MELLIFERA RESEARCH ARTICLE

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

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ABSTRACT

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

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

Introduction

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

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

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

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

Materials and Methods

Collection of materials

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

Preparation of preperats from material

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

Microscopic studies of pollen samples

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

Measurements of pollen samples

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

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

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

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

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

Microscopic analysis of honeys

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

Preparates from honey samples

Preparates to identify in 10 grams of honey are

obtained as follows:

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

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

Identification of honey sample preparates

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

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

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

Kjeldahl protein analysis in pollen samples

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

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

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

Results and Discussion

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

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

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

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

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

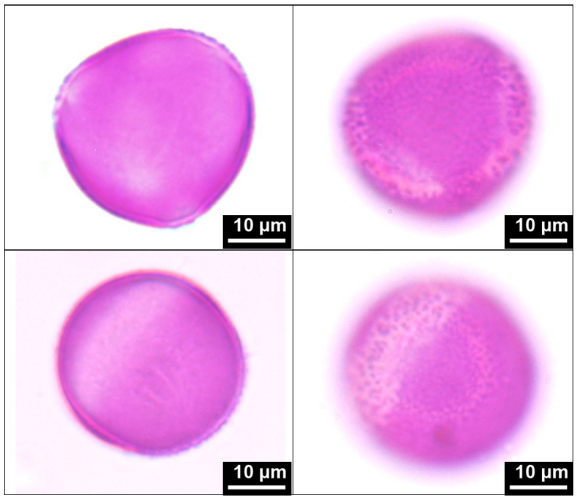

Figure 1. Pollen images of *Papaver somniferum* L. (10x100). (Photo: Aslı Özkök)

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

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

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

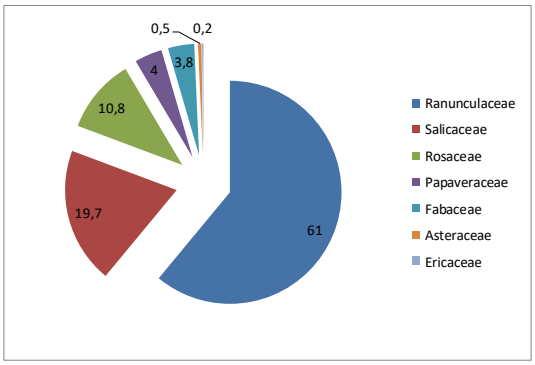

Figure 2. % Pollen tendency of honeybees before *Papaver somniferum* flowers blooming

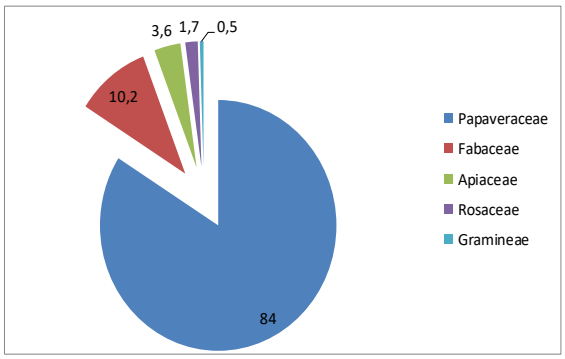

Figure 3. % Pollen tendency of honeybees after *Papaver somniferum* flowers blooming

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

Conclusion

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

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

ÖZ

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

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

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

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