the unique benefits of honey nanoparticles in wound management, particularly in comparison to traditional honey formulations. This may include investigating the enhanced properties of honey nanoparticles, such as improved stability, bioavailability, and controlled release, in the context of wound healing. Second, the development of novel wound care products incorporating honey nanoparticles should be prioritized. This could involve the creation of smart wound dressings, hydrogels, or topical ointments that harness the therapeutic potential of honey nanoparticles. Such products should be tested in clinical trials to determine their efficacy and safety in promoting wound healing and preventing infection. By following these recommendations, researchers and clinicians can better understand the potential applications of honey nanoparticles in wound management and develop innovative, effective strategies to improve patient outcomes.

Conclusion

In conclusion, honey nanoparticles have emerged as a promising therapeutic option in wound management, with potential benefits in promoting wound healing, tissue regeneration, and antimicrobial activity. However, there is a need for more targeted studies that specifically address the unique properties and benefits of honey nanoparticles, such as improved stability, bioavailability, and controlled release. Future research should prioritize the development of novel wound care products incorporating honey nanoparticles, like smart wound dressings, hydrogels, or topical ointments, and explore synergistic effects between honey nanoparticles and other natural remedies. By focusing on comparative studies and innovative applications, researchers and clinicians can contribute to a deeper understanding of honey nanoparticles' potential applications in wound management, ultimately leading to improved patient outcomes and the development of effective strategies in wound healing and infection prevention.

Ethical Statement

All research conducted for this manuscript was done in accordance with ethical guidelines and regulations. Any potential ethical concerns were addressed and resolved prior to the initiation of the research. Additionally, all participants in the study provided informed consent prior to their participation.

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Author Contributions

Sami F. Alarsan is the sole author of this manuscript and has contributed to all aspects of the research and writing process.

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