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MELLIFERA RESEARCH ARTICLE

Pollen Morphology of *Hedera helix* L.

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A B S T R A C T

Hedera helix (Ivy) is an everygreen dioecious wood and liana. It is one of the best species of *Hedera* (Araliaceae). In traditional use, many parts of this plant have been used for treatment of various diseases. In this study, pollen morphology of *H. helix L.* belonging to the genus *Hedera* (Araliaceae) were examined with light microscopy (LM). According to the investigation by LM, pollen grains of the species are monad, radially symmetrical, isopolar, tricolporate, oblate-spheroidal or prolate-spheroidal, colporate, reticulate and rugulate.

In my opinion, the palynological features of the taxon might be helpful to investigate the taxa various palynological, taxonomical, melissopalynological and pharmaceutical researches.

Keywords: Araliaceae, light microscope, palynology, taxonomy

Introduction

H. helix L. is one of the best species of *Hedera* (Araliaceae). This plant (Figure 1) has been used as medicinal plant for curing several human and animal diseases. Also, it has shown several biological activities which can be used for agricultural or medicinal purposes. It is woody, climbing or creeping perennials, the stems clinging by adventitious roots. Leaves simple, petiolate, elliptic to palmately lobed, exstipulate. Flowers are yellowish-green, in umbels which are arranged in racemose panicles or which are solitary. Its fruit resembles small berries and black as it matures. These rounded fruit contain 2-3 whitish seeds [1].

In the mostly production of autumnal honeys, usually, beekeepers determined that *Apis mellifera* L. visits flowers of *Ceratonia siliqua* L., *Erica multiflora* L., *Diplotaxis erucoides* (L.) DC., *Dittrichia viscosa* (L.) Greuter, *Hedera helix* L. and *Rosmarinus officinalis* L.[2]. This blooming time is good period because other food sources for honey bees are limited. *H. helix* blooms from mid-September to early November; one of the main pollinators is honey bee (*Apis mellifera*) [3].

H. helix (Ivy) honey is monofloral and rarely found in Spain, Italy, France, Croatia or Ireland [4, 5]. Besides, this honey is crystallizes in a short period of time. It is occurring mostly in consequence of the high content of glucose. The ivy honey contain usually up to 80% of it [2, 6]. The colour is dark (brown leather, toffee dark brown) and with an odour of liquorice and forest that is intense. The taste is acidic, bitter, medicines, roasted

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and coffee [2]. Kaya et al. (2005) determined that pollen grains of *H. helix* L. are dominant in Kırklareli [7]. Özler (2018) determined *H. helix* pollen in honey from Ereğli-Namık Kemal neighborhood [8].

Figure 1. Flowers of *H. helix*

H. helix grows dry and wet sclerophyll forest, woodlands, riparian vegetation, rocky outcrops and warm temperate rainforest. *H. helix* naturally grows in Giresun (A7) and Turkey [1]. In addition, it is grown as ornamental plant.

There is insufficiency of literature on pollen morphology of *H. helix* in Turkey. Therefore, the present study aims to fill this gap in literature by palynological analyses Consequently, it provides information that help plant taxonomy, mellisopalynological and aeropalynological analyses.

Materials and Methods

Locality

For the preparation of *Hedera* extract, flowers of the plant in October 2017 were collected and were identified at the Research Laboratory of Department of Health Care Services and Techniques of Giresun University, Giresun. It is located in the eastern part of the Black Sea region (40°54´K and 38°25´D) [9]. According to the grid system applied by Davis [1], Gure (Giresun) is located in the A7 frame (Fig. 1).

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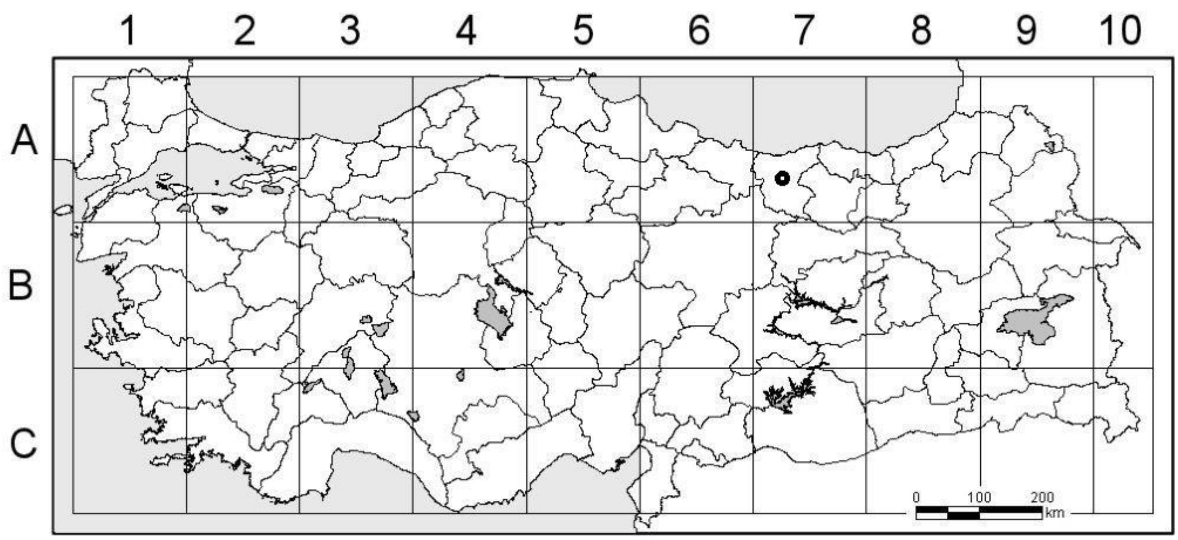

Figure 2. The picked flowers of *H.helix*

Pollen Sample

The light microscopy (LM) observations with their measurements were made on pollen from mature anthers, which have been prepared according to the Wodehouse method [10]. The measurements of the pollen grains of *H. helix* were taken on 30 pollen grains from the species. P: polar axis, E: equatorial diameter, Amb: diameter of pollen at the polar view, t: distance between colpi ends, were measured from 30 fully developed grains per sample under the Nikon Eclipse Ci microscope (1000×). Additionally, 15 measurements of Clg: length of colpus, Clt: latitude of colpus, Plg: length of porus, Plt: latitude of porus. Results are provided as minimum, maximum and mean±standard deviations. P/E ratios were also calculated. In addition, the ornamentation was established. All the statistical analyses of the palynological characters were made by the SPSS package program. The arithmetic mean, standard deviation and variation were calculated for sample. The statistical results are shown in tables. The terminology used is of Erdtman [11], Kremp [12] and Punt et al. [13].

Results and Discussion

Palynological description of *H. helix* was made based on the quantitative and qualitative morphologic results. It is monad, radially symmetric, isopolar, 3-colporate, medium-sized, Prolate-spheroidal or oblat spheroidal (P/E 0.93-1.01) (Fig. 3). Polar axis (P) 30.73±0.9 µm, equatorial axis (E) 30.23±0.77 µm. Amb trinagular-round, Exine 2.21±0.56µm thick, nexine is thinner than or as thick as sexine. Exine ornamentation is reticulate or rugulate; distance between colpi ends 12.23±1.86 µm. Apertural system 3-colporate: three colpus 21.37±1.45µm long, 4.56±0.89 µm wide, pore 6.87 ± 0.95 µm long, 6.43 ± 0.51 wide; distinct margin and terminal edges acute with margo, the pori situated at midpoint of colpus, are prolate-

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spheroidal(plg/plt 1.06) and with distinct margin. (Fig. 3, Table 1).

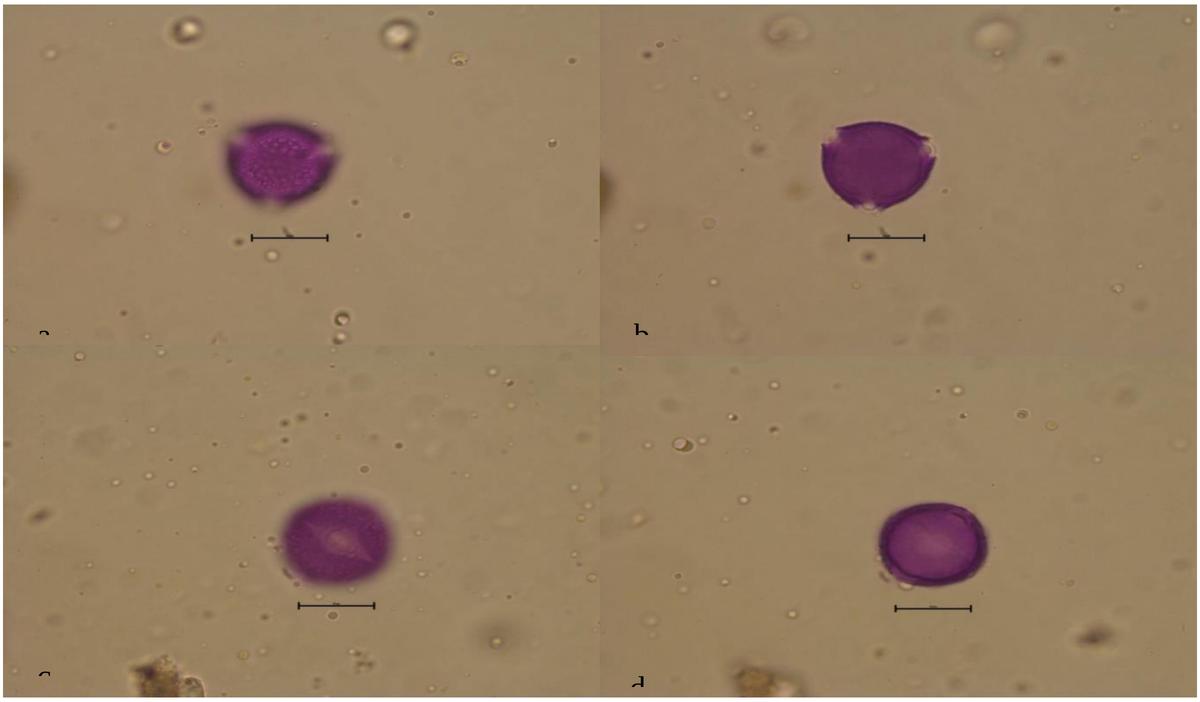

Figure 3. *H. helix* a-b: polar view; c-d: equatorial view (10x100) (20µm)

Table 1. The palynological measurements of *H.helix* (M: median, Var.: variation, S: standarddeviation).

P/E	Oblate spheroidal	0.99	Exine (µm)	M	2.21
				S	0.56
				Var.	1.5-3
P(µm)	M	30.23	Sexine (µm)	M	1.28
	S	0.77		S	0.48
	Var.	28-33		Var.	1-2
E (µm)	M	30.73	Nexine (µm)	M	0.85
	S	0.90		S	0.24
	Var.	28-32		Var.	0.5-1
Clt (µm)	M	4.56	Amb (µm)	M	29.16
	S	0.89		S	1.98
	Var.	3-7		Var.	26-33
Clg (µm)	M	21.37	t (µm)	M	12.23
	S	1.45		S	0.77
	Var.	20-24		Var.	9-16
Plt (µm)	M	6.45			
	S	0.51			
	Var.	6-7			
Plg (µm)	M	6.87			
	S	0.95			
	Var.	5-8			

In MediaWiki [14], *H. helix* pollens are 28 (26.2-31) µm (medium), tricolporatae and mit reticulate, microreticulate or fossulate. triangular, spheroid (0.96), isopolar, heterobrochate, lumens are getting small toward the colpus. Apertur membranen are no ornamentation. Pore wide is 5.7 (5.2-6.4) µm. Apocolpium is broad.

In Pal dat [15], *H. helix* pollens are monad, medium-sized (26-50 µm), isopolar, spheroidal, outline in polar view: circular, shape (dry pollen): prolate, outline in polar view (dry pollen): triangular, aperture 3 colporate, ornamentation reticulate, heterobrochate.

palynological results are concardant to previous research about *H. helix* pollen investigations. Pollen grains of *H. helix* are radially symmetric, isopolar, 3-colporate with margo, reticulate and regulate (LM).

Conclusion

H. helix has common name "ivy" and has been traditionally used as medicine all over the World. Pollen morphology of *H. helix* is determined. The remarkable property of this species, amb view is triangular-round and colpus margins regular, with a well-developed margo.

Hedera helix L.'nin Polen Morfololojisi

Öz: Bu çalışmada, *Hedera* L. (Araliaceae) cinsine ait *H. helix* L. polen morfolojisi ışık mikroskobu (LM) ile incelenmiştir. Yapılan incelemelere göre, bu taksona ait polenler tek, radyal simetrik, izopolar, oblat-siferoid ya da prolat siferoid, kolporat, retikülat and rugulat özellik göstermektedir.

Bu taksonlara ait palinolojik özelliklerin çeşitli palinolojik, taksonomik, melissopalinolojik ve farmasötik botanik çalışmalarında taksonların daha doğru teşhis edilmesine yardımcı olacağını düşünmekteyiz.

Anahtar Kelimeler: Araliaceae, ışık mikroskobu, palinoloji, taksonomi

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MELLIFERA REVIEW ARTICLE

Stress Factors on Honey Bees (*Apis mellifera* L.) and The Components of Their Defense System Against Diseases, Parasites, and Pests

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A B S T R A C T

Animal health is an important factor that may limit efficiency in beekeeping, as in other fields yielding animal products. After all, the diseases, parasites, and pests that pose a risk for honey bees do not only affect their health, but can decrease productivity, as well. The colony may collapse eventually unless measures are taken to improve the situation. Occasional epidemics of diseases, parasites, and pests cause producers to suffer great losses and harm beekeeping on a national scale in Turkey.

As with all living organisms, honey bees have a strong multicomponent defense (resistance, immunity) mechanism against diseases, parasites, and pests that threaten their health. However, various stress factors from those rooted in their genetic makeup to others resulting from the environment collapse their defense system and with the decline of bodily resistance, honey bees start getting ill and eventually die. Moreover, these stress factors or stressors interact with one another, with pathogenic microorganisms, parasites, and pests to create a synergic effect leading to exhaustion in bees, their offspring, and whole colonies. Therefore, it is critically important to eliminate stress factors that impair and collapse the defense system in honey bees.

Keywords: Honey bee, stress factors, components of defense system.

Introduction

I: Stress Factors on Honey Bees

1. Genetic Deficiency

Honey bee breeds show a wide variety in their genetic predisposition or resistance to certain diseases and parasites. Disease resistance of larvae is also a hereditary feature wildly varying across various breeds. In other words, each genotype has some specific inherited characteristics [1,2].

The hygienic behavior of various races differs wildly when it comes to practices like clearing honeycomb cells off dead brood after a disease such as American foulbrood, chalkbrood, or parasitic infection with the *Varroa destructor*. The heritability of hygienic behavior is $h^2 = 0.40$ to 0.67. Races with genetically poor hygienic behavior are more susceptible to certain diseases and experience more health problems [3,4]. For example, Caucasian and Anatolian honey bees carry more propolis to their nests to disinfect honeycomb cells as a protection against disease. However, Caucasian bees are more susceptible to Nosema disease than other races. On the other hand, Anatolian bees are susceptible to paralysis virus, but more resistant against acariasis and Nosema than Caucasian bees. Resistant lines developed against the parasite *Varroa destructor*

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through breeding and selection can also develop resistance against American foulbrood, tracheal mites, chalkbrood, Nosema, and even the wax moth [1,2].

African bees display more hygienic behavior against the chalkbrood disease. While Italian bees are resistant against acariasis, wax moths, and European foulbrood, the breed called European dark bee is susceptible to these diseases. Carniolan honey bees protect their nests well against parasites such as hornets or wax moths and their brood is almost never affected with disease especially in their native land. They are highly adaptable to environmental conditions, but susceptible to bee paralysis [1,2]. A genetic characteristic of Japanese honey bees allows them to raise their body temperature up to 48-50 ^{o}C in the event of an attack on their nests to almost burn the intruder to death. European honey bees display a similar behavior in the presence of wasps, but they cannot possibly survive a temperature rise at the same level [5].

Another important genetic deficiency negatively affecting bee survival is a result of continued inbreeding in apiculture. In nature, it is unlikely for a queen to mate with drones of her kin. However, kinship increases with inbreeding and systematic indoor beekeeping. In particular, using drones of the same breeding colony for artificial insemination and breeding a large number of sisters to distribute them as queens among colonies of the same apiary will rapidly increase kinship in future generations, thus narrowing down genetic variation and giving rise to inbreeding degeneration. As the queen mates with drones that share her sex alleles, the colony has more diploid eggs to turn into males with a low survival chance. Worker bees notice this type of genetically flawed broods at the beginning of the larval stage and eat them. This leaves the brood comb with a large number of empty cells, which in turn slows down colony development and makes the colony vulnerable to diseases [1,6,7,8,9].

Using old, disabled, or poorly queen bees also affects the development and strength of the colony, thus disrupts its dynamics. Weak colonies have low resistance against diseases [10].

2. Unfavorable Climate and Environmental Conditions

Since honey bees are diverse in their adaptability and will to survive, they respond differently to different environmental conditions. Temperature, wind, precipitation, humidity, altitude and other environmental factors are extremely important for the well-being and genetic efficiency of bees. As mentioned above, not all races thrive and realize their full genetic potential under the same circumstances. Some races thrive in high altitudes while others in low-altitude coastal areas, some have a healthier existence in humid regions with high levels of precipitation while others are better off in the desert. Therefore, it is important to provide the environmental conditions suitable for each race or, failing that, it is recommended to work with genotypes that do not have adaptation problems [1,7,8,9].

Since honey bees are cold-blooded insects, temperature is a crucially important factor in all their activities. Body temperature of honey bees changes according to ambient temperature and for survival they can get adapted to a range of 0±40 ^{o}C. However, the optimal ambient temperature for their regular activities is between 21-35°C. The farther the temperature moves away from this range, the more stressed and

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uncomfortable they get. Decrease in temperature slows down the metabolism, while increased temperature increases the respiratory rate and accelerates the metabolism. High metabolic rate increases energy consumption and speeds up physiological aging [1,2,7,8,9].

To compensate for the disturbed balance inside the hive due to suboptimal temperature, humidity, and CO_2 level, bees shrink the flight hole, cluster together and ventilate. However, for this purpose, there must be a sufficient number of bees and the hive should be suitable for airflow. Therefore, wooden beehives made of natural material that allows a better balance of temperature, humidity, and CO_2 should be preferred. Too high, too low, or continuously fluctuating body and hive temperatures, as well as excessive CO_2 levels in the hive stress out bees, negatively affect their lives, and accelerate physiological aging due to increased energy consumption. Rising hive temperatures cause eggs to dry and die, whereas insufficient incubation temperature will cause breeding to stop [11,12].

Especially in poorly ventilated hives honey absorbs the moisture in the atmosphere and this excess moisture reduces its acidity. Bees cannot compensate for this loss of acidity and high water content in honey activates yeast spores. Moisture accumulated in the hive starts fermentation in honey. The disrupted acidity balance, high water ratio, and fermentation of the honey weaken the natural defense mechanism of the bees. The moist environment in the hive not only causes digestive disorders in honey bees, but the rapid proliferation of microorganisms renders them vulnerable to fungal diseases, as well [1,2,10,13].

Unfavorable environmental conditions directly affect the life and health of bees. Continued exposure to wind, excessive cold, excessive rainfall and moist hinder the flight of bees. Not being able to fly for a long time is a strong stress factor on bees. Under circumstances of constant wind bees hardly fly and get disoriented, thus they cannot go on foraging. In regions with extreme heat, excessive precipitation or simoom, flowers stop secreting nectar and this gives rise to malnutrition. All these unfavorable environmental factors stress bees out, weaken their resistance, and leaves them vulnerable to illness [1,2,10,13,14,15].

3. Malnutrition

Bees obtain the nutrients they need to survive and thrive from nectar, honey, pollen, and water. They provide their carbohydrate and energy primarily from nectar and honey, while pollen is their only natural source of protein. And water is obviously essential for their life. Honey bees provide the energy they need for vital activities from carbohydrates. The nectar and/or pollen of some plants may contain compounds that are toxic or harmful for bees. Bees are not interested in some carbohydrates at all, but some of them shorten their lifespan, even have a toxic effect. A medium-sized colony consumes about 75 kg honey and 20-50 kg pollen per year. A honey stock in the hive under 9-10 kg (about 3 frames) any time of the year causes bees to suffer hunger stress. Malnutrition and starvation because of nutritional mistakes are important reasons leading to collapse of colonies. Therefore, it is essential to keep colonies well stocked above the stress line with sufficient and quality food at all times [1,2,16].

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Stationary beekeeping where colonies always remain in the same place, too many colonies in the same apiary, and closely spaced apiaries push bees into extreme competition to access food sources, resulting in malnutrition, hunger stress, and eventually poor resistance against diseases. Inadequate pollen accelerates physiological aging and shortens bee lives. The amount of pollen transported to the hive is closely linked to the queen's rate of spawning and worker bees' inclination to rear the young, to make wax and build honeycombs, to raise and feed the drones. Bees can accomplish these tasks only if they consume enough pollen to renew their fat tissue, which in turn increases their blood protein levels [12,13].

Pollen contains many fatty acids. Decanoic (capric), dodecanoic (lauric), myristic, linoleic, and linolenic fatty acids protect bees against bacterial and fungal diseases with their antimicrobial effects. For example, linoleic acid kills *Paenibacillus larvae*, the agent of American foulbrood disease; linoleic and lauric acids protect against *Bacillus cereus*, the soil bacteria whose toxins cause food poisoning; and lauric acid inhibits the development of *Helicobacter pylori* bacteria. Moreover, the fatty acids in pollens inhibit the development of other bacteria such as *Staphylococcus, Salmonella, E. coli* and *Bacillus anthracis* [1,2,16,17].

Nurse worker bees consume pollen to produce the royal jelly they feed the queen bee and larvae with. Royal jelly contains 10-hydroxy-2-decanoic acid (10-HDA), which has an antibiotic effect with very important lipid compounds and immunizes the larvae against infections. Bees process pollen to produce bee bread which helps larvae develop rapidly, protects the broods against disease, and extends the lifespan of bees. It is a natural probiotic with its content of lactic acid bacteria and bifidobacteria and is 3 times more nutritious than pollen, with a 6 times higher lactic acid content [1,2,16,17]. In the spring, colonies with an insufficient stock of pollen, bees must use the fat tissue in their bodies to compensate for the protein deficit, but this shortens their lifespan and diminishes incubation activity. Worker bees that lost 40% of their body protein reserve can live only for 20-25 days. Tissue renewal is disturbed unless the bee gets the necessary essential and antimicrobial fatty acids by consuming sufficient amounts of quality pollen, which again weakens immunity and shortens lifespan [1,2,18].

Vitellogenin is the protein found in the blood of honey bees. Juvenile hormone (JH) secreted by the corpus allatum gland on the rear part of the brain induces vitellogenin production. Vitellogenin is produced and stored in fat tissue cells covering the entire surface of the abdomen, and is delivered directly to blood from these cells [1,2,19].

The activity of the corpus allatum (i.e. production of secretions) and vitellogenin production are at lower levels in newly mated queen bees. In the first weeks the queen bee matured into adulthood, the blood vitellogenin level increases parallel with the JH level to reach its normal amount. Queen bees have a higher level of vitellogenin than worker bees. On the other hand, the blood vitellogenin levels of worker bees start to increase right after hatching and reach maximum in 7-10 days. From among the worker bees, foragers have less vitellogenin than nurse bees. Blood vitellogenin in the lowest in drones [1,2].

Vitellogenin has a role in the production of egg cells in the ovary and the transport of nutrients required for embryonic development. An in worker bees, it provides the nutrients and amino acids needed for the production of royal jelly. It is crucial for the immune system and lifespan, not to mention all activities of bees inside and outside the hive [1,2,19].

Since the blood vitellogenin level is lower in forager bees as compared to nurse bees, their lifespan is also shorter. Before wintering, old bees in the colony consume excessive pollen and increase their blood protein levels to 10 times the amount in young bees. Rising vitellogenin levels in autumn help extend the lifespan. Thus bees survive until spring and the colony lives on. Research suggests that forager bees carry various bacteria and other pathogens from nature to the hive, which then pass to the royal jelly produced by nurse bees and eventually to the queen bee feeding on it. In the body of the queen bee these bacteria are broken down to be bound by the vitellogenin in the blood and transferred to eggs produced. Consequently, the larvae are vaccinated even before hatching [1,19].

Malnutrition, starvation, scarcity of nectar and pollen stress out bees, thereby facilitating the growth of fungi, which in turn lays the groundwork for the activation of *A. apis*, which is an opportunistic fungus and the agent of chalkbrood. For this reason, bees consume large amounts of pollen in autumn to renew fat tissue, grow body and blood protein reserves, and boost immunity. All this physiological transformation they undergo secures their survival until spring [20,21]. Water is another essential food for bees. Daily water consumption in a colony can reach up to 4 liters, especially in warmer regions and during periods of intensive brood rearing [1].

4. Nutritional Mistakes

Natural foods (nectar, honey and pollen) are essential in the diet of honey bees. Digestive system and enzymes of the bees are adapted to absorb these natural nutrients. However, insufficient nectar and pollen resources, dependence on the nectar and pollen of a single type of plant, and inability to meet nutritional needs directly from nature are causes of nutritional deficiency. In this case, the diet of the bees can be supplemented with carbohydrate and protein feeds, even vitamin and mineral combos can be provided [1,16].

The nectar of some plants yields poisonous or bitter honey. Some of them are toxic only to humans, but some may be toxic and harmful to bees, as well. Nectar containing poisonous compounds is a major cause of food poisoning and death in bees. Moreover, berries that often attract bees may bear large amounts of pathogens, the larvae of carnivorous beetles, or mites and this may attack bees, rendering them weak and vulnerable [16,22].

Honey has antimicrobial effects owing to its high sugar and low water content, acidic structure, and hydrogen peroxide, various flavonoids, and phenolic acids it contains. However, boiling honey-added bee feed for a source of carbohydrates or using heated honey in feed preparation largely eliminates the antimicrobial effects of the honey in feeds. Therefore, sugar syrups and carbohydrate feeds prepared for bees should never be boiled or directly exposed to a heat source. Furthermore, treatment with heat breaks down monosaccharides, in particular fructose, and furthers the formation of a toxic compound called hydroxymethylfurfural (HMF). Depending on the level of their HMF content, heat-treated honey and syrups may cause

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poisoning, even mass deaths of bees [1,2,16,23].

Be sure that honey and pollen to be used in feeding did not come from colonies with disease. If mold is found in the honeycomb or honey becomes sour or fermented, it must not be used as food. Bees should be fed mature and high-quality honey during winter. Especially in regions with harsh and long winters, it is important to avoid using honeydew honey with a high content of mineral salts and dextrin. This is because bees wintered on immature or poor-quality honeydew honey produce more feces. When the amount of fecal matter exceeds the bearing capacity of the rectum, bees are forced to defecate in the honeycombs. This leads to contamination and by extension to bacterial growth and intestinal infections in honeys. Moreover, old bees in wintering colonies struggle to access high-carbon sugars [1,22].

After the honey harvest, colonies start winter without much loss and with a younger bee population and then they thrive again during the springtime with a fast paced and more efficient breeding. Excessive feeding, adding honey and pollen from diseased colonies in their feed, or using sour or poor-quality honey or moldy pollen in feeds are all risk factors for the health of bees. In recent years, vitamin and mineral combos can be found in the market in addition to ready-made feeds for bees. However, they should not be preferred since their methods of preparation, content, duration and conditions of storage, and the way they are marketed are hardly reassuring most of the time. It is advised to keep bees in regions rich in flora, to allow them meet their nutritional requirements directly from the nature by feeding on nectar and pollens [1].

Artificial feeding can never be considered as a substitute to nectar and pollen. In fact, it is usually a cause of digestive disorders and other health problems in bees. Brewer's yeast, milk powder, soya bean flour and similar additives used in the bee feeds to provide protein actually alkalize the digestive environment in the body of the bee and promote fungal development with their high protein content. Such mistakes in nutrition and some other environmental factors damage the immune system in larvae and adult bees, leaving them vulnerable to pathogens [24,25].

Excessive syrup feeding also promotes fungal development by increasing relative humidity inside the hive. When excess fructose is loaded into colonies in order to produce more honey, bees cannot convert all of it into honey sugars and end up storing a significant amount of fructose. This is yet another stress factor on the bees and it shortens the lifespan of worker bees, increasing the wintering losses [22,25].

Fructose and glucose sugars are produced in the industry by processing the starch from corn, potato, or rice. These industrial sugars with a high fructose content are mostly obtained from GMO corns and used in bee feeds. They also contain very high levels of HMF. Invert sugar syrup with a high HMF value kills bees within two to three weeks. For this reason, starch-based industrial sugars should better be avoided in bee feeds and traditional kinds of sugar obtained from sugar beets or sugarcanes should be preferred instead. It is also worth noting that in agricultural areas where GMO sunflower seeds are used, the honey yield is initially very high, but as years pass by, colony losses up to 75% are observed and the surviving colonies experience a parallel decrease in population. This shows that bacterial toxins in GMOs have a

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negative impact on the health of bees [1,2,16].

Honey bees obtain the lipid compounds they need from their feed and mostly from pollen. For this reason, fat is not used in bee feeding. In fact, the fat content of bee feed should not exceed 5%. Otherwise, bees may suffer from severe digestive problems. Excess mineral consumption is another factor that can shorten the lifespan of bees. The detrimental effects of excessive minerals are worse in long flightless periods. Salt rapidly decreases lifespan and water or feeds with a salt content higher than 1% causes bees to die within a few days. For this reason, honeydew honey with a high mineral content and salt must be avoided in bee feeds especially during long periods of wintering [1,2,16,22].

5. Using Poor Quality Honeycombs

It is best to use honeycomb made of pure wax. Since wax is a very important carrier vector for bacterial and fungal bee diseases, it should not be contaminated with disease pathogens and be completely free of naphthalene and any chemical residues. During the production of the honeycomb care should be taken to prevent wax from contamination with paraffin, ceresin, petroleum jelly, stearin, renin, lard or tallow, resins, mineral oils, or any other foreign matter. These substances neutralize the high acidity index of wax to prepare a suitable environment bacteria and fungi. Moreover, residual feces and pupae in old honeycombs provide favorable conditions for the development of fungi [1,2,16,22].

6. Mistakes in Colony Checks and Management

All kinds of practices to check and manage colonies must be organized in accordance with the biological needs of the bees. Colony checks, especially on cold, cloudy days in the early spring or autumn, weakens the larvae by bringing down the temperature in the incubation area and promotes fungal development. Unnecessarily frequent colony checks in bad weather conditions disturb the balance the bees struck between temperature, humidity, and CO_2 levels - especially if the hive is kept open for a prolonged time! Then, the bees have to spend a lot of time, consume even more energy, and stress out to restore the balance. In the hives with weak colonies, it is advisable to reduce the area in early spring and late autumn using a partition board [8,9,22].

Each colony forms its own population balance with groups of worker bees specialized in different tasks and their colony dynamics. Partitioning or joining, taking swarms, bringing in new brood or bees to colonies all disrupt this balance and stress the bees out. Once colony dynamics is disturbed through swarming, artificial swarming, or supplementing the brood, the number of nurse bees per colony declines. This leads part of the brood to be undernourished, while leaving others exposed to cold. As a result, larvae become more susceptible to chalkbrood disease [1,2,24,25].

The bees in a hive establish the optimum order and nest layout for themselves. The order they establish must be preserved and changing the location and direction of honeycombs during colony checks must be avoided unless there is an obligation to for technical reasons. Otherwise, bees spend days trying to adapt to the new layout deteriorating order and this waste of time and energy cause them extreme stress. Colony transfers in migratory beekeeping are always a major stressor for bees. In particular, transfers in extremely hot

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weather and without adequate ventilation may lead to mass deaths [1].

The following are not advisable practices with regard to the health and efficiency of bees: keeping colonies always in the same apiary, keeping an excessive number of colonies in the same apiary, not allowing for enough distance between colonies and apiaries, overfeeding, underfeeding, using unnecessary and excessive antibiotics, swarming from diseased colonies, using the honey and pollen of colonies contaminated with pathogens in feeds, exchanging bees, broods, honey, and pollen between healthy and diseased colonies, merging, using hives and other materials from diseased colonies in healthy ones without disinfection, ignoring rules of hygiene during colony checks, dividing a honeycomb base into several frames or not providing a base at all, leaving the honeycomb building entirely to the bees or using a plastic honeycomb, using poor quality or very old honeycombs contaminated with chemicals and pathogens, wintering colonies with an aged or insufficient bee population and/or with poor quality or inadequate food stock, or not providing suitable wintering conditions in general. In summary, mismanagement of the colony causes a decline in efficiency and productivity, because it stresses bees out, affects their health adversely, and facilitates the transmission and spread of diseases and parasites [7,26,27].

7. Chemical Pollution

The bee digestive system naturally contains a plethora of microorganisms, such as bacteria, viruses, fungi, yeasts, and protozoa. They live together on a symbiotic basis without causing any disease. Owing to their antagonistic relationship, bacteria and fungi inhibit the growth of each other in the same environment, thus mutually keeping populations below the threshold that could cause disease [1,2].

Antibiotics used to prevent bacterial bee diseases eradicate the bacteria in the digestive system cause new generations build antibiotic resistance, and accelerate fungi growth, eventually disturbing the biological balance between various microorganisms and laying the ground for fungal diseases. Furthermore, antibiotics do not only kill the harmful bacteria in the bee’s system, they annihilate the good bacteria that produce digestive enzymes and lactic acid, as well. As a result of such unnecessary and excessive use of antibiotics, the biological balance in the bee’s body is disturbed and nutritional disorders develop, resistance against pathogens declines and bacterial and fungal diseases start to affect the bee [22,28].

In fields where crops are grown, in silos, in shelters and other habitats for humans and animals, tons of insecticides are used annually. These chemicals cause serious pollution in natural resources such as soil, plants, and water. Biological or chemical substances that pollute the air, soil, water resources, and plants in and around apiaries create a serious problem for bees [1,2,29,30].

Chemicals contaminating nectar, pollen, propolis, and water are a huge adverse effect on nutrition and living of bees. In particular, systemic insecticides, mostly used for oilseed plants, can lead to food contamination and bee deaths. The chemicals forager bees carry to the hive on their bodies cause orientation and memory problems, as a result of which bees cannot find their way back home. They also affect nurse bees, their glands diminish because of these chemicals and they cannot feed the young, or the chemicals pass to the

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digestive system through honey, pollen, propolis, brood feeds, and honeycombs eventually to poison and kill both the bees and their offspring en masse [1,16].

Another harmful chemical is the naphthalene used against pests and especially wax moth to protect the honeycombs stored for reuse after harvesting, honeycomb bases, and recycled wax. The residue of naphthalene and other chemicals used for similar purposes are a serious threat not only for the bees, but for humans, as well [1,2,22].

Detergent and sewage wastes that cause chemical pollution in the soil, plants, and water sources, toxic waste and flue gases of industrial plants, exhaust gas, and agricultural fertilizers also affect bee life and health adversely, causing stress, poisoning, and mass death. Bees should be protected against these stressors that weaken them and leave them vulnerable to parasites and pests [24,25].

8. Electronic Pollution

Bee habitats are under increasing pressure from electronic pollution or radio frequency pollution created by base stations, electromagnetic fields, TV signals, radars, high-voltage lines, wireless internet access, and the like. Unfortunately, this type of pollution is underestimated as it is not visible. However, electronic pollution represents a serious stressor for bees causing them to get disoriented [1].

Because of the electromagnetic radiation sources in the vicinity of an apiary, 70% of the bees flying out cannot find their way back to the hives. Cell phones placed near a beehive emit a radiation of 900-1.800 megahertz and drives the bees away. Furthermore, the vibration frequency (220 vibrations/sec) generated by mobile phones is very close to the vibration frequency (190-250 vibrations/sec) bees use to communicate with each other and to find directions: yet another factor causing disorientation. Electromagnetic pollution affects the resistance and strength in bees and makes them vulnerable to diseases. It was demonstrated that a mobile phone receiver at a distance of 300 m to the apiary increased restlessness and belligerence by 38%, the tendency to cluster by 25% and eventually caused 63% of the colonies to collapse. Therefore, electronic pollution sources, known to have serious adverse effects also on human health, should be kept at least 500 m away from apiaries, as well as all human settlements [1,31].

All the unfavorable circumstances explained above are stressors for honey bees either on their own right or in interaction with one another and the stress bees experience results with the burnout syndrome, collapse of the immune system, illness, and eventually death. Like all other living beings, honey bees have a defense system against factors that may adversely affect their health and life in general. The collapse of this defense system is ultimately detrimental to their health. Therefore, an adequate knowledge of the bee defense system and a focus on protective medicine to maintain this system in a strong and effective state are of vital importance [1,2].

II: Components of the Honey Bee Defense System

1. Endurance of Worker Bees

Honey bees are genetically coded to instinctively display certain hygienic defense behaviors.

a. For instance, nurse bees decrease the pH value and increase the acidity of the nutrients they store in their stomachs to feed

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the larvae. This is a behavior to hinder bacteria and fungi growth, thus protect the larvae against pathogenic microorganisms [1,2,22].

b. Worker bees drive sick bees out and clear the hive off residue material, destroy parasites or groom each other to get rid of parasites, identify diseased larvae and young and clear the cells of the honeycomb off them and display other hygiene-oriented instinctual behavior [1,32,33,34,35,36,37].

c. Bees also cover the hatched areas of the honeycomb, narrow the flight hole, cluster together and air the hive to establish the ideal balance between temperature, humidity and CO_2 for hatching. This ensures that their offspring develop in healthy conditions and prevents the formation of an environment where fungi, yeast, or other pathogens could easily develop and reproduce [1].

d. Worker bees also effectively safeguard their nests against the possible raids or looting by robber bees and other pests. This keeps away any pathogenic microorganisms and parasites the robber bees might carry to colonies, as well as protecting the colony life against obvious dangers of predators, wasps, and other pests. A genetic characteristic of Japanese honey bees allow them to raise their body temperature up to 50 ^{o}C in the event of an attack on their nests to almost burn or suffocate the intruder to death. European honey bees display a similar behavior in the presence of wasps, but they cannot possibly survive a temperature rise at the same level [1,5,35].

2. Strength of the Larvae

The larvae have the following individual resistance mechanisms which are genetically coded and subject to change with age:

a. In the bee larvae, the midgut (stomach) and malpighian tubules are not connected to the hindgut. For this reason, digestive and nitrogenous metabolic residues are stored in the body and the food around the larvae is thus protected from fecal contamination. Once larvae complete their feeding period and start weaving the cocoon (before the prepupal stage), the stomach and malpighian tubules form a connection with the anus to dispose of the fecal material, which is then stored on the cell floor. This anatomical change in the digestive system of larvae ensures hygiene and prevents the food from turning into a breeding ground for microorganisms [1,2,16].

b. Before the prepupal stage, owing to the anaerobic conditions in the digestive tract due to lack of connection between the stomach and the hindgut, younger larvae are far more resistant against disease than older larvae [1,22].

c. The larval digestive system is not suitable for the growth of vegetative forms of bacteria. As the larva develops, the peritrophic membrane lining the inner surface of the ventriculus thickens and this prevents vegetative forms of bacteria to turn infective for older larvae [1,22].

d. Honey bee larvae are vaccinated against bacteria before hatching with the help of yolk protein (vitellogenin). The bacteria forager bees carry into the hive on their bodies pass to royal jelly and to the queen bee who feeds on it. The queen bee breaks down the bacteria in the royal jelly and the protein vitellogenin in her blood binds these bacterial fragments and transports them to the eggs she lays. Thus, the larvae are vaccinated against these bacteria even

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before hatching. Based on this scientific process, it seems possible that a renewable vaccine can be developed in the future, for example against the spores of *P. larvae*, the bacterium causing American foulbrood disease [19,38,39].

e. The venom and hemolymph of many species contain various peptides (e.g. secropins, defensins, abaecins, gomesins, attacins, sarcotoxins, diptericins, and coleoptericins). Most of these are of low molecular weight and rich in amino acids such as proline, arginine, glycine and cysteine. They are effective on antibiotic-resistant gram-positive and gram-negative bacteria and protect the organism against bacterial infections. Some antimicrobial peptides do not only work well against bacteria, but they are also highly effective against viruses, fungi, protozoa, yeasts, and even parasites [1,2].

Some of these antimicrobial peptides are found in the bee venom and hemolymph. Bees are protected against infections through synthesizing peptides such as apidaecins, abaecins, or defensins. These peptides with antimicrobial action play an important role in the immunity of honey bee larvae against bacterial infections and *P. larvae*, the agent of the American foulbrood disease [1,2,16].

3. Life of Symbiotic Microorganisms and Spore Filtering

a. Microorganisms naturally occurring in the digestive system of bees are in a symbiotic relationship, preventing one another from reproducing beyond a limit and causing disease [1,2,22].

b. The digestive tract of bees filters out spores that bear disease agents. Useful probiotic bacteria in the intestinal flora of honey bees protect the intestinal wall against pathogens and harmful substances, remove pathogens from the body. The spores expelled with the feces cannot survive in the sun [1,2,22].

c. Useful lactic acid bacteria that live in the ventriculus and produce lactic acid inhibit the reproduction of *Paenibacillus larvae* and other pathogenic bacteria, help toxins removed from the body, and reinforce the immune system [1,2,22].

4. Strength Based on Nutrition and Produce

The structure, content, and antimicrobial properties of propolis and natural foods bees consume, such as honey, pollen, bee bread, and royal jelly are important components of the immune systems of bees and larvae.

a. Honey has an antimicrobial effect owing to its high sugar content, low water content, acidic structure, and hydrogen peroxide and various flavonoids and phenolic acids it contains. It has an important part to play in the bee defense system, preventing the development of bacteria and other pathogenic microorganisms [1,2,40].

b. Pollen protects bees against bacterial and fungal diseases with the antimicrobial effects of capric, lauric, myristic, linoleic, and linolenic acids it contains. For example, linoleic acid has an inhibitory effect on the development of the bacterium *Paenibacillus larvae*. Bee bread bees obtain by processing pollen is a food for larvae helping them develop better. It also protects the offspring against diseases and increases the lifespan of bees [17,40.41].

c. Especially during autumn, bees expand their protein reserves by renewing their fat tissues. Vitellogenin is the protein found in the blood of honey bees. It is crucial for the immune system and lifespan, not to mention the production of royal jelly and all

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other activities of bees inside and outside the hive. Bees that renew their fat tissues and increase blood vitellogenin levels before wintering have better immunity and live longer [1,2,19].

d. Royal jelly contains 10-hydroxy-2-decanoic acid (10-HDA) which protects larvae against infections and is very important as an antibiotic. An environment of approximately pH=6.6 is required for bacterial sporulation. However, the royal jelly used by nurses to feed the larvae is acidic (pH=3.6-4.2). Thus, the acidic nature and antimicrobial properties of royal jelly make larvae and bees resistant to bacterial and fungal disease agents [2,22].

e. Bees use propolis to narrow the flight hole as a protection against cold, robber bees and other natural enemies, to cover up holes and cracks in the hive, and to polish and disinfect the inner surfaces of the hive and honeycomb cells. Bees thus protect their nests from exposure to adverse external conditions, provide heat insulation, establish a balance between temperature and humidity in the beehive, and have protection against pathogens such as bacteria, viruses, and fungi. Moreover, they cover the remains of intruders that they killed, but could not remove from the hive. Organic remains buried under propolis do not decompose to stink or spread disease [30,42,43,44].

Conclusion

Honey bees developed a rather complex and strong defense system against pathogens, parasites, and pests through the structure of the digestive tract in larvae and adults. Microflora and probiotics in a symbiotic relation in their digestive system, vitellogenin and antimicrobial peptides in their blood, various hygienic and defensive behaviors of adult bees, and finally the structure, content, and antimicrobial properties of the natural food and propolis they consume.

However, bees are subjected to stress because of genetic insufficiency, unfavorable climate and environmental conditions, malnutrition, mistakes in nutrition and colony management in apiculture, as well as chemical and electronic pollution in their natural habitat. The result of such stress is the collapse of their defense system and rapid deterioration of their health. Therefore, it is of vital importance to eliminate these stressors and maintain the effectiveness of the immune system in order to protect the health and well-being of bees.

Bal Arılarında (*Apis mellifera* L.) Stres Faktörleri ile Hastalık, Parazit ve Zararlılara Karşı Savunma Sisteminin Bileşenleri

Öz: Hayvan sağlığı, diğer hayvansal üretim dallarında olduğu gibi, arıcılıkta da üretim etkinliğini sınırlayan önemli bir faktördür. Çünkü bal arısı hastalık, parazit ve zararlıları arıların sadece sağlığını olumsuz olarak etkilemekle kalmayıp, verimi düşürmekte ve hatta önlem alınmadığı taktirde koloni yaşamına son verebilmektedir. Zaman zaman salgın şeklinde ortaya çıkabilen hastalık, parazit ve zararlılar üreticilerin büyük kayıplara uğramasına yol açarak ülke arıcılığını çıkmaza sokmaktadır.

Diğer bütün canlılar gibi bal arıları da sağlıklarını olumsuz etkileyen hastalık, parazit ve zararlılara karşı çok bileşenli

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güçlü bir savunma (direnç, bağışıklık) mekanizmasına sahiptir. Ancak bal arılarının sahip olduğu savunma sistemi, genetik yapı kaynaklı ve çevresel birçok faktörün arılar üzerinde oluşturduğu stresin etkisiyle yetersiz kılıp çökmekte ve vücut direnci düşen arılar sağlıkları bozularak hastalanıp ölmektedir. Üstelik söz konusu stres faktörleri birbirleriyle, patojen mikroorganizmalarla, parazit ve zararlılarla sinerjik etki oluşturarak arılar, yavrular ve kolonilerde tükenmişliğe yol açmaktadır. Bu nedenle öncelikle bal arılarında savunma sisteminin etkisiz kalıp çökmesine neden olan bu stres faktörlerinin elimine edilmesi şarttır.

Anahtar kelimeler: Bal arısı, stress faktörleri, savunma sisteminin bileşenleri.

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MELLIFERA RESEARCH ARTICLE

An Evaluation on Bee Bread: Chemical and Palynological Analysis

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A B S T R A C T

Beebread is important protein source for honeybees in early spring. Chemical composition of beebread may vary according to geographical origin. In this study, proximate and fatty acids composition of five Citrus beebread samples were determined obtained from different geographical origins. Moisture content of citrus beebread samples varied between 11.0-16.4 %, ash 1.86-2.4 %, fat 7.0-13.4 %, and protein between 18.6-21.6 %. A total of thirty-seven fatty acids (FAs) were identified and of these palmitic, stearic, arachidic, oleic, eicosenoic, erucic, and linoleic acids were the most abundant in all of the samples. Beebread sample ratios of unsaturated/saturated FAs were ranged between 1.28 and 2.23 indicating that citrus beebread is a good source of unsaturated FAs. The pollen, proximate and fatty acid composition of beebread may vary significantly according to its geographical origin and citrus beebread is good source of unsaturated fatty acids.

Keywords: Citrus, beebread, pollen, fatty acid, pollen analysis, chemical composition

Introduction

Pollen, which is one of the bee products, is the reproduction units that occur in male organs of flowering plants [1]. When pollens are collected from plants by bees, they are usually adhered to with some saliva to form pellets. The pollen is carried in the corbicula and brought to the hive. This new product differentiated from flower pollen is called “bee pollen”. Bee pollen is the most important nutrient that provides the protein, lipid, sterol, vitamins and minerals necessary for honeybees to grow sufficiently after larvae and for the adequate development of their tissues, muscles, glands and other organs [2]. When honeybees carry the pollen they collect into the beehive, they store it in the form of a bee bread (perga) in the honeycomb cells. While the bee bread is produced in the hive, it is mixed with pollen, honey and other bee secretions and subjected to lactic acid fermentation. The mixture turns into bee bread in about two weeks, so that the bee bread, which is a fermented product, can be kept in the hive for a long time [3,4]. Bee bread is the source of protein, fat and vitamins for the feeding of bees. Although the contents of bee pollen and bee bread are similar, there are some differences. Bee bread contains less protein than bee pollen, but bee bread proteins are easier to digest [4,5].

Lipids are important to honeybees primarily as a source of energy with some components of lipids involved in the synthesis of reserve fat and glycogen and the membrane structure of cells [6]. Pollen is virtually the only source of lipid in the honeybee diet. Lipid components such as fatty acids are important in honeybee

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development, nutrition and reproduction. The bee pollen and bee bread have been studied for fatty acid composition. Soldberg & Remedios [7] reported that ether extracts of pollen consisted mainly of linoleic, linoleic and arachidonic acids. Human & Nicolson [8] investigated the amino acid and fatty acid composition of fresh, bee collected and stored pollen. In another study, Ceksteryte et al. [9] identified twenty-four fatty acids in the bee bread collected in spring and summer, and oleic and arachidonic acids were found to be the most abundant unsaturated fatty acids. Moreover, Ceksteryte, & Jansen [10] studied fatty acid composition in bee bread collected from various floral origins and linolenic acid was reported to be the highest within fatty acids identified in spring bee bread.

The Citrus genus is the most important of all Turkish plants to the beekeeping industry. At the beginning of spring, beekeepers migrate to the Mediterranean region to prepare their bees for the season. Also, in early spring, Citrus nectar and pollen have great importance for the development of bees in the region [11]. Bees collect plenty of citrus pollen and use them to produce bee bread.

Bee bread is an important nutrient for bees and recently gained importance in human nutrition due to its rich nutrient content and useful biological properties such as hypolipidemic, antimicrobial, and antioxidant [12-14]. People nowadays takes into account the nutritional activities as versatile, to be protected from certain diseases and natural nutrition for treatment has come to the fore. Studies in the literature about the bee bread are quite a few. It is the first study on fatty acid composition of citrus bee bread from different geographical origin. Therefore, the aim of this study is to investigate the pollen content, fatty acid and proximate composition of Citrus bee bread samples collected from various geographic regions.

Material and Methods

Bee bread samples

A total of five bee bread samples were collected from apiaries located in two major citrus growing provinces, Adana and Mersin (Mediterranean Region of Turkey) during 2014. Two of the samples were from Adana (Seyhan and Kozan) and three samples from Mersin (Erdemli, Silifke and Tarsus). Bee bread samples were collected manually from honeycombs and stored in a deep-freezer at -20 ^{o}C before analyses.

Chemicals

All chemical reagents were purchased from Sigma-Aldrich-Fluka Co. Ltd. (Steinheim, Germany), unless otherwise stated. N,O-Bis (trimethylsilyl) trifluoroacetamide, trimethyl chlorosilane, 5α-cholastene-3-β-ol and fatty acid methyl esters (FAME) mixture was obtained from ABCR (Karlsruhe, Germany), Merck (Darmstadt, Germany), Alfa Aesar (Karlsruhe, Germany) and Supelco (Bellefonte, U.S.A), respectively. Potassium hydroxide and anhydrous sodium sulphate were purchased from Merck (Darmstadt, Germany).

Pollen analysis

A 10 g of bee bread sample was weighed and dissolved in 20 ml of distilled water (20-40 ^{o}C). This solution was centrifuged for 10 min at 1.000 g. The supernatant liquid was discarded. The sediment was redisolved in 20 ml of distilled water to

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completely dissolve the remaining sugar crystals then centrifuged for 5 min at 1.000 g. The excess water in the sediment was removed by placing it on an absorbent paper. Then it was spread on a slide over an area of about 20 mm. The slide with the sediment of pollen was dried on a heating plate at 40 ^{o}C. The glycerine jelly was liquefied at 40 ^{o}C. The cover slips (22x22 mm) were warmed on the heating plate. One drop of glycerine jelly was united onto the cover slip and placed on the slide. The pollen grain exine and shape were visualized under light Microskope Nicon Eclipse E 600 and photographed. Pollen grains were identified using reference collection and with the help of microphotographs from the literature. About 500 pollen grains were counted in each sample. The frequency of pollen grains of each taxon is expressed as percentage of the total pollen sum [15].

Chemical analysis

Determination of ash, crude fat and crude protein in bee bread samples was carried out using standard analytical procedures, Association of Official Analytical Chemists [16], 920.153, 991.36, and 960.52 respectively. Moisture content was determined using a vacuum oven at 60 °C and weighing until a constant weight. The results were expressed in grams per 100 g of fresh weight. The ash content was determined gravimetrically following incineration in an oven at 550°C and weighing until constant weight. Nitrogen determination was performed using micro-Kjeldahl method. Then a conversion factor of 6.25 was used for converting percentage of nitrogen in the sample into percentages of protein. All analyses were carried out in triplicate.

Determination of oil content in bee bread samples was performed using the standard method of ISO 659 [17]. The bee bread samples were homogenized in a stainless steel Waring blender. A of 2 g of sample was weighed accurately into a glass beaker and 100 mL 4 N HCl were added. Then the content was heated at 100 ^{0}C and stirred for 15 minutes. The sample solution was then cooled to room temperature and washed with 25 mL distilled water for three times. Sample was filtered through filter paper and the filter paper was dried at 105 ^{0}C in an oven for 1 hour. Diethyl ether was used for the extraction of oil from bee breads at 50 ^{0}C for 3 h using automated Soxhlet extractor (VELP Scientifica Ser 148, Italy). Oil extracted from bee bread samples was kept in amber vials prior to fatty acid analysis.

Determination of Fatty Acid Composition

The ISO 12966-2 [18] standard method was used for the determination of fatty acid methyl esters (FAME) in oils of bee bread samples. In brief, 0.1 g bee bread oil was weighed into test tube with screw cap and 0.5 mL of methanolic 2 M KOH and 5 mL heptane were added and vortexed. Then, the upper layer was dried with anhydrous sodium sulphate for gas chromatography analysis. Chromatographic analysis was carried out by a Perkin Elmer Clarus 500 (Perkin-Elmer, Shelton, CT, USA) gas chromatograph equipped with an auto sampler, split-splitless injector and a flame ionization detector (FID). Chromatographic separation was achieved on a Supelco 2380 capillary column (100 m x 0.25 mm i.d., 0.2 µm film thickness). Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The injector and detector temperatures were set at 250 ^{0}C and 260 ^{0}C respectively.

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The temperature programme was started and held at 165 ^{0}C for 5 min then increased to 240 ^{0}C at a rate of 5^{0}C/min and finally held at 240 ^{0}C for 10 min. The injection volume was 1.0 μL and the split ratio was set at 1:50. Identification of the peaks was performed by comparing their relative retention times with those of the standard FAME mixture. The results were expressed as percent of total FAMEs.

Statistical analysis

All chemical assays were carried out in triplicate and the data were expressed as means ± standard deviations (SD). One-way analysis of variance (ANOVA) followed by least significant difference (LSD) was used to comparison of the data. Differences between means at the 95 % ($P < 0.05$) confidence level were considered statistically significant.

Results

The results of pollen analyses showed that bee bread samples contained Citrus spp. pollen at a range of 62.87-98.44 %. Other pollens belonging to Asteraceae, Fabaceae and Brassicaceae families were observed in the samples at minor and rare levels (Table 1)

Table 1. Results of palynological analysis of citrus beebread samples (n=5)

Sample	Geographical origin	% pollen
SeC	Seyhan (Adana)	78.02
KC	Kozan (Adana)	85.43
TC	Tarsus (Mersin)	62.87
EC	Erdemli (Mersin)	98.44
SiC	Silifke (Mersin)	89.39

The moisture content of bee bread samples collected from Adana region were between 15.82-16.43 % and the samples from Mersin region contained lower moisture ranging between 11.01 and 12.34 %. Similarly, Adana bee bread samples contained higher fat content ranged between 10.47 and 13.46 % than Mersin samples ranged between 7.06 and 9.22 %. The ash and protein content of Adana samples were between 1.86-2.03 and 19.71-19.83 respectively whereas Mersin samples had slightly higher content of ash and protein at a range of 2.03 to 2.42 and 18.60 to 21.60 respectively (Table 2). Compared to the bee bread samples from different geographical origin, statistically significant differences were determined in moisture, protein and fat content ($p> 0.05$).

A total of 37 fatty acids identified and quantified in Citrus bee bread samples (Table 3). The quantities of these twelve fatty acids determined were similar and there was no statistically significant difference between the samples ($p>0.05$). The saturated fatty acids including palmitic, stearic and arachidic determined in high amounts were common to all samples. The common unsaturated ones were oleic, cis eicosenoic, erucic and linoleic acids. Palmitic acid to saturated fatty acids was found in the range of 17.83-29.59 % in the bee bread samples studied. Of these unsaturated fatty acids, the amount of oleic acid ranged from 11.75 to 18.86%, cis eicosenoic acid from 2.24 to 8.51 %, erucic from 0.39 to 5.80 % and linoleic acid ranged from 14.19 to 32.41% in all of the citrus bee bread samples. There were no differences in terms of palmitic, stearic, erucic and oleic acids in the samples from different region. However, butyric, arachidic and oleic acids were found in

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greater amounts in Adana samples. Unsaturated/saturated ratio of fatty acids in all samples was determined in the range of 1.28 and 2.23.

Table 2. Chemical compositon of citrus bee bread samples (n=5)

Bee bread samples	Moisture	Ash	Protein	Fat
SeC	15.82±0.01[d]	2.03±0.01[b]	19.71±0.01[b]	10.47±0.04[c]
KC	16.43±0.02[e]	1.86±0.01[a]	19.83±0.02[c]	13.46±0.03[d]
EC	11.01±0.04[a]	2.42±0.01[c]	21.60±0.02[e]	7.06±0.34[a]
MC	12.34±0.02[c]	2.42±0.01[c]	20.09±0.02[d]	9.15±0.03[b]
SiC	11.86±0.01[b]	2.03±0.01[b]	18.60±0.02[a]	9.22±0.07[b]

Data: Arithmetical mean ±SD. [a-e] The groups in the same row with different letters are statistically significant ($p < 0.05$).

Discussion

Citrus honey is regarded as one of the best unifloral honey due to its unique taste and floral aroma [19]. It is also economically important for migratory beekeeping in Anatolia as well as local beekeeping in the Mediterranean region. In a several studies, citrus pollens found as "under represented" in citrus honeys from different countries [11,20]. It is attributed to anther maturity does not always coincide with the maximum secretion of nectar [21]. However, Citrus pollen was represented predominantly in bee bread samples analyzed in this study. The honeybees need to increase their population, in early spring in order to have a productive season. In this season, the flora is not diverse and rich yet and therefore, the vast majority of the beekeepers move their hives to the Mediterranean region where plenty of citrus pollen and nectar available for the development of bees.

Bee bread samples showed differences in the chemical analysis. Despite being both Mersin and Adana provinces located in the Mediterranean region with high-density citrus orchards, moisture content of the bee bread samples collected from Mersin was lower than the samples collected from Adana. This can be explained as the influence of environmental factors on the chemical composition of bee bread. Similarly, fat content of bee bread samples from Adana was found lower than Mersin samples. Nevertheless, the protein content of citrus bee bread samples collected from Mersin was higher than the beebread samples of Adana. In a study, the moisture contents ranged between 18.8 and 28.0%, protein content between 19.3 and 26.5%, ash content ranged from 2.1 to 3.2 % and lipid content changed between 3.9 and 6.7% of bee bread samples [22]. In another study, HTF (hot-water fraction), WSF (water soluble fraction) and ESF (ethanol soluble fraction) of bee bread from Lithuania were extracted and the protein contents of these fractions were 2.29 mg/ml, 9.59 mg/ml and 1.94 mg/ml, respectively [11]. A review by Roulston and Cane [23] of ether extractable material from dry pollen of 62 plant species showed the percent lipid in pollen to vary from 0.8 % (*Eucalyptus marginate*), to 18.9 % for dandelion (*Taraxacum officinale*). According to the obtained data, differences in the chemical composition of bee bread samples may vary depending on botanical origin, environmental and geographical factors and extraction methods.

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Table 3. Fatty acid composition of citrus beebread samples from different regions.

Saturated Fatty Acids		Fatty acid contents (%)				
		Adana Province		Mersin Province		
		SeC	KC	TC	EC	SiC
C4:0 Butyric acid		1.34+0.01^{c}	1.87+0.03^{d}	0.37+0.01^{a}	0.83+0.01^{b}	1.30+0.07^{c}
C6:0 Caproic acid		0.36+0.00	0.09+0.01	-	0.13+0.01	-
C8:0 Caprylic acid		0.09±0.02^{b}	0.09±0.01^{b}	0.02±0.00^{a}	0.29±0.01^{c}	0.06±0.01^{b}
C10:0 Capric acid		0.05±0.01	0.67±0.06	0.16±0.01	-	0.11±0.01
C11:0 Undecanoic acid		-	0.09±0.00	-	-	0.08±0.00
C12:0 Lauric acid		0.28±0.00^{abc}	0.60±0.02^{d}	0.11±0.01^{ab}	0.08±0.00^{a}	0.47±0.02^{bc}
C13:0 Tridecanoic acid		-	0.07±0.00	-	-	-
C14:0 Myristic acid		0.52±0.01^{ab}	0.65±0.02^{ab}	0.22±0.01^{a}	0.56±0.01^{ab}	0.85±0.04^{b}
C15:0 Pentadecanoic		0.20±0.01^{bc}	0.09±0.06^{a}	0.14±0.01^{ab}	0.28±0.01^{c}	0.13±0.01^{ab}
C16:0 Palmitic acid		29.59±0.18^{b}	17.83±8.39^{a}	22.49±0.29^{a}	23.20±0.06^{a}	27.18±1.34^{a}
C17:0 Heptadecanoic acid		0.49±0.01	0.49±0.00	0.33±0.01	0.24±0.00	0.32±0.00
C18:0 Stearic acid		6.56±0.05^{b}	3.27±0.06^{a}	2.35±0.03^{a}	2.57±0.01^{a}	3.34±0.00^{a}
C20:0 Arachidic acid		3.35±0.01	6.08±±0.04	1.07±0.01	0.91±0.00	1.40±0.06
C21:0 Heneicosanoic		0.45±0.02^{b}	0.08±0.00^{a}	1.80±0.01^{c}	0.08±0.01^{a}	0.24±0.01^{ab}
C22:0 Behenic acid		0.09±0.00^{a}	0.60±0.05^{b}	0.41±0.02^{ab}	1.25±0.02^{c}	0.36±0.07^{ab}
C23:0 Tricosanoic ac		0.43±0.01^{a}	2.16±0.08^{a}	5.79±0.12^{b}	0.51±0.02^{a}	0.55±0.02^{a}
C24:0 Lignoceric acid		0.13±0.01	1.29±0.08	0.04±0.00	-	0.28±0.01
Total		**43.93**	**36.02**	**35.3**	**30.93**	**36.67**
Unsaturated Fatty Acids	ω	**AC**	**KC**	**MC**	**EC**	**SC**
C14:1 Myristoleic acid	ω-5	1.18±0.01^{b}	1.17±0.06^{b}	0.76±0.01^{ab}	1.58±0.01^{b}	0.67±0.01^{a}
C15:1 cis-pentadecanoic acid		0.16±0.01	0.40±0.02	0.09±0.01	0.11±0.01	0.48±0.02
C16:1 Palmitoleic acid	ω-7	0.12±0.01^{a}	0.12±0.01^{a}	0.15±0.00^{b}	0.22±0.00^{c}	0.13±0.00^{a}
C17:1 cis-Heptadecanoic acid		4.21±0.01	8.77±0.08	0.27±0.01	0.15±0.02	0.26±0.02
C18:1n9t Elaidic acid	ω-9	0.07±0.03	0.27±0.02	0.03±0.01	0.06±0.01	0.17±0.04
C18:1n9c Oleic acid	ω-9	16.29±0.19^{ab}	18.86±0.05^{a}	11.75±0.06^{a}	21.53±0.03^{b}	13.06±0.63^{a}
C20:1 cis-Eicosenoic	ω-9	8.51±0.12	8.58±0.06	2.24±0.59	2.57±0.01	2.32±0.09
C22:1n9 Erucic acid	ω-9	3.69±0.02^{b}	4.22±1.66^{b}	5.54±0.05^{b}	0.39±0.02^{a}	5.80±0.31^{b}
C24:1 Nervonic acid	ω-9	0.34±0.01^{ab}	0.34±0.01^{ab}	0.23±0.01^{a}	0.84±0.03^{c}	0.40±0.01^{b}
C18:2n6t Linolelaidic acid	ω-6	0.18±0.02	1.67±0.02	0.16±0.01	0.18±0.01	0.05±0.01
C18:2n6c Linoleic acid	ω-6	14.90±0.42^{a}	14.19±0.07^{a}	24.45±0.28^{ab}	32.41±0.09^{b}	28.53±1.38^{b}
C18:3n6 g-Linolenic acid	ω-6	2.80±0.05^{b}	0.08±0.01^{a}	0.06±0.01^{a}	0.07±0.01^{a}	0.10±0.00^{a}
C20: 2 cis-11,14-Eicosadienoic	ω-6	0.09±0.01^{a}	1.40±0.69^{bc}	0.96±0.06^{ab}	0.81±0.03^{ab}	2.14±0.09^{c}
C20: 3n6 cis-8,11,14-Eicosatrienoic acid	ω-6	0.07±0.01^{a}	0.51±0.03^{a}	0.03±0.01^{a}	1.85±0.20^{b}	0.02±0.01^{a}
C20:4n6 Arachidonic acid	ω-6	1.14±0.61^{b}	1.10±0.06^{b}	0.05±0.01^{a}	2.98±0.02^{c}	0.19±0.01^{a}
C22: 2 cis-13,16 Docosadienoic	ω-6	1.24±0.06^{d}	0.68±0.06^{b}	0.46±0.03^{a}	0.91±0.03^{c}	0.63±0.02^{ab}
C18:3n3 a-Linolenic acid	ω-3	0.11±0.03^{a}	0.71±0.03^{a}	17.22±0.19^{c}	0.29±0.07^{a}	6.78±0.11^{b}
C20: 3n3 cis-11,14,17-Eicosatrienoic acid	ω-3	0.52±0.05^{ab}	0.21±0.00^{a}	0.58±0.02^{ab}	1.11±0.07^{b}	0.88±0.02^{b}
C20: 5n3 cis-5,8,11,14,17-Eicosapentaenoic acid	ω-3	0.52±0.05^{ab}	0.88±0.02^{b}	0.21±0.00^{a}	0.58±0.02^{ab}	1.11±0.07^{b}
C22:6n3 Docosahexaenoic acid	ω-3	0.07±0.01^{a}	0.11±0.05^{ab}	0.04±0.00^{a}	0.43±0.02^{c}	0.23±0.01^{ab}
Total		**56.21**	**64.27**	**65.28**	**69.07**	**63.95**
Unsaturated/saturated		1.28	1.78	1.85	2.23	1.74

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The lipid content of pollen is quite variable with values ranging from 0.8-18.9 % [23]. One important component of lipids for development of honeybees is fatty acids [6]. The nutrition aspect of fatty acids to honey bees is well known. Of the seven common fatty acids found in all bee bread examined in our study, palmitic, stearic, and arachidic acids are saturated fatty acids, while oleic, erucic and eicosenoic acids are mono saturated fatty acids (also known as omega 9) and linoleic acid (polyunsaturated, omega 6). In honeybees, pollen stimulates the development of the hypopharyngeal gland that is responsible for producing royal jelly used to feed larva [24]. Several studies were made on fatty acid related to royal jelly and the honeybee body. Royal jelly was found to be dominated by palmitic, oleic, linolenic and myristoleic acids all in amounts greater than 10 % of the total lipid [25]. In addition, they also showed that adult honeybee tissue had large amounts of oleic acid, whilst for 6 days old larvae, oleic and palmitic acids were equally dominant. Manning [26] reported that linoleic acid was present in merged bees and in adult bees, linoleic acid was the second highest fatty acid by concentration.

Studies on fatty acid content of pollen and bee bread are limited. Serra Bonvehi and Escola Jorda [27] stated that bees select pollen with a high level of unsaturated fatty acids, which are more adequate for honey metabolism. For example, oleic acid an important fatty acid honey bees. Some fatty acids such as linoleic, linolenic, myristic and dodecanoic acids are highly antimicrobial [28,29]. Ceksteryte et al. [9] reported oleic and arachidonic were found to be the most abundant unsaturated fatty acids, constituting around 15% of total fatty acids. Moreover, Ceksteryte, & Jansen [10] showed linolenic acid (n-3) was reported to be the highest within twenty-two fatty acids identified in spring bee bread.

In our study, although having the same botanical origin, the geographical origin affected the quantity of fatty acids particularly. Citrus samples from both Mersin and Adana contained all the following palmitic, stearic, arachidic (saturated), oleic, linoleic, cis Eicosanoic, and erucic (unsaturated) acids at significantly different percentages. Therefore, the differences in the compositions of the samples from these locations are not only related to the intensity of plant populations, but also with the different chemical composition of species of Citrus genus and preferences of honeybees. Sczcesna [30] published similar findings to ours for Eucalyptus bee pollen, namely, Australian eucalyptus pollen contained linoleic and linolenic acid, whereas Italian eucalyptus pollen contained a higher proportion of linoleic acid.

The total of unsaturated fatty acids was higher than the sum of saturated fatty acids found in all the samples. The fatty acid content of bee bread is very important because deficiency of polyunsaturated fatty acids on insects' diet may result in slow body development, deformed wings and lower productivity. Also, fatty acids release the required energy on the flight muscles during the flight [31]. However, unsaturated fatty acids are not just essential for bees but also for human nutrition. Unsaturated fatty acids have many beneficial health effects such as reducing the level of serum triglycerides [32]; possessing cardio protective properties through reducing blood cholesterol, triglyceride level and exerting an anti-arrhythmic, anti-thrombotic, anti-inflammatory impact [33]. In addition, linoleic acid is an essential fatty acid required by humans and external source. It is reported in helping lower the ratio of low-density lipoproteins which carry such lipids as cholesterol from the body via the liver [34].

As a conclusion, the changes of the pollen content, fatty acid composition, and chemical composition of Citrus bee bread samples originated from different geographical origin were determined. The results revealed that Citrus plants preferred or readily available for bees as pollen resource was detected extensively in the bee bread samples. It is clearly seen from the results of current study that the proximate composition and the fatty acid composition of Citrus bee bread samples from different geographical origins varied significantly.

Arı Ekmeği Üzerine Bir Değerlendirme: Kimyasal ve Pallinolojik Analiz

Öz: Arı ekmeği, ilkbaharda bal arıları için önemli bir protein kaynağıdır. Arı ekmeğinin kimyasal bileşimi coğrafik orijine göre değişebilir. Bu çalışmada, farklı coğrafi orijinlerden elde edilen beş narenciye arı ekmeği örneğinin kimyasal ve yağ asitleri bileşimi belirlenmiştir Narenciye arı ekmeği örneklerinin nem içeriği % 11.0-16.4, kül % 1.86-2.4, yağ % 7.0-13.4 ve protein % 18.6-21.6 arasında değişmiştir. Toplamda otuz yedi yağ asidi (FA) tanımlanmış ve bunlardan palmitik, stearik, araşidik, oleik, eikosinoik, erüsik ve linoleik asitler tüm örneklerde en çok oranda bulunmuştur. Arı ekmeği örneklerinde doymamış/doymuş yağ asitleri oranları, 1.28 ile 2.23 arasında değişmiştir ve bu sonuç, narenciye arı ekmeğinin iyi bir doymamış yağ asidi kaynağı olduğunu göstermiştir. Arı ekmeğinin polen, kimyasal ve yağ asidi bileşimi, coğrafik orijine göre önemli ölçüde değişebilmektedir ve narenciye arı ekmeği iyi bir doymamış yağ asitleri kaynağıdır.

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MELLIFERA REVIEW ARTICLE

Beekeeping Activities in Turkey and Algeria

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ABSTRACT

The present review consists of highlighting the different aspects of one of the most important sectors of agriculture, beekeeping, of two countries; Turkey and Algeria.

We gave a historical overview about beekeeping of both countries and also the flora and fauna (more especially on honey bee species) components of the two countries and their impacts on the beekeeping. In addition, position of the beekeeping in each country's economy and its possibilities as well as the most important problems that face the beekeepers have also been cited.

Keywords: Beekeeping, Turkey, Algeria

Introduction

Beekeeping, which means rearing bees, has been practiced since antiquity and still widely widespread in the world. Practiced on all continents, this activity differs according to the climate, flora, the variety of bees and the level of economic development.

Turkey forms a bridge between Europe and Asia, surrounded by the Black Sea in the north, the Aegean in the west, and the Mediterranean in the south. It is bounded by the Caucasus mountains in the north-east, and the Middle East in the south-east. Turkey is a mountainous country and it has many rivers, lakes and dams. The climate ranges from subtropical to arid upland steppe. Turkey is endowed with a diverse and rich flora [1]. It has about 10.000 plant species and 3.506 of them are endemic to this country. About 500 of them provide large amount of nectar and pollen for bees [2]. Beekeeping is conducted in all the provinces of Turkey. On the Mediterranean coast: from the end of autumn until spring, *Erica*, *Eriobotrya japonica*, *Prunus amygdalus*, *Citrus*; In spring in mountains and arcs: *Trifolium*, *Thymus*, *Astragalus* and *Salvia officinalis*; in the Thrace region: *Helianthus annuus*; in the South (Southeast and East) regions: *Gossypium*, are seen as the most important source of nectar and pollen for bees. Besides that, in the southwestern region they grow an important source of nectar which are the pine trees. In addition, in nature there are trees such as *Acacia*, *Tilia*, *Rhodendron*, *Castanea sativa* which grow wildly and contribute importantly to beekeeping [3]. In addition, almond trees (*Amigdalus*), *Castanea sativa*, *Castanea vulgaris*, *Salix alba*, *Robinia pseudoacacia* and *Erica* are the most important plants contributing in the enrichment of the beekeeping in Turkey with an interesting pollen yields [4, 2].

Algeria is located in North Africa and with an area of 2.4 million km^2 it is the largest

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country in the African continent. It includes 4000 plant species. There are three geographical regions: the tell in the north, the steppe and the Sahara. The tell covering 5% of the territory spreads on 1600 km of the coast and includes towards the interior many plains and the different mountains of the Tellian atlas. It is a region under the influence of the Mediterranean climate and presents the most diversified flora of the country with 2500 species of which 10% are endemic. Citing as an example: the species *Erysimum cheiri* of the *Brassicaceae*'s family and *Hypochaeris saldensis* of the *Asteraceae*'s family, two endemics of Gouraya National Park in the area of Bejaia [5].

Many trees, cultivated crops and wild plants, like eucalypt (*Eucalyptus camaldulensis* and *E. globulus*), orange trees (*Citrus* spp.), sunflower (*Helianthus annuus*), clover (*Trifolium* species), French honeysuckle (*Hedysarum coronarium*), Rosemary (*Rosmarinus officinalis*) and thyme (thymus sp.), provide nectar and pollen for the bees. Also, natural forests, including pine trees, are good sources for the bees and it is possible to obtain honey all year round [6, 7]. After passing the south side of the Tellian atlas, it is the steppe. It covers about 15% of the territory and includes the highlands whose attitude varies between 600-1200 m. It is under an arid Mediterranean climate and has a less rich flora than the tell with about 1100 species with few endemics.

As an example, *Saccocalyx satureioides* (steppic thyme). When we pass the southern side of the Saharan Atlas it is the Sahara. It covers 80% of the territory. It is under the influence of an extremely arid climate and thus presents the less rich flora with only 600 species and less favourable for beekeeping. On the other hand, the rate of endemics is high; 25%. From the most well known we can cite the Saharan Myrtle (*Myrtus nivellei*) and *Olea europea* subsp. *lapperrinei*. [5].

Beside this richness in flora of the two countries, we can observe a great diversity of the honeybees *Apis mellifera* breeds. In Turkey, the presence of at least five different races of *Apis mellifera* has been investigated: *A. m. anatoliaca*, *A. m. caucasica*, *A. m. meda*, *A. m. syriaca*, *A. m. carnica* [8, 9] and the recently found to be existed in the Greek border region *A. m. macedonica* [10]. While in Algeria, there are two races: Tellian *A. m. intermissa* and the Saharan *A. m. sahariensis*.

With these two most indispensable components, an abundant and varied flora and the bee, the beekeeping becomes a necessity to be cared out. That, not only because it is a very important activity in the development of agriculture for its various productions, noting that the bee is a source of incomparable wealth for the whole world. Honey, pollen, royal jelly, venom, larvae and the bee itself are used in dietetics and pharmacy, but also, since a third of what we eat would not exist if the bees disappeared. Beekeeping is involved, through the pollination process, as an element of integration in the development of fruit growing, not to neglect the fact that it intervenes in the process of income formation of farmers located in agro-zones ecologically difficult (mountain areas and foothills, oases, steppes) [11].

By the aim of this review, further information about history, general structure and position of the beekeeping in each of the two countries' economy and the most important studies done in this field have been underlined.

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I. Brief history of beekeeping

I. 1. Turkey

"Turkey is on the intersection of three continents and also located on two important trade routes of the past, namely the Spice and Silk Roads. Thus, it played a very important role bridging Asia, Europe and Africa. Indeed, Turkey was also the place where very important civilizations such as the Roman, Hittite, Byzantine, Ottoman and finally the modern Turkish Republic became established. Covering all of these civilizations, beekeeping can be divides into three main periods, supported by archaeological findings, the written laws of Ottomans and the present period of the new Republic. The history of beekeeping in Turkey is well documented in many books and articles (Crane, 1983; Crane and Graham, 1985; Kandemir, 2003; Akkaya and Alkan, 2007)." [12].

According to Kandemir, 2018 the three periods of Turkey's beekeeping history are:

Before Ottoman Empire

They have been found in central Anatolia, Boğazköy (Çorum) and Hattusa, many beekeeping remains like hives, bees, bees wax and some laws tablets showing how the honeybee thief was punished, going back to the period of Hittite Kingdom ([13]; [14]).

Furthermore, in Çatalhöyük, located in the Northeast of Konya and represent from the most important archaeological sites in Turkey and in the world, they have been observed, in a form of wall paintings and motifs on objects such as rugs, the presence of honey, beeswax, bee figures as well as the domestication of many animals and the daily life in this city which its presence goes back to B.C. 80007000 [15, 16].

In addition, dating back to the period of the Hellenistic and Romans, bee figures on different objects such as coins have been found in the excavations in Ephesus and Torbalı [17].

Moreover, about the beekeeping history in this period, about 2500 years ago, an intoxication incident has been reported while a group of soldiers during their passage by the villages of Trabzon consumed honey from hives. This was due to the "mad honey" in which till the present time is the cause of a lot of intoxications in these regions [12].

Ottoman Empire Period

The Ottoman Empire recorded an important plus for the history of beekeeping in Turkey.

"Many Ottoman Sultans used honey as a sweetener. During the period of Ottoman Sultan "Fatih Sultan Mehmet", more than 3 tons of honey was consumed in Topkapı Palace according to the records. In the Ottoman Empire period, beekeepers had to pay tax for their hives (Öşr-ü- kovan meaning hive tax) and honey (Öşr-ü asel meaning honey tax), until the end of 18^{th} century, all sweets were made from grape molasses and honey.

Beeswax was also used in Ottoman Empire for document seals and also candles as light sources. At the end of the Ottoman period (the beginning of 1900's) beekeeping books and leaflets were published and the first modern beekeeping book was translated but not published for a long time. [12].

Modernization Period

In this period the beekeeping stayed stable for some time.

After 1923, the government put an important step in order to develop the activity of beekeeping by teaching it in schools. In the other hand, the use of

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primitive hives was still dominant. In addition, the production of honey was also law. However, this period has been considered as a transition period from using the primitive beekeeping to the modern beekeeping.

The modern beekeeping started to be developed significantly after the establishment of the Development Foundation (TKV) in 1969 and far away in 2003, the foundation of the Turkish Beekeeping Association. The aim is to relate the beekeepers to the government and to solve their problems.

For the same aim, many institutes (Ardahan İnstitute and Ordu Institute) and nongovernmental organizations (like Al Nihat Gökyiğit [ANG] foundation) were established, as well as the contributions of universities are not neglectable [12].

I. 2. Algeria

The ancient Algeria passed by many dynasties and empires such as the ancient Numidians, Phoenicians, vandals, Carthaginians, Byzantines, Abbasids, Umayyads, Idridis, Rustamis, Aghlabid, Ziris, Fatimids, Hammadids, Almohads, Ottomans,

Almoravids and the French colonisation empire. In the period of French occupation, many archaeological sites have been destroyed to build new cities or establish prisons, as it has also burned or hidden many state archives.

Beekeeping is an ancestral practice in Algeria. However, its origin is lost in the mists of time. Furthermore, data are very few.

According to [18], the history of Algerian beekeeping can be divided into two important stages:

During French colonisation

Traditional beekeeping was important, but modern beekeeping was largely at the hands of settlers without knowledge transfer to indigenous peoples.

[19] site the statistical data of 1891, there were 27,885 beekeepers including 260861 Algerians owning together 231,329 traditional hives. The 1000 French beekeepers operated about 10,000 hives with frames. - Before the national liberation war, the French authorities estimated at 150,000 traditional hives in Algeria but other information estimates the double 300,000 traditional hives and 20,000 hives to frame.

In 1954 came the national liberation war which contributed to the destruction of a large part whose situation was critical to independence [20- 22].

During the war of liberation, a large part of the traditional hives was destroyed by the French army which considered that each hive could serve as a hiding place for weapons.

After independence

During this period, the State looked into the problem of the beekeeping sector by implementing development programs. It has focused on the growth of livestock, moving towards the importation of foreign bees and also to build a hive called Algerian [18, 23] and the creation of beekeeping cooperatives.

II. Current beekeeping situation of the two countries

II. 1. Turkish beekeeping

Turkey has a great natural potential for beekeeping; various and economically

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precious bee breeds, richness in floral resources and the appropriate climate conditions [1]. As it showed a remarkable progress in recent years, in which almost in all cities of Turkey, beekeeping unions have been founded and gathered under one main union (Central Turkish Beekeeping Association [TAB]). In addition, each beekeeper has an identification number in order to be able to control all what concerns the production and the problems that may happen [10].

The number of beekeepers in Turkey increased from 40.000 [24] to 83.210 in 2017 (Statistical İnstitute of Turkey [TÜİK]). One from the important development images of the Turkish beekeeping as well is in term of number of beehives. Turkey is considered among the countries having a high number of beehives in the world [25].

The country's colony presence was 4,2 million between 2000-2002, it reached 6,6 million in 2013 [26].

In an agriculture fair held on 27. 09. 2017 in Samsun, the Ministry of Food, Agriculture and Livestock Deputy Undersecretary Hasan Özlü, in his speech: "As of 2017, there are 7,9 million of beehive units" said.

Langstroth type hives are the most applied in the beekeeping of Turkey and only about 4,2 % as traditional hives are still used [25].

Honeybee races

In turkey there are at least five different races of the honeybee *Apis mellifera*:

Apis mellifera anatoliaca (central Anatolia) it has large spread in lot of regions in Anatolia. They are generally small bees and have a light brown colour. The most important characteristic of these bees that they are vey adapted to the geography and climate of Anatolia. They are more resistant to winter conditions and diseases [27, 28]. Thus, they have many ecotypes adapted to different regions of Turkey, in which the most common are Muğla, Thrace and Central Anatolian ecotypes [29, 30].

A. m. caucasica (North east of Turkey) The second important honeybee in Turkey. They are seen in the eastern Anatolian plateau and in the border regions of Caucasus. They are adapted to highlands and temperate climates. They are dark grey in colour and have the longest tongue between the other bee races. They give stronger colonies during the summer. They use lot of propolis and the honey yield that can produce is far superior than that of the Anatolian honeybee. It is found that they are sensitive to Nosema disease [25; 27].

A. m. carnica (Black Sea region) called as well grey bees, they are slim and have a long tongue. They are the calmest bee breeds. Their giving puppies' yield is very good and they use less propolis. Besides that, in harsh climatic conditions they have a very good wintering skill. It is observed that they are not sensitive to puppies' diseases [27].

A. m. meda (southeastern of Anatolia) and *A. m. syriaca* (southwestern of Anatolia) It was observed that they are characterized by less honey storage and their aggressivity comparing to the other bee races and ecotypes of Turkey [31].

Bee products

The most important beehive product in Turkey is honey. Honey production has increased from 81.115 in 2010, 107.665 in 2015 to 114.471 ton in 2017. Yearly honey production is reported as 14 kg per colony in the years 2012-2015 [10, 32]. Turkey offers a great diversity of honey types with different taste, colour and aroma [2].

“As unifloral honey there are produced numerous kinds of honey including 'yonca' (alfalfa), 'Anzer', 'kestane' (chestnut), 'deli' (crazy), 'okaliptus' (eucalyptus), 'ihlamur' (linden), 'nane' (mint), 'portakal' (orange), 'çam' (pine),'kuşdili/biberiye' (rosemary), 'ayçiçeği' (sunflower), 'kekik' (thyme) honey.” [33].

Anzer honey is the most expensive honey in Turkey and it is produced in Anzer highland located in Rize [34].

Deli honey is produced by taking nectar from Rhododendron plants which contains high level of grayanatocin which makes the honey poisonous [33].

Also, the honeydew produced in Turkey represent 85 % of it in the world [35].

The average consumption of honey in Turkey estimated 1,2 kg, in which almost all the honey produced in the country per year in consumed locally [36].

“In 2014, Turkey exported approximatively 5.000 MT (20 million $) honey mostly to Germany, United States, Jordan, Hungary, Iraq, Saudi Arabia, Austria, Northern Cyprus, Belgium and Spain”. [36]

In the other hand, “Turkey has no import ban on honey, the Turkish Ministry of Economy implemented a high custom tariff on imports, thus making it difficult for Turkish business to import honey”. [36]

Beeswax, pollen, propolis, royal jelly, bee venom, bee swarm, package bee, queen bee and bee hives are also a part the beekeeping economy of Turkey [37].

Beeswax production is 4750 ton in 2015 [38], which is not enough to cover the demand. In consequence, in the last years it was obliged to import the wax.

There is no sufficient data about the yearly production of pollen, royal jelly, propolis and bee venom [10].

Queen production is about 100.000 which is reported that it is less sufficient.

Furthermore, because of the huge importance of bee pollination, it has a crucial role for any country’s economy. “In Turkey, the added value to agriculture from honeybee pollination is over $ 2.3 billion annually” [39].

Most faced problems

The most serious problem is presence of honeybee diseases;

Marketing, trading and quality of the products;

Environmental factors (like climate);

Education of beekeepers;

“Beekeepers have to pay fees to the farmers instead of being paid for pollination services”; - “Beekeepers can not enter to some places in Turkey with scientifically unknown reasons”; in addition to the underestimated pollination [25].

Uncontrolled use of pesticides in agricultural areas affects so badly the productivity of bees and the yield of colonies [40].

Resistance and residue problem as a result of overusing the chemical treatments [41].

Migratory beekeeping

Migratory beekeeping is widespread in Turkey, in which, the beekeepers transport their hives from one place to another in order to reach a sufficient and the desired level of honey production [39]. The transfer is generally done to citrus and thyme areas in spring, to the fire forests in June, to cotton areas in summer, clover and

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sunflowers areas in August and to pine forests in September and October [42]. It is first started about 35-40 years ago from the Black Sea region [1].

80 % of total honey production in Turkey is operated by the main of migratory beekeeping [43], and around 75 % of total Turkish honeybee colonies are concerned in this practice. It provides the country with an important revenue. The average honey yield resulted after a long distance migratory beekeeping can reach 30 Kg per colony [44]. However, it has been reported that the migratory beekeeping is a main cause of lot of beekeeping problems. It affects seriously the natural genetic diversity of bee races in Turkey. Moreover, it causes the distribution of resistant varroa mites and other diseases to the other colonies in areas visited, which results huge colony losses [10].

II. 2. Algerian Beekeeping

Algeria has within its possibilities that conditions the success of this sector the mild climate, especially in the north, and the diversity of honey resources well adapted to the three levels of climate present in the country.

With the National Plan of Agrarian Development (N.P.A.D.) that the State set up in 2000, beekeeping in Algeria has undergone a certain development in which the evolution of the beekeeping continues to increase.

During the period 2000-2008, the beekeeping sector underwent a major improvement thanks to this plan, such as, the apiarian livestock increased from 360.000 in 2000 to nearly one million colonies in 2008. Thus, the production of honey has been tripled; it has changed from 10.500 quintals in 2000 to 33.000 quintals in 2008 [45].

In addition, a very large number of traditional hives have been replaced by modern hives; 95.000 was the number of traditional hives in 2000 and it became 25.000 in 2008 [45].

The Agricultural and Rural Renewal (ARR) strategy applied in the country has contributed as well to the development of the beekeeping sector. The number of colonies has increased by more than 30 % to reach 1.3 million between 2008- 2014. In the same period, the production of honey was estimated around 600.000 tons, as it was noted a diversification of bee products (pollen, royal jelly, propolis, beeswax) [45].

Moreover, in the framework of the country's Human Capacity Building and Technical Assistance Program (HCBTA), more than 40.800 beekeeping trainings, provided by different institutions, have been launched. This resulted the presence of more than 40.000 beekeepers distributed in 43 wilayas from 48 (present in different geoclimatic and agricultural zones: mountains forests, steppe and Saharan areas) [45, 46].

The Langstroth hive type is the most modern hive used in Algeria. This type of bee hive has undergone some modifications in order to protect the bees from winds and hot weather (especially in the warmer regions) and to promote a better production of honey. In the desert areas of Algeria where temperatures are very high and winds are violent, traditional hives made of stone and clay have also been found [11].

Honeybee races

Algeria has two main breeds of bees. *Apis mellifera intermissa* and *A. m. sahariensis*.

a. *A. m. intermissa* (Tellian bees) Called Tellian bee relative to the Tellian Atlas Mountains and named by some the

"Phoenician bee" compared to the ancient Phoenicians who settled on the coasts of North Africa, spreads in the northern regions of

Algeria, Morocco and Tunisia. It's a medium sized bee compared with the other bee breeds and its colour is black [47].

"Generally, more than 100 queen cells are built during the swarming period and several virgin queens can coexist until the fertilization of one of them, an observation that is made in other Mediterranean breeds. The colonies swarm lot, sometimes seven times in a season. They are nervous bees and have a strong defensive character. Moreover, they propolis a lot" [48] and it is characterized by a good production of honey [49].

A. m. intermissa is very sensitive to brood diseases but resists well to that of adults [50].

b. *A. m. sahariensis* (Saharan bee) It is the most threatened bee breed because of its low interest in the Algerian economy. Its pollinating activity is not negligible and its scientific interest lies mainly in its ability to adapt to the desert climate of the Sahara [47]. "This bee spreads in the oases of southern Morocco and western Algeria. In the oases of the Eastern Algerian Sahara, the presence of *A. m. intermissa* was registered. It can survive in extreme weather conditions, with temperatures ranging from -8 ° to 50 °. The colonies are not very populous. *A. m. sahariensis* is smaller than *A. m. intermissa*. It seldom swarms, makes few royal cells and virgin queens are eliminated during swarming. Moreover, the colony propolis little and is not very defensive although a little nervous" [48]. Its colour is yellow and its production of honey is good.

"Many foreign breeds have been introduced to Algeria, such as common bees of Europe, Italian, Caucasian, etc…" [47].

Bee products

In Algeria, as well, honey is found to be the most important and the most consumed compared to other hive products. According to one from the beekeepers of the city of Constantin, there are 10-12 kinds of honey in Algeria; the most famous are: eucalyptus honey, orange honey (the cheapest honey in Algeria), wild carrots honey, white clover honey, lavender honey, wild strawberry honey, mountains honey, various flowers honey…

However, there are those who confirm that there are more than that number; referred to the Sahara beekeepers, they produce about eight other types of honey like, thyme honey, absinthe honey (*Artemisia* sp.), desert wild chamomile (*Anacyclus valentinus*) honey, *Thapsia garganica* honey, *Euphorbia helioscopia* honey, *Peganum harmala* honey, *Aristida pungens* honey besides the cedar honey which raises the number to more than 20 kinds of honey.

Cedar honey is the most expensive and famous honey in Algeria. This is due to the spread of cedar trees more in the desert, which requires the beekeepers to move their hives for long distances which cost them lot of expenses, especially transportation and renting lands of farmers, in addition to its various health benefits [51].

Although the production of honey in Algeria has undergone a remarkable evolution in recent years, the consumption of honey by the Algerian remains insignificant, because it is generally only used for the needs of a remedy. The annual consumption has been estimated around 90 g [52].

Moreover, the country fails to achieve selfsufficiency for this product where it has to rely on imports. This latter of honey reached the 150 thousand tons in recent years. Hence the main supplier countries are: Saudi Arabia, Thailand, Turkey, USA, Germany, Bulgaria and Hungary [53].

In addition to honey, there are also other bee products such as wax, pollen, propolis and royal jelly, hence their production is very law, because many beekeepers either do not produce them or provide them in small quantities and use them for their own needs.

Algeria also imports wax, beehive wood, beespecific veterinary products and biological materials (queens, swarms).

All raw materials (either imported or supplied by beekeepers) are sent to the cooperatives which transform them into final products for producers (embossed wax, beehives, etc).

Most faced problems

Despite the efforts to improve the beekeeping sector in Algeria, the latter still suffers from problems related mostly to organizational issues, plus to climatic conditions characterized by fluctuation.

One of the most important problems among beekeepers in Algeria is due to the problem of marketing for local products, which is due to various reasons: Lack of coordination between beekeepers, including cooperatives, Dumping local markets with foreign products, Lack of publicity to local products, Problem of packing in which the beekeeper still cannot expose his products in a way that satisfies the consumer. Colony losses due mainly to honeybee diseases; Problem of coordination between farmers ad beekeepers at the time of use of pesticides [51]. Lack of accredited honey analysis laboratories [45].

Migratory beekeeping

In order to increase their productions, beekeepers have adopted the practice of migratory beekeeping, which means transporting hives to areas where flowering occurs at different times.

The development of the sector in recent years as a result of governmental programs and the efforts of beekeepers has led to increased production and diversification of hive products; beekeepers crisscross approximatively the entire national territory and do up to eight migrations per year [54].

However, this practice remains expensive (despite the quality of products offered by this possibility), limited (lack of resources and professionalism) and poorly organized (overload on the same sites) [45].

Türkiye ve Cezayir'deki Arıcılık Aktiviteleri

Öz: Bu derleme, Türkiye ve Cezayir'deki en önemli tarımsal sektörlerden biri olan arıcılığın farklı yönlerinin vurgulanması amacıyla oluşturulmuştur.

İki ülkenin arıcılığı hakkında tarihi bir bakış açısı sunulmuş, ayrıca iki ülkenin flora ve fauna (daha özel olarak bal arısı türleri üzerinde) bileşenleri ve bunların arıcılık üzerindeki etkileri hakkında bilgi verilmiştir. Ayrıca, arıcılığın her ülkenin ekonomisindeki konumu ve olanakları ile arıcıların karşılaştığı en önemli sorunlardan bahsedilmiştir.

Anahtar Kelimeler: Arıcılık, Türkiye, Cezayir

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