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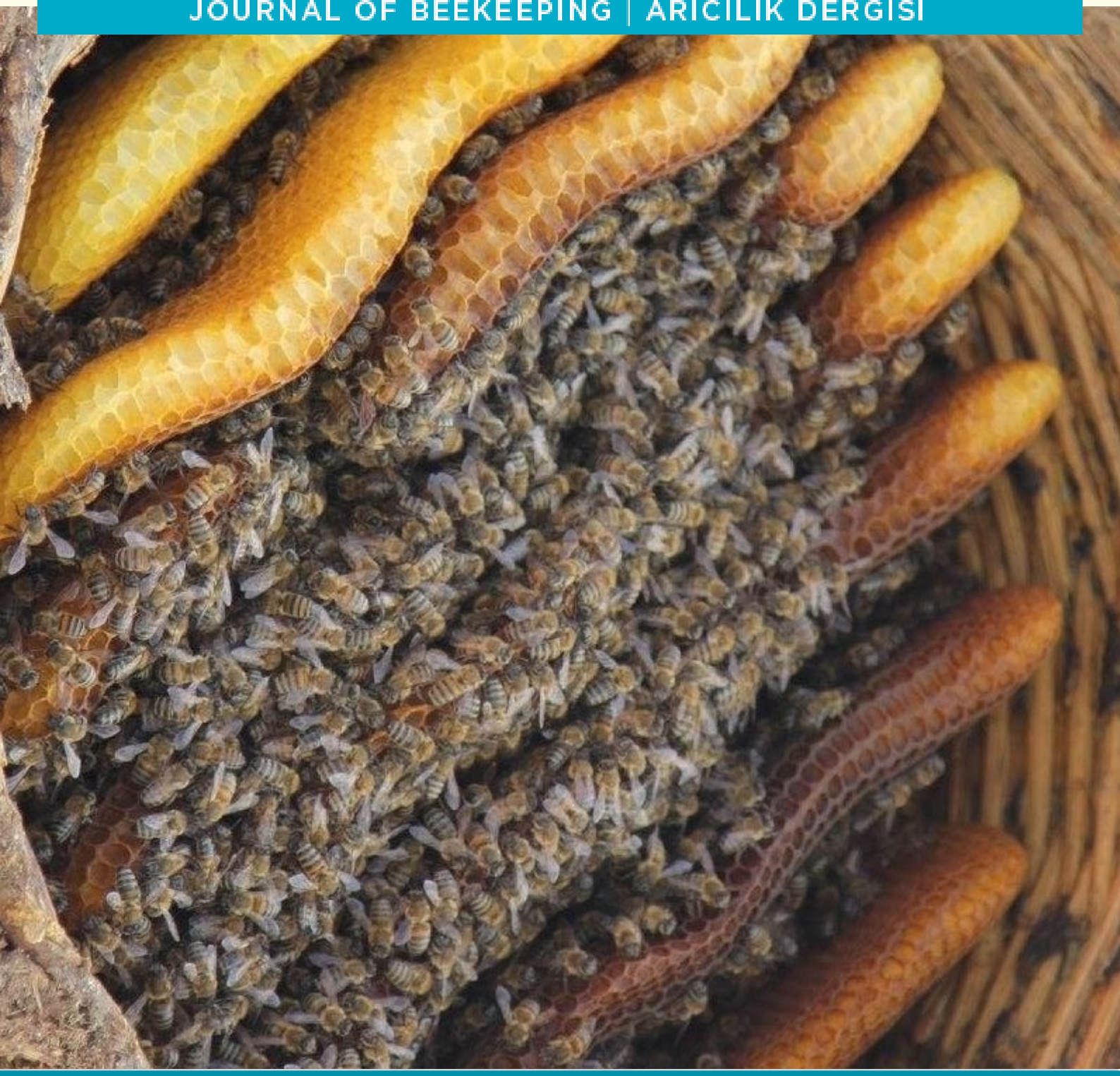

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MELLIFERA REVIEW ARTICLE

Importance of Pyrrolizidine Alkaloids in Bee Products

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A B S T R A C T

Pyrrolizidine alkaloids are one of the groups of harmful chemicals of plants, which are natural toxins. Pyrrolizidine alkaloids found in about 3% of all flowering plants of widespread geographical distribution are known as one of the components of the hepatotoxic group of plant origin and referred as hepatotoxic pyrrolizidine alkaloids. According to researches, bee products is regarded as one of the main food sources in the exposure of people to pyrrolizidine alkaloids. Consumption of pyrrolizidine alkaloids containing bee products, such as honey, pollen, propolis and royal jelly, is a potential threat to human health, especially for infant and fetuses. Besides the acute toxic effects, the genotoxic effects and tumorigenicity potential of pyrrolizidine alkaloids was demonstrated. This manuscript gives an overview about bee products containing pyrrolizidine alkaloids and toxification processes in humans.

Key words: Pyrrolizidine alkaloids, honey, pollen, bee products, human health.

Introduction

Plants which are primary producers are defenseless to consumers and they have evolved toxic substances as a defense system for protection. Honey is a complex mixture of these numerous substances. One such group of toxic substance that threaten public health like grayanotoxin is pyrrolizidine alkaloids (PAs) [1, 2]. Indicating widespread distribution, PAs are secondary metabolites of plants and presumedly the most important natural toxins affecting livestock and humans. Mainly located in the *Fabaceae* (genus *Crotalaria*), *Boraginaceae* (all genera) and *Asteraceae* (tribes *Senecioneae* and *Eupatorieae*) families, PAs are estimated to be present in approximately 6000 plant species, over 350 PAs have been identified. The flowering and the seeds portion of the plants contains toxins at the highest rate [3]. Despite their rich variety they all share a common general molecule formula which composed of a necine base esterified to one or more necic acids. Taking into account chemosystematics and biogenetical analysis, almost all pyrrolizidine alkaloids can be classified into five different large structural types (Fig. 1) [4].

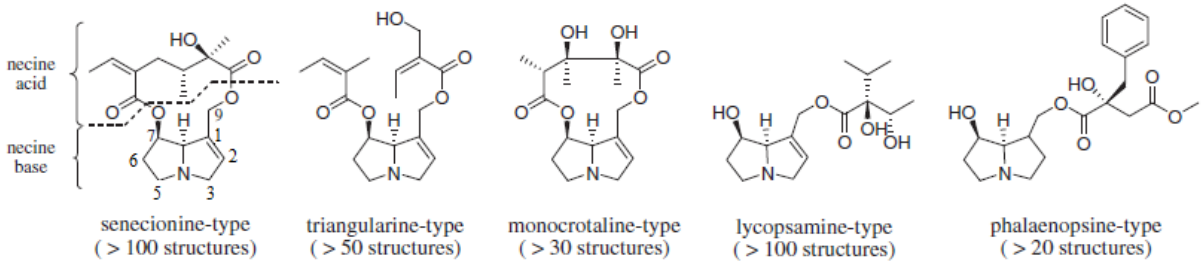

Figure 1. Major structural types of naturally occurring plant PAs [4].

As with the majority of toxic chemicals, PAs also require metabolic activation to show their toxicity. However, the presence of a carbon-carbon double bond in 1,2-unsaturated position of the necine base is a pre-requisite for toxicity. An ester functionality in location C-7 or C-9 or both positions is also required. If these requirements are met, PA structures can converted into 6,7-dihydropyrrolizine ester metabolites (pyrroles), mainly by liver cytochrome P-450 monooxygenases located primarily in hepatocytes. These pyrroles are initial toxic metabolites and highly electrophilic compounds that irreversibly and readily react with negatively charged nucleophilic functional groups on proteins, DNA or amino acids to form adducts or are hydrolysed to produce more stable 6,7-dihydropyrrolizine alcohols. Alcohols are common and ultimate toxic metabolites, and are thought to play an important role in PAs toxicity [5]. PAs are known to cause damage primarily in the liver, lungs and blood vessels, secondary in the kidney, gastrointestinal tract, pancreas and bone marrow. According to cases of poisoning it is seen that especially children and neonates are affected to PAs intoxication due to their high sensitivity [6].

Among the bee products, one of the most important source of dietary exposure to PAs is honey. According to published risk assessments when PAs containing-honeys are regularly consumed about 15–25 g which are recommended serving sizes or at higher consumption levels, acute and chronic toxicity may occur in risk group of humans. Genotoxicity, carcinogenicity and teratogenic effects were also seen due to the consumption of contaminated foods with PAs [5, 7].

The detection of pyrrolizidine alkaloids in a sensitive and reliable manner is of great importance in terms of public health. Chromatographic methods used in determination of PAs are Thin-layer chromatography, Gas chromatography, Gas chromatography–mass spectrometry (GC–MS), High-performance liquid chromatography (HPLC) and High-performance liquid chromatography-mass spectrometry (LC–MS). However HPLC is more preferred since the determination of both the free base form and the *N*-oxides can be done more practically [8].

Pyrrolizidine Alkaloids in Honey

Beehives are placed around the most efficient flowering plants. While it is expected that the nominated floral sources represent these plants, other flowering plants in the area also contribute to the honey produced. This situation causes a variation in the level of pollen from the nominated source in the honey and results in the transfer of pollen of undesirable plants, such as PAs containing plants, into the honey. On the other hand apiarists utilize voluntarily a series of PAs-containing plants for honey production in many countries. These plants consist of plant species of *Echium, Senecio, Eupatorium, Heliotropium, Borago, Myosotis, Chromolaena, Petasites, Ageratum, Cynoglosum, Crotalaria, Tussilago* and *Symphytum* [5, 9].

In a study conducted by Betteridge et al. [10], nine floral honeys were analyzed and five of which were attributed to *Echium vulgare.* As published by Beales et al. [11], 63 honey samples which attributed to floral sources known to produce pyrrolizidine alkaloids were analyzed. As a result of the analysis 13 samples were *Echium plantagineum*, 9 samples were *Echium plantagineum* mix, 4 samples were *Heliotropium amplexicaule*, 2 samples were *Heliotropium europaeum* and 35 samples were attributed to floral sources with no known association with PAs. Levels of pyrrolizidine alkaloids differ from 800 to 2000 ppb in the samples. There were no pyrrolizidine alkaloids in 30% of the

samples. Moreover five retail samples of honey were analyzed. The two are derived from bees foraging on *Eucryphia lucida* and *Echium vulgare*, respectively. According to Dubecke et al. [12] a total of 3917 honey samples were analyzed for PAs which were found in 66% of the raw honeys and in 94% of retail honeys available in supermarkets. In a study by Griffin et al. [13], the 50 retail samples surveyed were categorized by their origin. Eight samples tested positive for one or two PAs, predominantly *lycopsamine* and *echimidine*. Detected PAs concentrations ranged from 182 to 4078 µg/kg. In a study researched by Martinello et al. [3], the amounts of PAs that consumers are exposed to were investigated whether it is dangerous to humans or not. The nine PAs were determined simultaneously in 70 retail honey samples purchased in local supermarkets. 64% of honey samples were found positive for at least one of the PAs. The concentrations detected ranged from 1 to 169 mg PAs/kg. This study reveals that many samples tested would exceed the tolerable daily intake suggested for these substances and they could be a hazard to human health. According to Orantes-Bermejo et al. [14] the incidence and concentration of PAs from *Echium spp*. have been defined in 103 Spanish honey samples. PAs were found in 94.2% of the raw honey samples analyzed, in the range of 1 to 237 µg/kg. The study conducted by Bodi et al. [15] comprised the analysis of 87 honeys which originate from German/Austrian beekeepers, brand products, discount products and other forms of sale in the Berlin retail market. The total PAs concentration ranged from 6.1 to 14 µg/kg honey in samples from beekeepers and discount products, while 15 µg/kg in branded honeys. According to EFSA (European Food Safety Authority) report [16], 14604 honey samples were analyzed for PAs. 13280 of the samples are concerned bulk honey which has been found to be positive in terms of PAs, predominantly *echiumine* (71%), followed by *echimidine* (56%) and *lycopsamine* (49%). For the samples of which 1324 covered retail honey, the situation was slightly different in terms of positive PAs, predominantly *echiumine* (45%), followed by *lycopsamine* (36%) and *echimidine* (16 %).

Pyrrolizidine Alkaloids in Other Bee Products

Pyrrolizidine alkaloids in pollen

Most of plants produced pyrrolizidine alkaloids does not affect the pollinators. This situation raises the idea that nectar of the plants does not contain PAs or contain only small concentrations of PAs. This rationale allowed the hypothesis that PAs in honey originate not from nectar but from pollen [1]. In a study conducted by Boppre et al. [9] pure pollen collected from the anthers of *Echium vulgare* were investigated in terms of PAs and detected 8000 to 14000 ppm. Kempf et al. [17] investigated the question whether honey filtration method can be reduced the level of PAs or not. But there is showed no decrease in PAs levels. It is appeared that when pollen grains come into contact with nectar, PAs rapidly transfer from pollen to honey due to the fact that PAs are very soluble in water. The study by Boppre et al. [18] freshly collected pollen from *Senecio ovatus, Senecio jacobaea, Echium plantagineum* and *Eupatorium cannabinum*, and pollen loads from bees that foraged on those plants was examined in terms of PAs. In PAs levels of collected samples from pollen loads lower than the PAs level (8000-14000 µg) of pure pollen collected directly from plants. The difference is based on the dilution effect of regurgitate fluid used excreting pollen from their honey stomachs by nectar collecting bees.

Pollen collected by honeybees is consumed as food supplements for its nutritional and health benefits. According to Kempf et al. [19] 55 commercially available pollen products were investigated in terms of PAs. 31% of

samples were found PAs positive which are range from 1.08 to 16.35 mg/g. In another study 119 pollen samples collected by honeybees were analyzed for PAs. 60% of the samples contained PAs [12].

Pyrrolizidine alkaloids in products containing honey and bee products

In recent years, there has been a steadily growing number of published data on PAs in honey and pollen due to the use of honey and pollen as ingredients in food processing. In the survey, several food types containing honey between 5-37%, which are mead, candy, fennel honey, soft drinks, power bars and cereals, jelly babies, baby food, supplements and fruit sauce were investigated. Positive samples are mead, candy and fennel honey [17]. A study conducted by Cao et al. [20] has investigated the PAs level in mead, or honey wine. In this study 1 kg of honey and two bottle of mead made from the honey, have been analyzed. While PAs level of honey is found 780 ng/g, level of PAs in meads are measured 236 ng/ml and 540 ng/ml.

Honey and bee pollen are not only consumed for breakfast but also used in alternative medical treatments. In recent years, there has been an increasing interest in antibacterial use of honey for wound treatment [1]. Several recent studies demonstrated that honey for human consumption was contaminated with natural occurring, plant derived pyrrolizidine alkaloids. In a study by Cramer and Beuerle [21] 19 different medical honey samples were analyzed for PAs. All medical honeys, except one, were found PAs positive (from 10.6 to 494.5 μg/kg).

According to EFSA report [22], a total of 29 samples of food supplements containing bee products are analyzed for the presence of PAs. The samples consist of pollen, propolis and royal jelly which are 12 samples, 9 samples and 8 samples, respectively. The samples were collected in supermarkets, retail shops and internet in France, Germany, Greece, Italy, the Netherlands and Spain. While concentrations of PAs were found 576.0 μg/kg in 11 of the 12 pollen products, 0.6 μg/kg and 15.5 μg/kg are quantified in propolis and royal jelly products. Bee products mainly contained PAs of the *lycopsamine*-type.

Toxicity of Pyrrolizidine Alkaloids

Toxicity caused by pyrrolizidine alkaloids has become more common in developed countries because of the increase in interest in alternative medicine methods in the last 25 years, while it pose a problem in developing countries in previous years. Using of herbal products containing PAs increased the number of fatal intoxication in the European Union (EU), The United Kingdom (UK) and The United States Of America (USA) [23]. After the consumption of PAs containing products, toxic effects begin to appear and toxicity of PAs occur via cytochrome P450 [5]. It is know that PAs causes damage primarily in the liver, lungs and blood vessels; secondary in the kidney, gastrointestinal tract, pancreas and bone marrow. Depending on the amount of intake PAs poisoning in humans occurs in three forms: acute, sub-acute and chronic. Acute toxicity has been observed in very rare cases. It is often occurred in newborns and infants due to their high sensitivity. This is characterized by ascites, hepatomegaly and hemorrhagic necrosis. Death occurs as a result of liver failure. Sub-acute poisoning is characterized by endothelial proliferation and medial hypertrophy which causing obstruction of the hepatic veins and resulting in hepatic sinusoidal obstruction syndrome (VOD). Furthermore hepatomegaly and recurrent ascites is also seen in this forms. VOD is proceed with epigastric pain associated with abdominal swelling originated from ascites. Other organs may be also affected by the PAs as well as liver. The pyrrolic metabolites can

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pass from the liver into pulmonary arterioles where they can produce damage similar to the VOD-changes in the liver. The first changes seen in pulmonary vessels are thrombus in vessels, acute inflammation and thickening of the vessel wall causing obstruction. These effects lead to hypertension inter alveolar and septal fibrosis. As a result of weakening of pulmonary blood flow, the operation of the right ventricle increases. This situation leads to the hypertrophy and congestive heart failure eventually. It is estimated that the pulmonary damage are observed in the low dose exposure for prolonged periods [4, 6, 24]. Although there are a few cases which recorded exposure level varied between acute and chronic levels in people exposed to PAs, there are no reports of cancer associated with such exposures by far. But the carcinogenic potential of some pyrrolizidine alkaloids has been proven in rodents, and the National Toxicology Program recently has accepted *riddelliine* as a human carcinogen. Some plant species are known to cause cancer in rodents: *S. longilobus, Petasites japanicus Maxim, Tussilago farfara L., Symphytum officinale, Farfugium japonicum, Ligularia dentata* and *S. cannabifolis* [7, 24].

PAs intoxication in humans is not only related to exposure time and amount of taken, but also age and sex are important. Men are more susceptible than women. Fetuses and children (especially newborns and infants) show the highest sensitivity. There are some cases about PAs intoxications. For example a pregnant woman consumed herbal spices contaminated with PAs about 7 mg daily, during her pregnancy in 2003. While it is not seen any toxic effects in the mother's liver, newborns died 2 days later after he born due to damage in the fetal liver. The toxic compounds leads to the formation of hepatic failure in the fetus through the placenta due to the highly lipophilic properties of this compounds. Another VOD case has been reported in newborn baby of pregnant woman who consume brewed herbal tea with leaves of *T. farfara* containing 0.6 mg/kg *senecionine* [6, 24, 25]. However none of the cases of toxicity in humans are associated with PAs toxicity based on honey consumption despite the statistics [5].

Tolerable Levels of Pyrrolizidine Alkaloids

Although some cases of acute intoxication in humans have been reported after consumption of high doses of PAs containing foods, the amount of cases is still not enough to suggest whether how much concentrations of PAs ingestion would pose a health risk to consumers. Rarity of information on pollen markets and lack of chemical screening of commercial bee product samples currently makes risk assessments uncertain. Because of that there is no legal limit about the concentration of PAs in bee products. However there are several countries which have regulations about the amount of PAs in some other food products or food supplements [26, 27].

According to Germany the Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung – BfR) both acute and chronic toxic effects of PAs have been taken into account. Therefore ingestion of PAs containing foods is to be kept as low as possible [27]. In 1992 The German Federal Ministry of Health established regulations about the sale of herbal products containing PAs. While use of herbal products containing PAs which have no demonstrated health benefits are banned by the German regulations, the dose of herbal products which provide some health benefits is restricted to 1µg of PAs per day for oral administration or 100 µg of PAs per day for external use. However such use is applied just for a maximum of six weeks per year. If the herbal medicine is to be taken for longer than 6 weeks per year, the allowed dose for pyrrolizidine alkaloids is reduced to 0.1 µg per daily for oral use or 10 µg for external use. On the other hand The German regulations have mandated a

zero tolerance for PAs containing herbal products by women in the lactation period and pregnant women. They also require prescribed level and legal tag which is "Not to be used in pregnancy and during the lactation period" for all PAs containing herbal products [28].

The Netherlands Food and Consumer Product Safety Authority (NVWA) advise limit value of 1 μg/kg for consumption of PAs. The same limit value also carries out to beverages containing herbal extracts contaminated with PAs, such as soft drinks or sweets containing, as well as herbal teas and food supplements [29]. According the assessment published by the Australian New Zealand Food Authority (ANZFA) it is stated that hepatocellular injury, liver cirrhosis and VOD are the major effects in humans, but information related to the formation of cancer causing PAs ingestion remains insufficient. Therefore, according to available human data, the PAs level which does not cause VOD is stated about 10 μg/kg. Thus the ANZFA proposed a provisional tolerable daily intake of 1 μg/kg for PAs [30]. On the other hand in Belgium, use of *Borago officinalis* plant in foods and food supplements was accepted if the final product does not contain PAs [3].

The investigations are stated that consumption rates of *echimidine* and related alkaloids (equivalent to 9 μg *heliotrine*/kg per day) above 15 μg/kg per day over a period of four to six months may cause acute or sub-acute liver disease. Therefore International Programme on Chemical Safety of The World Health Organization (WHO-IPCS) conclude that a dose equivalent to 10 μg *heliotrine*/kg per day may lead to disease in humans. In terms of equivalent doses of *heliotrine*, the total doses in the known outbreaks or cases of VOD, nonfatal cases and fatal cases are estimated to range from 1000 to 167,000 μg/kg, 1000 to 120,000 μg/kg and 6000 to 167,000 μg/kg, respectively [16].

Conclusion

Honey is known as natural food with high nutritional value and safe for human consumption. In addition to providing nutritional benefits of honey, it is also vital importance in terms of medical terms. Bee pollen which is another bee product is consumed as a positive food support recently. Although it has been known that hazardous levels of PAs may be presented in honey, importance of using PAs containing honey and pollen as food additives is not yet fully comprehended. Recent chemoecological research has shown that pollen from PAs producing plants contains very high levels of PAs and it carries significant health risks for consumers if this pollen is consumed as a health food supplement; the possibility that PAs in honey originate from contaminating pollen is also suggested by this finding. Consumers who take pollen based food supplements should be aware that such products can contain PAs in higher concentrations and regular consumption of them would pose a health risk to consumers. When such products are mainly consumed by children and pregnant women, a dangerous situation emerges. Because studies have revealed that fetuses and children show the highest sensitivity. Multidisciplinary action is required at international level; however, it should avoid creating public concern about honey and bee products.

Arı Ürünlerinde Bulunan Pirolizidin Alkaloidlerinin Önemi

Öz: Pirolizidin alkaloidleri bitkilerde bulunan doğal toksinlerdir ve zararlı bitki kimyasalları gruplarından birini oluşturmaktadır. Dünyadaki çiçekli bitkilerin %3'ünde bulunan bu alkaloidler bitki orijinli komponentlerin en hepatotoksik gruplarından biri olarak bilinirler ve hepatotoksik pirolizidin alkaloidleri olarak adlandırılırlar. Yapılan

araştırmalara göre, arı ürünleri insanların pirolizidin alkaloidine maruz kalmasında temel gıda kaynaklarından biri olarak kabul edilmektedir. Bal, polen, propolis ve arı sütü gibi pirolizidin alkaloidi içeren arı ürünlerinin tüketilmesi başta fetuslar ve yenidoğan bebekler olmak üzere insanlar için sağlık tehlikesi oluşturmaktadır. Pirolizidin alkaloidlerinin akut toksik etkilerinin yanı sıra genotoksik ve tümörejenik etkilerinin olma potansiyelleri olduğu da bildirilmiştir. Bu makale, arı ürünlerinde bulunan pirolizidin alkaloidleri ve bunların insanlarda meydana getiridiği toksisite ile ilgili genel bilgi vermektedir.

Anahtar kelimeler: Pirolizidin alkaloidleri, bal, polen, arı ürünleri, insan sağlığı

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MELLIFERA RESEARCH ARTICLE

Pollen morphology of *Tussilago farfara L.* pollinated by Honeybees

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A B S T R A C T

In this study, pollen morphology of *Tussilago farfara* L. belonging to the genus *Tussilago* (Asteraceae) were examined with light microscopy (LM). According to the investigation by light microscope (LM), pollen grains of the species is monad, radially symmetrical, isopolar, oblate-spheroidal, colporate and echinate. In our opinion, the palynological features of the taxon might be helpful to investigate the taxa various palynological, taxonomical and pharmaceutical researches.

Key words: Asteraceae, *T. farfara,* light microscope, palynological, taxonomical researches.

Introduction

Asteraceae comprises more than 1700 genera and 25000 species, and is considered the largest family among the flowering plants [1]. The tribe Senecioneae is one of the largest tribe of the Asteraceae with c. 3000 species and 150 genera, distributed in central and south America, south eastern Africa, central and east Asia but not common in Mediterranean type areas [2-3].

Tussilago farfara L. is a perennial plant widespread in Giresun on embankments (soil banks), wet shores, screes, sewers and abandoned places on clay (loamy) soils. Its english name is "Coltsfoot". It is known in Germany as "Huflattich/horse-hoof", in France "coughwort/ feuilles de tussilage", in Bulgaria as "podbel", in Chinese "Kuan Dong Hua", in Turkey ""kabalak/öksürük otu" [4-7]. *Tussilago farfara* L., starting its blooming in early spring, supplies colonies of wild Apoidea and honeybees with pollen and nectar flow under good weather conditions. Under laboratory conditions at 20°C and relative humidity 60%, full opening of 7 tagged inflorescences of *T.farfara* was completed after one and half hour. Therefore, it is considered as a honey plant with importance in Europe [8].

Ethnobotany provided data for *T. farfara* as a valuable medicinal plant that has been used in folk remedies as herbal tea for a wide range of disorders, such as throat, catarrh, bronchitis, laryngitis, pulmonary emphysema, silicosis and tubercular coughs [9].

There is no enough literature on pollen morphology of *T.farfara* in Turkey. Therefore, the present study aims to fill this gap in literature by palynologically analyzing the *T. farfara*. Consequently, it provides information that help

mellisopaygnological and aeropalynological analyses.

Material and Methods

Locality

Materials of this study were collected in February 2016 from Giresun-Güre–Batlama River. Giresun is located in the eastern part of the Black sea region (40°54′K and 38°25′D). According to the grid system applied by Davis [10], Gure (Giresun) is located in the A7 frame (Fig. 1).

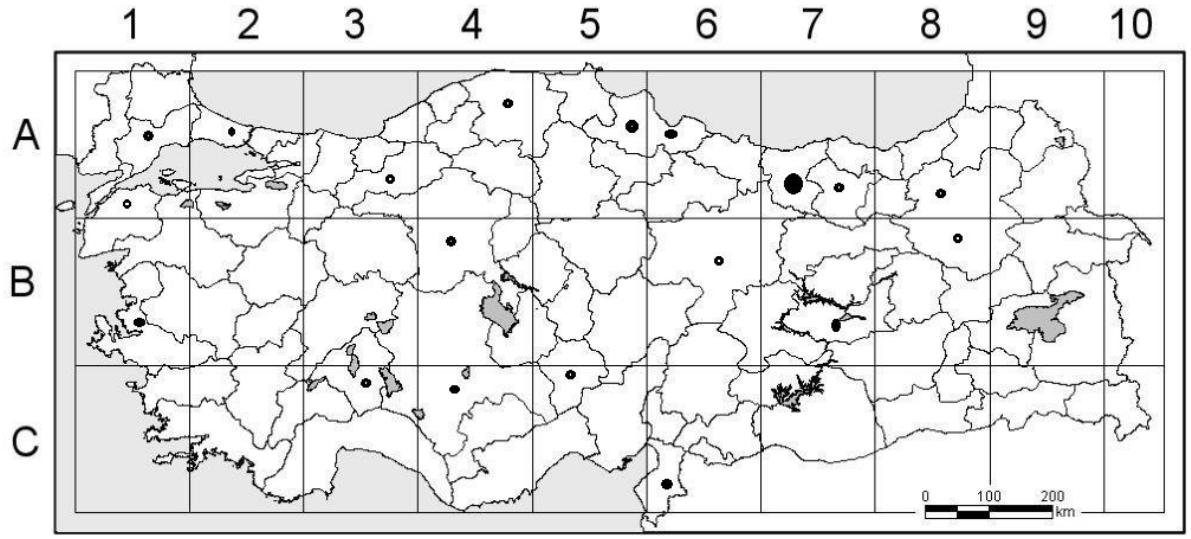

Figure 1. Geographical distribution of *T.farfara* in Turkey

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 [11]. The measurements of the pollen grains of *T. farfara* were taken on 30 pollen grains from the species. P: polar axis, E: equatorial diameter, Amb: diameter of polen 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, 12 measurements of Clg: length of colpus, Clt: latitude of colpus, Spin length, base of length, 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 were 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 [12], Kremp [13] and Punt et al. [14].

Result and Discussion

Palynological description of *T. Farfara* (Fig. 2) was made based on the quantitative and qualitative morphologic results. It is monad, radially symmetric, isopolar, 3-colporate, medium-sized, oblate-spheroidal (P/E 0.99) (Fig. 3). Polar axis (P) is 33.63±1.60 µm, equatorial axis (E) is 33.96±2.09 µm. Amb is circular. Exine is 2.45±0.52µm thick; nexine is thinner than or as thick as sexine. Exine ornamentation is echinate: length of spine

4.27±0.78 µm, spin base 2.45±0.52 µm, spines acute, concave-conic, distance between colpi ends 20.20±1.47 µm. Apertural system 3-colporate: three colpus 17.93 ±1.34µm long, 10.25±1.48 µm wide, pore 10.61 ± 1.19 µm long, 10.25 ± 1.48 wide; distinct margin and terminal edges acute, the pori situated at midpoint of colpus, are circular and with distinct margin. (Fig. 3, Table 1).

Figure 2. *Tussilago farfara*'nın genel görüntüsü

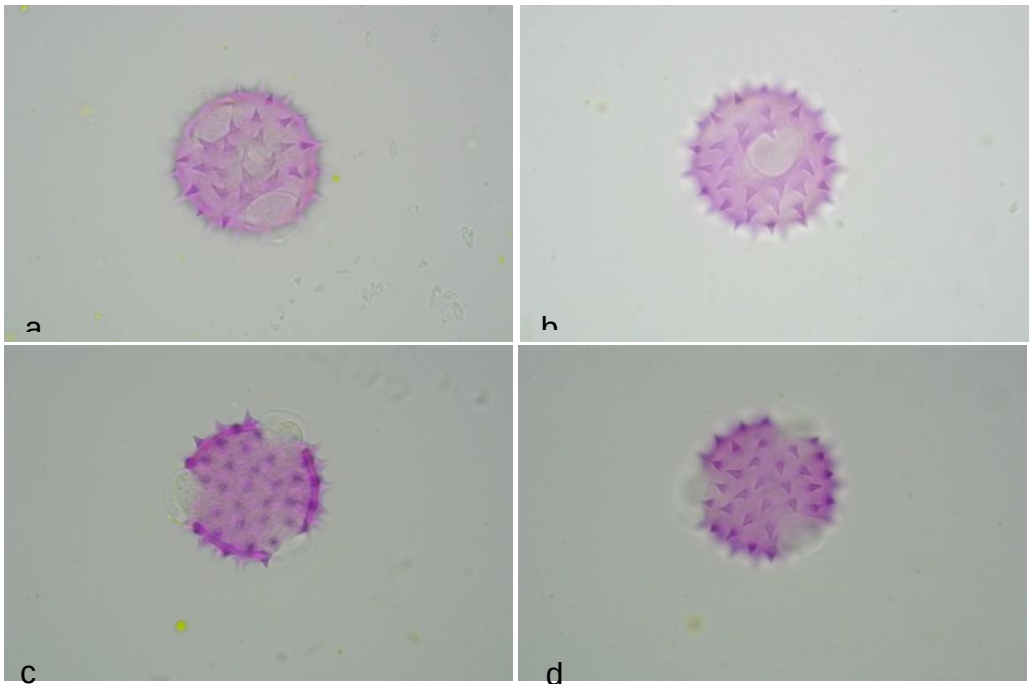

Figure 3. *T. farfara* a-b: equatorial view; c-d: polar view (10x100)

Table 1. The palynological measurements of *T. farfara* (M: median, Var.: variation, S: standart deviation).

P/E	**Oblate spheroidal**	0.99	Exine (µm)	M	2.45
				S	0.52
				Var.	2-3
P(µm)	M	33.63	Sexine (µm)	M	1.45
	S	1.6		S	0.52
	Var.	31-236		Var.	1-2
E (µm)	M	33.96	Nexine (µm)	M	1
	S	2.09		S	0
	Var.	30-37		Var.	1
Clt (µm)	M	10.25	Length of spine (µm)	M	4.27
	S	1.48		S	0.78
	Var.	8-13		Var.	3-5
Clg (µm)	M	17.93	Spine base (µm)	M	2.45
	S	1.34		S	0.52
	Var.	15-20		Var.	2-3
Plt (µm)	M	10.25	Amb (µm)	M	31.13
	S	1.48		S	1.45
	Var.	8-13		Var.	28-34
Plg (µm)	M	10.61	t (µm)	M	20.20
	S	1.19		S	1.47
	Var.	9-13		Var.	17-24

İnceoğlu and Karamustafa [15] claimed that pollen grains of senecioneae are tricolporate, oblate-spheroidal, spheroidal, Ambs circular, spines conic or concav-conic. Pollen grains of *T. farfara*, heteromorphous pollen grains nonaperturate and spheroidal, 13-50 µm (E) in diameters, Exine is 3,1 µm thick, echinate. Some spines are conic shape with acicular or rounded ends, others dome-shaped. Spines 2,6 µm in length with base diameter of 4,3 µm.

Warakomska and Kolasa [8] put forward the idea that *T. farfara* (Coltsfoot) pollen grains were isodiametric, trizonocolporate and echinate. Their diameters are without echinate, ranged from 28.5 µm to 29.5 µm.

The grains covered with pollen kit easily adhered to insect's body.
In MediaWiki [16], *T.farfara* pollens are 36 (33.4-39.8) µm (Medium), Tricolporatae and echinate, Senecio-Typ, spheroidal, isopolar, pore width 11 µm, spin 4 µm lang, plentiful pollenkit
In Pal dat [17], *T.farfara* 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 LM: echinate, SEM: echinate, perforate, TEM tectum: eutectate.

Our palynological results are concordant to previous research about *Tussilago* and *T. farfara* pollen investigations. Pollen grains of *T. farfara* are radially symmetric, isopolar, 3-colporate, oblate-spheroidal, echinate (LM)

Conclusion

In Turkey, *T. farfara* has a common name "öksürük otu" and has been traditionally used as medicine all over the World. Pollen morphology of *T. farfara* is determined. The remarkable property of this species is colpus as wide as porus and porus big.

Acknowledgements

We would like to thank Giresun University's Scientific Research Unit (Project No. FEN-BAP-A-250414-49) for the financial support.

Bal arısı ile Tozlaşan *Tussilago farfara*'nın Polen Morfolojisi

Öz: Bu çalışmada, *Tussilago* L. (Asterraceae) cinsine ait *T. farfara* L. polen morfolojisi ışık mikroskobu (LM) ile incelenmiştir. Yapılan incelemelere göre, bu taksona ait polenler tek, radyal simetrik, izopolar, oblat-siferoid, kolporat, ekinat özellik göstermektedir.

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

Anahtar kelimeler: Asteraceae, *T. farfara*, ışık mikroskobu, palinolojik, taksonomik araştırmalar.

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Appendix

Pollen morphology of Tussilago farfara L.

Leaves petiolate, lamina 10-20(-30) cm diam., with rather acute lobes, margin irregularly toothed, at first white-floccose on both sides, becoming glabrous above. Scapes 4-15 cm (lengthening in fruit to c. 30 cm), with numerous purplish scale-leaves, floccose, erect in bud, nodding after anthesis. Capitula 1.5-2.5 cm broad. Phyllaries linear, obtuse, often purplish and white-hairy, sometimes with black glandular hairs. Achenes 3-4 mm. Pappus 10-15 mm. Fl. 3-4. Waste and sandy places, damp ground, s.1.-2400 m.

Described from Europe (Hb. Linn. 995/10, photo!).

Widespread except in E. Anatolia; commonest in N. Turkey

Europe except extreme N., N. & W. Asia, N. Africa. Euro-Sib. element.

Introduced in N. America. The leaves of this species are medicinal [10].

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MELLIFERA RESEARCH ARTICLE

Pollen morphology of *Lythrum salicaria* L.

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A B S T R A C T

In this study, pollen morphology of *Lythrum salicaria* L. belonging to the aromatic genus *Lythrum* (Lythraceae), which has widely medicinal benefits were examined with light microscopy (LM). According to the investigation by light microscope (LM), pollen grains of the species is radially symmetrical, isopolar, oblate-spheroidal, heteroaperturate (3 colpori-3 pseudocolpi) and psilate.

In our opinion, the palynological features of the taxon might be helpful to investigate the taxon in various palynological, taxonomical and pharmaceutical researches.

Key words: Lytraceae, *L. salicaria*, light microscope, palynological, taxonomical researches.

Introduction

The Lythraceae consist about 24 genera and 500 species which mostly tropical herbs, occasionally shrubs or trees comprising [1]. The genus *Lythrum* L. (Lythraceae) is spread throughout the world. It is represented by around 30 species, 10 of which are found in Europe. In Turkey, Lythraceae family is represented by two genera (*Lythrum* and *Ammannia* L.) and nine species belong to *Lytrum* [2].

L. salicaria is Euro-Sib. Element [2]. *L. salicaria* is originally Eurasian, but during the 19th century it was spread via the ballast of European ships not only to Europe but also into North and South America, as well as Australia. Its English name is "blooming sally", "purple willow-herb", "rainbow weed" and "purple loosestrife". It is known in German as "Blutweiderich", in French "Salicaire", in Romanian "răchitan" and in Swedish "fackelblomster" , in Turkey "Tıbbi hevhulma"[3- 6]. *L. salicaria* is known as a medicinal plant from the ancient Greek and Roman times and it has been an important drug for centuries [7].

The flowers are visited mainly by bumble bees, but also by lepidoptera, honey bees, solitary bees and syrphid flies [8].The bees visit *L. salicaria* are in bloom between June and August [2]. Furthermore, it grows in small, discrete patches on sand bars and gravel banks in canopy

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openings of flood plain forests with *Populus alba* L. but is also observed on lake banks, in dry river beds, ditches, and among rocks [9].

Antioxidant, antimicrobial, and hypoglycemic effects of *L. salicaria* have been reported [10-13].

There are no reports on the pollen morphology of *L. salicaria* from Turkey.

Materials and Method

Locality

Materials of this study were collected in 2014 from Giresun-Güre–Batlama river. Giresun is located in the eastern part of the Black sea region (40°54´K and 38°25´D). According to the grid system applied by Davis [2], Gure (Giresun) is located in the A7 frame.

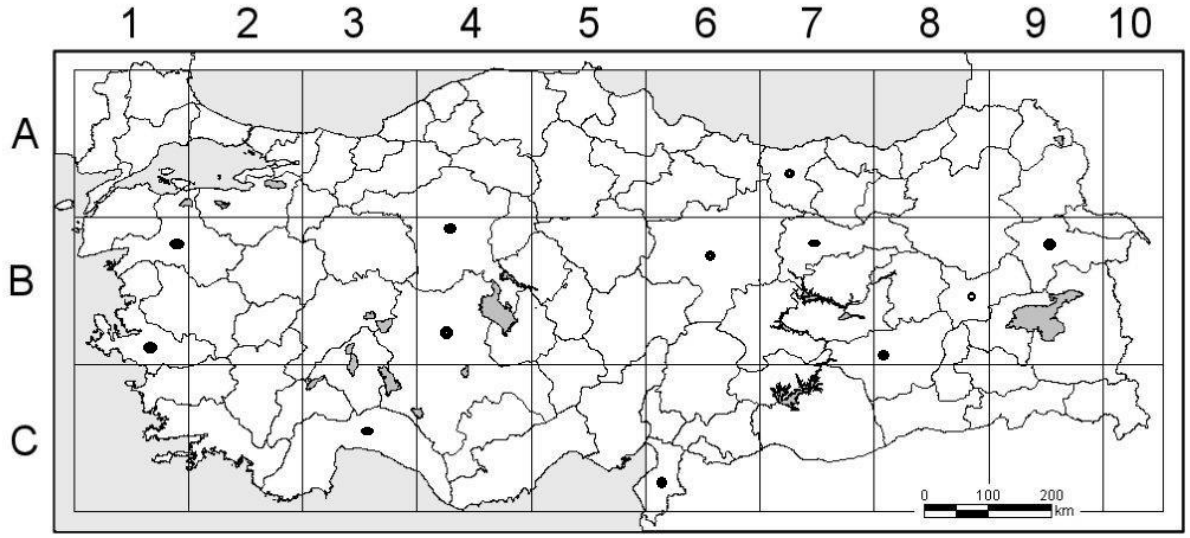

Figure 1. Geographical distribution of *L. salicaria* in Turkey

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 [14]. The measurements of the pollen grains of *L. salicaria* were taken on 30 pollen grains from the species. P: polar axis, E: equatorial diameter, Amb: diameter of polen at the polar view, Clg: length of colpus, Clt: latitude of colpus, PClg: length of pseudocolpus, PClt: latitude of pseudocolpus, Plg: length of porus, Plt: latitude of porus, t: distance between colpi ends, were measured from 30 fully developed grains per sample under the Nikon Eclipse Ci microscope (1000×). 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 terminology used is of Erdtman [15] and Punt et al. [16].

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Results and Discussion

Palynological description of *L. salicaria* (Fig. 2) was made based on the quantitative and qualitative morphologic results. It is radially symmetric, isopolar, heteroaperturate, oblate-spheroidal (P/E 0.93) (Fig. 3) and polar axis (P) 19.33±1.70 µm, equatorial axis (E) 20.76±1.81 µm. Amb hexagonal. Exine 2µm thick, nexine is thinner or as thick as sexine. Exine ornamentation is psilate. Distance between colpi ends 2.73±0.73 µm. Apertural system 6-zono-heteroaperturate: three pseudocolpi 12.03±0.66 µm long, 2.5±0.50 µm wide, three colpori 15.26 ±0.82 µm long, 8.13±1.07 µm wide, pore 3.43 ± 1 µm long-wide; margins of the pseudocolpi and colpori granulate, distinct margin and terminal edges acute, The pori situated at midpoint of colpus, are circular and with distinct margin. Annulus thickness is 1 µm, slightly protruding with granulations frequently visible on side of annulus ((Fig. 3, Table 1).

Figure 2. *Lythrum salicaria*

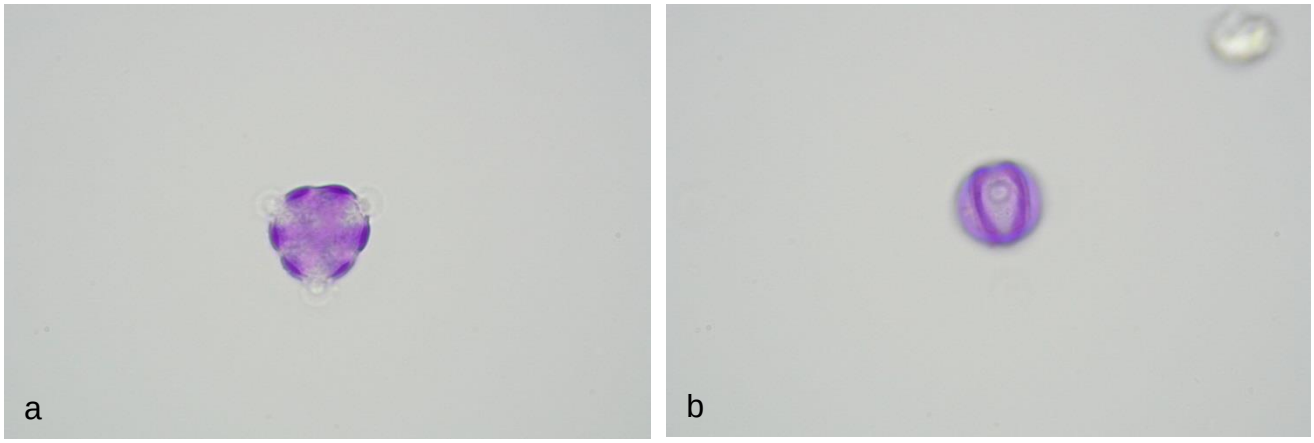

Figure 2. *L. salicaria* a: Polar view; b: equatoral view

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Table 1. The palynological measurements of *L. salicaria* (M: median, Var.: variation, S: standart deviation)

P/E	**Oblate spheroidal**	0.93	Exine (µm)	M	2
				S	0
				Var.	2
P(µm)	M	19.33	Sexine (µm)	M	1.35
	S	1.7		S	0.23
	Var.	15-25		Var.	1-1.50
E (µm)	M	20.76	Nexine (µm)	M	0.65
	S	1.81		S	0.23
	Var.	17-25		Var.	0.5-1
Clt (µm)	M	8.13	PClt (µm)	M	2.5
	S	1.01		S	0.5
	Var.	7-10		Var.	2-3
Clg (µm)	M	15.26	PClg (µm)	M	12.03
	S	0.82		S	0.66
	Var.	14-17		Var.	11-13
Plt (µm)	M	3.43	Amb (µm)	M	19.93
	S	1		S	1.46
	Var.	2-5		Var.	18-23
Plg (µm)	M	3.43	t (µm)	M	2.73
	S	1		S	0.73
	Var.	2-5		Var.	2-5

Lythraceae is a europalynous family [15]. General pollen characters of the family Lythraceae are radially symmetrical, isopolar, colporate or heterocolpate, sub-prolate or prolate often oblate-spheroidal. Sexine thicker than or as thick as nexine. Tectum reticulate-rugulate or scabrate to sub-psilate.

Perveen and Qaiser [17] examined species representing 5 genera of the family Lythraceae from Pakistan by light and scanning electron microscope. According to this research, Lythraceae is an eurypalynous family. Pollen grains are generally free, radially symmetrical, isopolar, colporate or heterocolpate. Shape of pollen grains are sub-prolate or prolate often oblate-spheroidal. Sexine thicker than or as thick as nexine. Tectum reticulate rugulate or scabrate to sub-psilate. The pollen morphology of the family Lythraceae is significantly helpful at generic and specific level. On the basis of apertural types 2 distinct pollen types viz., *Lagerstroemia indica*-type and *Ammannia baccifera*-type are recognized. Pollen grains of *L. salicaria* is oblate-spheroidal and in terms of aperture types belonginig to *Ammannia baccifera* type.

Graham et al. [18] investigated 12 genera of the Lythraceae is described, using light microscopy(LM), scanning (SEM) and transmission electron microscopy (TEM). According to them, the pollen of *Lythrum* is uniform and distinguished by the three prominent granular pseudocolpi alternating with the three apertures in which the colpi are also granular. The tectum is finely striate with the long axis of the striae mostly parallel to the polar axis.

Guers [19] has established that, within the genus Rotala (Lythraceae), species ocur which are characterized respectively by three colporate apertures with three indistinct pseudocolpi, and by three colporate apertures with three distinct pseudocolpi.

In Paldat [20] *L.salicaria* pollens are monad, medium-sized (26-50 μm), isopolar, spheroidal, circular, prolate, lobate, 3 colporate, heteroaperturate, aperture membrane ornamented, ornamentation SEM: striate, TEM tectum: eutectate, columellate, compact-continuous.

Our palynological results are concardant to previous research about Lythraceae and *Lythrum* pollen investigations. Pollen grains of *L. salicaria* are radially symmetric, isopolar, heterocolpate, oblate-spheroidal, psilate (LM).

Conclusion

In Turkey, *L. salicaria* has a common name "Tıbbi hevhulma" and has been traditionally used as medicine all over the World. Pollen morphology of *L. salicaria* is determined. The remarkable property of this species separating from other species is heteroaperturate (3 colpori-3 pseudocolpi).

Acknowledgements

We would like to thank Giresun University's Scientific Research Unit (Project No. FEN-BAP-A-250414-49) for the financial support

Lythrum salicaria L. Polen morfolojisi

Öz: Bu çalışmada, geniş medikal kullanımı olan aromatik *Lythrum* L. (Lythraceae) cinsine ait *L. salicaria* L. polen morfolojisi ışık mikroskobu (LM) ile incelenmiştir. Yapılan incelemelere göre, bu taksona ait polenler radyal simetrik, izopolar, oblat-siferoid, heteroapertür (3 kolporat-3 pseudokolpat), psilat özellik göstermektedir. Bu taksonlara ait palinolojik özelliklerin çeşitli palinolojik, taksonomik ve farmasötik botanik çalışmalarında taksonların daha doğru teşhis edilmesine yardımcı olacağını düşünmekteyiz.

Anahtar kelimeler: Lytraceae, *L. salicaria*, ışık mikroskobu, palinolojik, taksonomik araştırmalar.

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Appendix

L. salicaria L., Syn: *L. tomentosum* DC,; *L. cinereum* Gris. Stout, ± densely pubescent perennial; stems 20-180 cm, winged, sparingly branched. Leaves 10-70 mm, ovate to narrowly lanceolate, truncate to subcordate at base, sessile. Inflorescence a ± dense verticillate spike. Flowers 3-8, in axillary cymose whorls, trimorphic; hypanthium 4-5 mm, broadly tubular in flower and fruit; epicalyx segments 2.5-3-5 mm, subulate; sepals 0.5-1 mm, deltate; petals 8-12 mm, purple: stamens 12. Capsule 3-4 mm, ovoid, included within the hypanthium.
FIowering time: 6-8.
Habitat: Wet places by lakes and streams, dry river beds, etc., 100-2000 m.
Distribiton: Europe [2].

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MELLIFERA RESEARCH ARTICLE

Protein Analysis of Anzer Bee Pollen by Bradford Method

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A B S T R A C T

The aim of this work is to determine the plants that are foraged by honeybees in Anzer Plateau and to evaluate the protein content of the pollen samples. Within the context of the study, pollen samples were collected from two different bee farms located in the Anzer Plateau during the period June-August, 2013 by using traps in honey bee hives.
The pollen loads of the bees were firstly classified according to their color. The origin of plants was determined in family and/or genus level according to microscopical analyses. Total protein content of pollen samples was determined by using Bradford method. As a result of palynological analysis, it was found that the protein content of the Anzer pollen samples were found between the range of 3.2-17.6 g/100g. Geraniaceae family was found as the mostly preferred taxon as a foraged plant. According to our findings, its protein content is determined to be found between 5-7.8 g/100g according to the collection date and location.

Key words: Bee pollen, protein analysis, Bradford method, palynological analysis, Geraniaceae

Introduction

Bee pollen which is an important source of protein in bee colonies are in fact male reproductive microspores of flowering plants collected by honey bees that are later on mixed with nectar and bee salivary glands secretions [1-2]. The pollen is used for feeding, especially for larvae growth and development [3]. Honey bees use pollen as their nutritional source of protein, fatty acids, lipids, sterols, vitamins, minerals and certain carbohydrates [4].
Pollen is a fine powder-like material [5]. Depending of the plant species pollen grains, differ in shape, color, size, and weight. The color of pollen varies, ranging from bright yellow to black [6].

Chemical composition of pollen depends on the availability of bee pastures and species of plants visited by bees. Pollen is an essential source of compounds with health protective potential like phytosterols and phytochemicals such as phenolic compounds [7]. Bee pollen is rich in sugar, protein, lipid, vitamin and flavonoid [1,8]. Besides, it includes minerals, some antioxidant vitamins, namely C, E, β-carotene, and also vitamins from the B-complex [9] and it is also a rich source of

flavonoid glycosides [10]. Almaraz and Naranjo (2004) reported that this bee hive product can be an important source of natural flavonol antioxidants [11]. However, the composition of bee pollen depends strongly on the plant source and its geographic origin, together with other factors such as climatic factors, soil type, and beekeeper activities [12] .

Bee pollen has antimicrobial, antifungal, antioxidant,anti-radiation,hepatoprotective, chemopreventive, antioxidant, anticancer and anti-inflammatory effects [12]. Such uses suggest that bee pollen could be useful in the prevention of diseases associated with free radicals. These therapeutic and protective effects, especially antioxidant activities, may be related to polyphenols. Antioxidant activities of bee pollen may be associated with the floral species [13]. According to the results in a study with 200mg/kg pollen fed white New Zealand rabbits, the body weight had increased and biochemical profiles of blood had improved [14]. It has been shown that bee pollen fed chickens revealed a better development of small intestine villi from the duodenum, jejunum and ileum. These findings suggest that bee pollen could promote the early development of the digestive system [15].
Honey bee collected pollen can be considered as a potential source of energy and proteins for human consumption [4]. Bee pollen has also been used for many years in traditional medicine and supplementary nutrients, primarily because bee pollen has nutritional and health benefits [13]. In recent years people's interest in natural foods have gained increased attention. Due to its rich content, bee pollen is preferred by people as a natural food supplement [16].

Pollen provides a rich source of easily digestible protein and essential amino acids for humans. Protein content in pollen depends on the origin of the plant. These values vary between a large range from 3.8 to 40.8%, with 25% being the average [17]. Cane and Buchmann (2000) indicates these values as 2.5% to 61% [18-20]. According to Bonvehi et al., pollen from insect-pollinated species are richer in protein content compared to that from anemophilous plants [21]. Quite the contrary, Cane and Buchmann (2000) supported that zoophilous species are not statistically richer in protein content than anemophilous species and both mass of protein per pollen grain and pollen grain volume were correlated with stigma-ovule distance [22]. They suggested that the need for growing pollen tubes probably plays a more important role in determining pollen protein content than rewarding pollinators. are essential for life and the organism cannot synthesize them by itself [6].

Bogdanov, (2012) reported the that total protein content of the pollen to be no less than 15g/100g [18].

Anzer plateau is located in Rize province at the Eastern Black Sea region at an altitude above 2300m. With its rich flora, this plateau is famous for its honey which provides beekeepers as an important source of income. Anzer honey is the most popular and expensive honey in Turkey, and it is traditionally used for medical purposes [23], explaining why so much attention has been paid to this honey, [23-25] but not on other bee products, with only one study conducted on pollen so far [26].

The aim of this work is to determine the plants foraged by honeybees in Anzer Plateau and also to evaluate their protein content by using Bradford method.

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Materials and Methods

Collection of Pollen samples

Pollen samples were collected from two different bee farms located in the Anzer Plateau during a period of June-August of 2013. The samples were kept at -18°C until the analysis.

A total of nine mixed samples were obtained for investigation. The collection times and the locality of the samples are given in Table 1.

Table 1. Sample no, code, collection time, location, plant family, plant taxon, pollen color and protein content of pollen samples

Sample No	Sample code	Collection time	Location	Plant Family	Plant Taxon	Protein content (g/100g)
Sample 1	1-1	25.06.13	Plateu 1	Campanulaceae		7.6
	1-2			Rosaceae	*Sarcopoterium* spp.	4.4l
	1-3			Geraniaceae		5
	1-4			Fabaceae	*Onobrychis* spp.	6
	1-5			Ranunculaceae	Type1	7
	1-6			Asteraceae	*Bellis* spp.	5.4
	1-7			Brassicaceae		11.8
	1-8			Asteraceae	*Carduus* spp.	5.4
Sample 2	2-1	11.07.13	Plateu 1	Ericaceae		7.8
	2-2			Cistaceae		3.2
	2-3			Geraniaceae		6.2
	2-4			Ranunculaceae	Type 2	5.6
	2-5			Fabaceae	Trifolium repens	9.2
	2-6			Caryophyllaceae		17.6
	2-7				*Buxus sempervirens*	10
Sample3	3-1	28.07.13	Plateu 1		*Astragalus* spp.	7
	3-2			Cistaceae		5.4
	3-3			Cistaceae	*Cistus* spp.	4.2
	3-4			Asteraceae	*Taraxacum* spp.	3.6
Sample 4		14.08.13	Plateu 1			
	4-1			Geraniaceae		5
	4-2			Poaceae		9.6
	4-3			Campanulaceae		6.4
	4-4			Dipsecaeae	*Scabiosa* spp	7.8
	4-5			Asteraceae	*Carduus* spp	7.4
	4-6			Cistaceae	*Cistus* spp.	6
Sample 5	5-1	28.08.13	Plateu 1	Geraniaceae		5
	5-2			Gentaniaceae		11.2
	5-3			Dipsecaeae	*Scabiosa* spp	8
	5-4			Asteraceae	*Taraxacum* spp	1.6
	5-5			Asteraceae	*Carduus* spp	11.2
	5-6			Campanulaceae		7.4
Sample6	6-1	11.07.13	Plateu 2	Caryophyllaceae		7.8
	6-2			Rosaceae	Type 1	12.4
	6-3			Geraniaceaae		7.2
	6-4			Asteraceae	*Taraxacum* spp	4.6
	6-5			Rosaceae	Sarcopoterium	10.2

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Sample 7	7-1	28.07.13	Plateu 2	Geraniaceae		7.8
	7-2			Asteraceae	*Taraxacum* spp.	6.4
	7-3			Caryohyllaceae		6.2
	7-4			Campanulaceae		14.2
	7-5			Buxaceae	*Buxus sempervirens*	11.4
Sample 8	8-1	14.08.13	Plateu 2	Asteraceae	Centaurea spp	7
	8-2			Geraniaceae		7.8
	8-3			Gentaniaceae		1.8
	8-4			Campanulaceae		11.4
	8-5			Ranunculaceae	Type1	7.6
Sample 9	9-1	28.08.13	Plateu 2	Gentianaceae	*Centaurium* spp.	5.8
	9-2			Geraniaceae		7.6
	9-3			Campanulaceae		5.4
	9-4				*Scabiosa* spp	12.4
	9-5			Asteraceae	*Carduus* spp	4.8
	9-6			Ranunculaceae	Type 1	10.4
	9-7			Asteraceae	*Taraxacum* spp.	6.6

Microscopic Analysis of Pollen samples

Analyses were carried out assuming that a pollen load was collected from only one plant species [27-29]. The mixed pollen samples were firstly classified according to their color. After this process, pollen slides were prepared for examination by light microscopy according to the Wodehouse method [30].

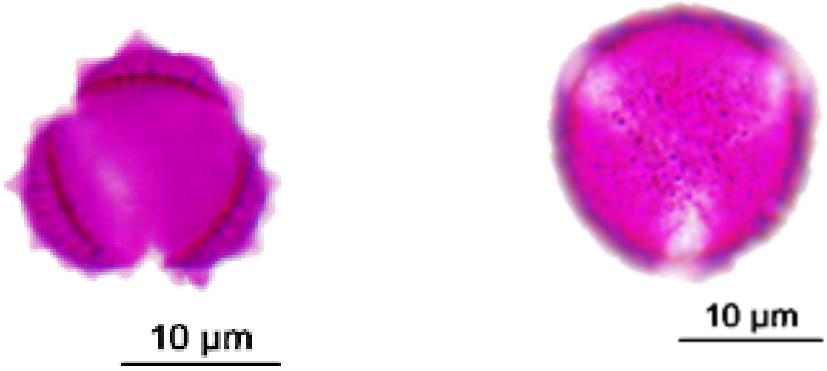
Protein Concentration Measurement

Protein content of pollens were determined by using Bradford [31]. Three replicates from each pollen sample was pooled. Briefly, 4 mg of each pollen sample was deffatted in 80 µl of dichloromethane for two hours and this step was repeated. Defatted pollens were grinded by using liquid N_2. The obtained pollen powder was mixed with 80 µl of urea lysis buffer [(7 M urea (Bio-Rad, ABD), 2 M thiourea (Bio-Rad, USA), % 4 CHAPS (Amresco, Ohio), % 1 DTT (Fluka, Switzerland), % 2 carrier ampholytes (pH 3-10, Fluka, Switzerland), 1 tablet protease inhibitor cocktail (Roche, Switzerland)] and were homogenized by sonication. Following homogenization, each pollen lysate was centrifuged at 17000 g for 1 hour at +4°C. The supernatant was used for further analysis while the pellet was discarded. 200, 400, 600, 800 ve 1000 µg/ml of Bovine Serum Albumin (BSA) were used as standards. BSA standards and 1:10 diluted samples were loaded on 96-well microplate as 4 replicates. 245 µl of Coommassie Brilliant Blue G-250 dye was added on each well and the microplate was incubated for 15 minutes in dark at room temperature. Lastly, the samples were measured spectrophometrically at 595 nm. Values were obtained in g/ 100g.

Results and Discussion

Microscopical analysis of pollen samples

According to the results from microscopic analysis, it is determined that the pollen samples collected by honey bees belong to 15 different families (Asteraceae, Brassicaceae, Campanulaceae, Cistaceae, Rosaceae, Geraniaceae, Fabaceae, Ranunculaceae,Ericaceae,Caryophyllaceae, Poaceae, Campanulaceae, Dipsecaceae, Gentianaceae, Buxaceae) (Table 1, Figure1-4).

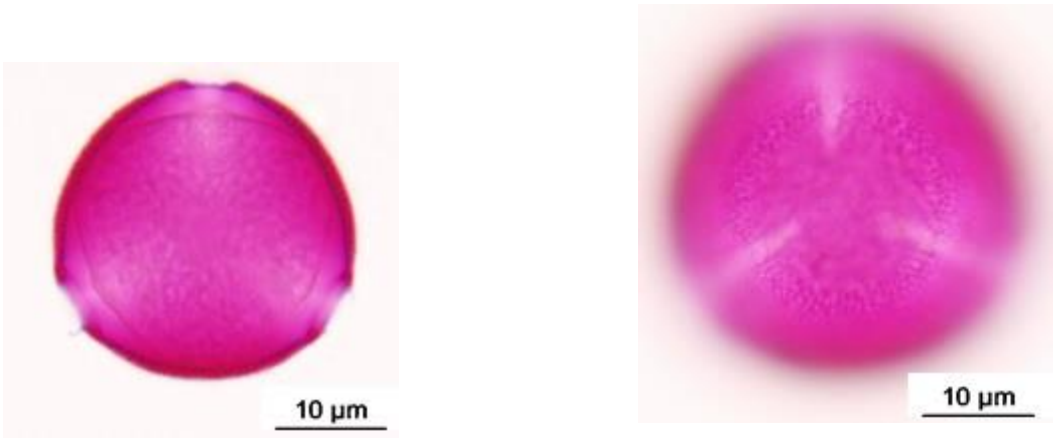


a) b)

Figure 1. Microphotograph of a) *Carduus* spp. pollen (x100) b) Ranunculaceae pollen (x100)

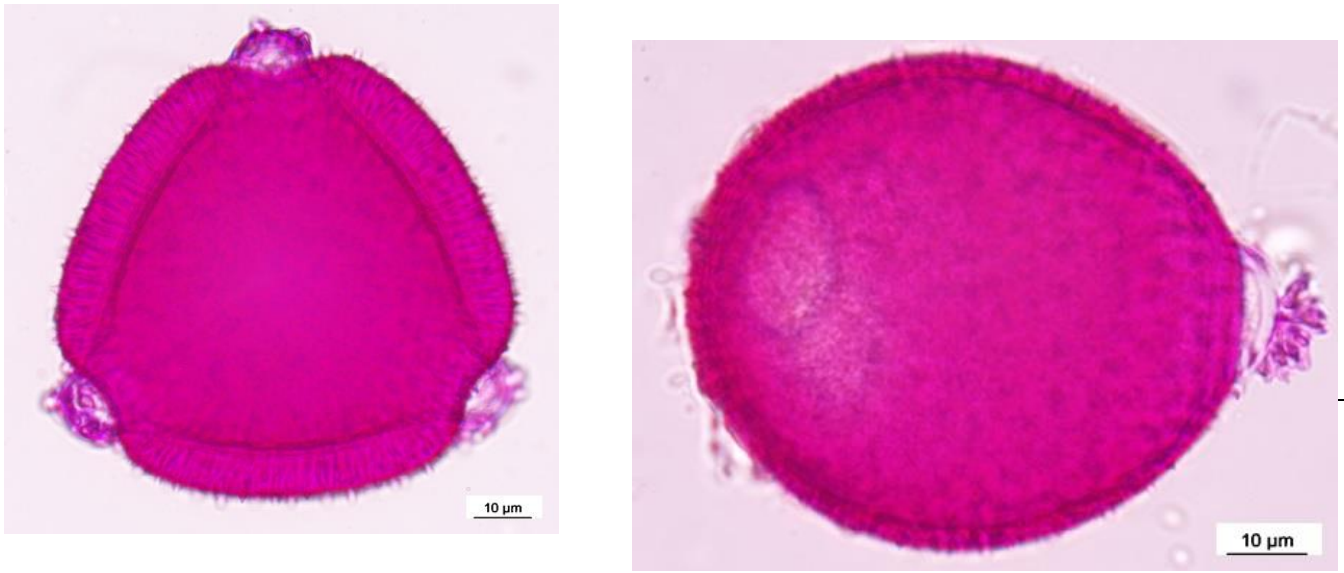
a) b)

Figure 2. Microphotograph of a-b) Geraniaceae pollen (x100)



a) b)

Figure 3. Microphotograph of a-b) Cistaceae pollen (x100)



a) b)

Figure 4. Microphotograph of a) Dipsacaceae pollen- polar view b) Dipsacaceae pollen- equatorial view (x100)

Protein content analysis of pollen samples

According to the results obtained by using Bradford method, the protein content of the pollen samples were found between the range 3.2-17.6 g/100g. The lowest value was found for the Cistaceae pollen (sample code 2-2), collected from first bee farm in July, 2013.

The highest value was found for the pollen sample (sample code 2-6) collected from the first bee farm in July, 2013, which belongs to the Caryophyllaceae family.

Bee pollen is the result of the agglutination of flower pollens; it is made by worker honey bees with nectar and salivary substances and stored at the hive entrance [12].The pollen should not contain impurities like bee parts, wax, plant

articles or other extraneous matter. Some countries as Brazil, Bulgaria, Poland and Switzerland have national standards about pollen but there is no international standard [18]. Also Turkey has a pollen standard but it is not detailed so much.

In this study we have determined the protein content of nine different pollen samples collected from hives of honey bees foraging in Anzer plateau. There have been two main approaches for quantifying the protein content of pollen samples. The micro-Kjeldahl method which was developed back in the late 1880`s, is based on the estimation of the nitrogen content of the sample of interest. However due to limiting factors such as quantification of nitrogen containing compounds of nonprotein origin, colorimetric techniques are preferred over this method [32]. One of the most commonly used colorimetric methods in determining protein concentration is Bradford (1976). Compared to Lowry method, it is faster, more accurate and shows minimum interference with reagents [33]. It is based on the color transition of the red Coomassie Brilliant Blue G-250 dye to the color blue, upon binding to protein. Bradford method is a widely used for protein quantification of pollen samples [19,34-35].

According to the results from microscopical analysis,, honey bees have mostly preferred gathering pollen of Geraniaceae family, since we identified this pollen in eight of the nine investigated samples. Following Geraniaceae, the second mostly preferred pollens are from is the Campanulaceae family (six of nine samples). *Taraxacum* spp., Cistaceae and *Scabiosa* spp. are following preferences. Kaya et al. examined the pollens of Anzer honey and similar to our results they found the pollen of Campanula, as secondary pollen, *Geranium, Lotus, Salvia, Heracleum, Myosotis, Lamium, Thymus* as minor and *Cardamine, Silene, Centaurea, Veronica, Helianthemum, Rumex, Scabiosa, Tragopon, Teucrium, Anemone, Draba, Chaerophyllum, Onobrychis* as rare pollen in the honey samples according to microscopic analysis [36].
Sorkun and Doğan (1995) also investigated the Anzer honey microscopically. They had investigated 28 honey samples and identified the pollen from 35 plant taxa mostly in minor (6-20%) or rare (0-5%) ratios. The only pollen that was identified in all 28 samples belonged to the taxa of Myosotis which was observed as minor or rare ratios in the investigated samples [25].

The only research about Anzer pollen was conducted by Ulusoy and Kolaylı [26]. They investigated the phenolic composition and antioxidant properties of Anzer bee pollen. They found the mean content of identified total phenolics from 0.5 mg/100 g pollen to 2.6 mg/100 g pollen. They also declared that the antioxidant activities showed a marked correlation with total phenolics.

Previous studies have shown that the protein content of pollen from the same species may vary depending on environmental factors (climatic conditions [37].

According to the results, the protein content of the Anzer pollen samples were found between the range 3.2-17.6 g/100g. As there is no data available on the protein content of Anzer pollen, we compared our results with previous studies conducted in different regions. We have found that our results show similarities with previous findings.Szczęsna, 2006 found the protein content of Caryophyllaceae pollen as 21.02%, Ranunculus as 17.83%, Brassica 24.08%, Campanula patula 23.60% [17]. In the study it is pointed out that pollen belonging to ruderal plants, especially *Sinapis arvensis, Sinapis alba* and *Chelidonium maius* is an important source of protein and amino acids for bees and for humans. Bonvehi and Jorda investigated

the protein content of 20 pollen samples collected from Spain and they found the protein content values between 12.6and 18.2g/100g [4]. Szczęsna,(2006) investigated the protein content of pollen samples collected from Poland (13 samples), South Korea (9 samples) and China (5 samples). For Poland samples they found the protein content values between 15.8-24.14%, for Korea 17.63-24.51%, for China 17.83-26.13% [17].

Feás et al. (2012) examined the botanical origin, and nutritional value of some bee pollens. According to their results, pollens of pine, corn and bulrush contain 13.45; 20.32 and 18.90% proteins respectively. It is also reported that many factors are known to affect the nutrient content of bee pollen, including climate, geography, apicultural practices and the genetic composition of the plant species [12].
Nutritional value of the bee pollen can be influenced from the storage conditions [38].
As seen from Table 2, the protein content of the pollen samples collected on the same date and belonging to the same taxa, varies quite much according to the location. This may indicate that climatic factors can be one of the parameters that effect the protein content of pollen. Besides, harvesting methods and storage conditions may have an impact on the protein content too. Further researches are necessary to say certainresults.

Table 2 .Comparing of protein contents pollen samples collected in the same time and belong to the same taxa according to the location

Collection time	**Plant Family**	**Plant Taxa**	**Code of Plateau 1 samples**	**Protein content of Plateau 1 samples(g/100g)**	**Code of Plateau 2 samples**	**Protein content of Plateau 2 samples(g/100g)**
11.07.13						
	Geraniaceae		2-3	6.2	6-3	7.2
	Caryophyllaceae		2-6	17.6	6-1	7.8
28.07.13						
	Asteraceae	*Taraxacum* spp.	3-4	3.6	**7-2**	6.4
14.08.17						
	Geraniaceae		4-1	5	**8-2**	7.8
	Campanulaceae		4-3	6.4	**8-4**	11.4
28.08.13						
	Geraniaceae		5-1	5	9-2	7.6
	Gentaniaceae		5-2	11.2	9-1	5.8
	Dipsecaeae	*Scabiosa* spp	5-3	8	9-4	12.4
	Asteraceae	*Taraxacum* spp	5-4	1.6	9-7	6.6
	Asteraceae	*Carduus* spp	5-5	11.2	9-5	4.8
	Campanulaceae		5-6	7.4	9-3	5.4

Conclusion

Results of the present study suggest that honey bees have preferred to collect the pollen of taxa belonging to Asteraceae, Brassicaceae, Campanulaceae, Cistaceae, Rosaceae, Geraniaceae, Fabaceae, Ranunculaceae, Ericaceae, Caryophyllaceae, Poaceae, Campanulaceae, Dipsecaceae, Gentianaceae, Buxaceae families in Anzer Plateu. In contrast to previous findings, it

can be concluded that honeybees may not always prefer the pollen types that have higher protein content, owing to the fact that the highly preferred Geraniaceae pollen has a protein content between the range 5-7.8 g/100g, while Caryophyllaceae pollen which is the least preferred pollen sample has a relatively higher protein content level (7.8-17.6 g/100g) Another observation will be the fact that locality may be an effective
parameter for the protein content of pollen samples.

Anzer Poleninin Bradford Metoduyla Protein Analizi

ÖZ

Bu çalışmanın amacı Anzer yaylasında bal arıları tarafından tercih edilen bitkileri saptamak ve bu bitkilerin polenlerinin protein içeriklerinin değerlendirilmesidir. Bu kapsamda, polen örnekleri Anzer yaylasının iki farklı arılığından 2013 yılı Haziran-Ağustos zaman peryodunda kovanlara konulan tuzaklarla toplanılmıştır.

Polen topakları öncelikle renklerine göre sınıflandırılmıştır. Mikroskobik analiz ile polenler familya ve /veya cins düzeyinde tanımlanmıştır. Polen örneklerinin toplam protein miktarı ise Bradford metodu kullanılarak saptanmıştır.

Protein analizlerin sonucunda, Anzer polen örneklerinin protein içerikleri 3.2-17.6 g/100g değerleri arasında bulunmuştur. Geraniaceae familyası en çok tercih edilen takson olarak saptanmıştır. Bulgularımıza göre, protein içeriği ise 5-7.8 g/100g değerleri arasında toplanma tarihi ve yerine göre değişiklik göstermektedir.

Anahtar Kelimeler: Arı poleni, protein analizi, Bradford metodu, palinolojik analiz, Geraniaceae

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