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Anatomical and palynological studies on economically important *Peganum harmala* L. (Zygophyllaceae)

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Abstract

The genus *Peganum* L. (Zygophyllaceae) is comprised of five species one of which is found in Turkey. As much as it is used in Anatolia in cosmetics production, it is particularly used as a fumigant. In this study, the anatomical properties of the species *Peganum harmala* L., of the Zygophyllaceae family were studied in detail. The plant has a woody root anatomy and the stem is herbaceous. The leaf is amphistomatic.

In this study, the detailed morphological structure of the pollen of the taxon *P. harmala* L. was observed under light microscope for the first time. The results of the light microscope investigation revealed that the pollens of *P. harmala* taxon were tricolporate. The exine is striate-rugulate.

Key words: *Peganum harmala*, Anatomy, Pollen morphology, Light microscope, Turkey.

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Ekonomik olarak önemli *Peganum harmala* L. (Zygophyllaceae) üzerinde anatomik ve palinolojik çalışmalar

Özet

Peganum L. cinsi (Zygophyllaceae) Dünyada 5 türden oluşmaktadır, bunlardan biri Türkiye'de bulunmaktadır. Anadolu'da kozmetik üretiminde olduğu kadar özellikle fumigant olarak kullanılmaktadır. Bu çalışmada, *Peganum harmala* L. (Zygophyllaceae)'nın anatomik özellikleri ayrıntılı olarak incelenmiştir. Bitki odunsu kök anatomisine sahiptir, gövde otsudur. Yaprak amfistomatiktir.

Bu çalışmada *P. harmala* L. taksonunun ayrıntılı polen morfolojik yapısı ışık mikroskobu altında ilk kez incelenmiştir. Işık mikroskobu inceleme sonuçları *P. harmala* taksonunun poleninin trikolporat olduğunu ortaya koymuştur. Ekzin striat-rugulat'tır.

Anahtar kelimeler: *Peganum harmala*, Anatomi, Polen morfoloji, Işık mikroskobu, Türkiye.

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1. Introduction

The genus *Peganum* L. (Zygophyllaceae) is comprised of five species, one of which is found in Turkey. As much as it is used in Anatolia in cosmetics production, it is particularly used as a fumigant (Standley and Williams, 1987).

Due to the absence of any detailed anatomical and palynological studies, the present study was undertaken to provide a detailed account of the anatomical and palynological characters of *P. harmala* L. in Turkey.

2. Materials and methods

The species *P. harmala* L. was collected from Emirdağ (Afyon) and its environs for use in the study: B3 Emirdağ: Yeniköy, near the village, grassy limestone slopes, 39° 04' 23.6''-031° 28' 24.1'', 780m, 26.06.2006, OUFE 13413. In order to ensure a methodical study of the material obtained, herbarium samples were prepared and stored at the Eskişehir Osmangazi University Herbarium (OUFE 13413). For the anatomical study of the root, the stem and leaf were fixed in 70 % alcohol. From the Herbarium sample, the detailed morphological characteristics of the species were established and pollen preparation arrangements were employed according to the designated species. For the anatomical investigations, samples were removed from the alcohol both manually and by scalpel. The Prior marker was investigated under light microscope and microscope photographs were taken with a Spot In-SIGHT Colour Digital camera and an Olympus type microscope. A variety of foundation anatomical books and previously conducted studies were used as sources for identification of the plant (Metcalfe & Chalk, 1972; Esau, 1967; Fahn, 1967; Özörgücü ve ark. 1991; Özörgücü, 1993; Yentür, 1995).

The pollen samples were obtained from dried plants, found at the Osmangazi University Science Faculty, Department of Biology's Herbarium. The pollen morphology of the taxon in the study was investigated by light microscope. Faegri and Iversen's (1975) terminology for the names of the exine layers were used. In the light microscope investigations, the pollen obtained from the samples were set according to the method of preparation described by Wodehouse (1935). The Prior marker of the pollen investigation was conducted by light microscope. Apochromatic oil immersion objective (x100) and micrometric ocular (x10) were used. One space on the micrometric rule used was calculated to be 1 µm. Pollen measurements of the taxon for P and E were conducted until the Gaussian curve was obtained. Prepared according to the method given by Wodehouse (1935), the exine and intine thickness pertaining to taxon is to be measured a minimum of 20 and a maximum of 50 times. From these obtained measurements, a natural mathematical mean is obtained.

Identification and pollen count was achieved. For the counts, a x10 ocullar, and x10 and x40 plan objectives were used; for the purpose of identification, a x 100 plan oil-immersion objective was used. Pollen identification and count was obtained through Prior binocular microscope. The spacing between each ocular micrometer was 0.98 µm. Microphotographs were taken at the Osmangazi University Science Faculty, Department of Biology by Spot In-SIGHT Color Digital camera and an Olympus type microscope. The photograph dimensions were 10 µm, 25 µm and 100 µm.

Information from a variety of foundation palynological books and previously conducted studies were drawn from for identification of the pollens, (Wodehouse, 1935; Erdtman et al. 1954; Pokrovskaia, 1958; Kuprianova, 1965; Erdtman, 1966; Erdtman, 1969; Kapp, 1968; Aytuğ ve ark. 1971; Charpin et al. 1974; Faegri and Iversen, 1975; Moore et al. 1991; Pehlivan, 1995).

3. Results

3.1. Anatomical Features

For the anatomical investigations, samples were taken from the plants' root, stem, and leaves.

3.1.1. Root

Due to the longevity of the plant, epiderma tissue had taken root on the very outer surface (Figure 1). The cortex covers a very small area, and underneath the ring-shaped scleranchyma and this encompasses the entire transporter bunch to the scleranchyma. The rings of the scleranchyma perform the plant's endurance and resistance functions against external influences. Following this, the transporter sheaf is situated from the xylem to the root and is filled with root xylem components. Two different rings can be distinguished in the xylem. Situated on the outside of the xylem is an even thinner cell wall made up of a small, internal dense wall and larger parenchyma cells. In the cross-section captured, we could vaguely pick out the phloem scattered between the scleranchyma rings and the xylem, constituted by 2-3 layer cells.

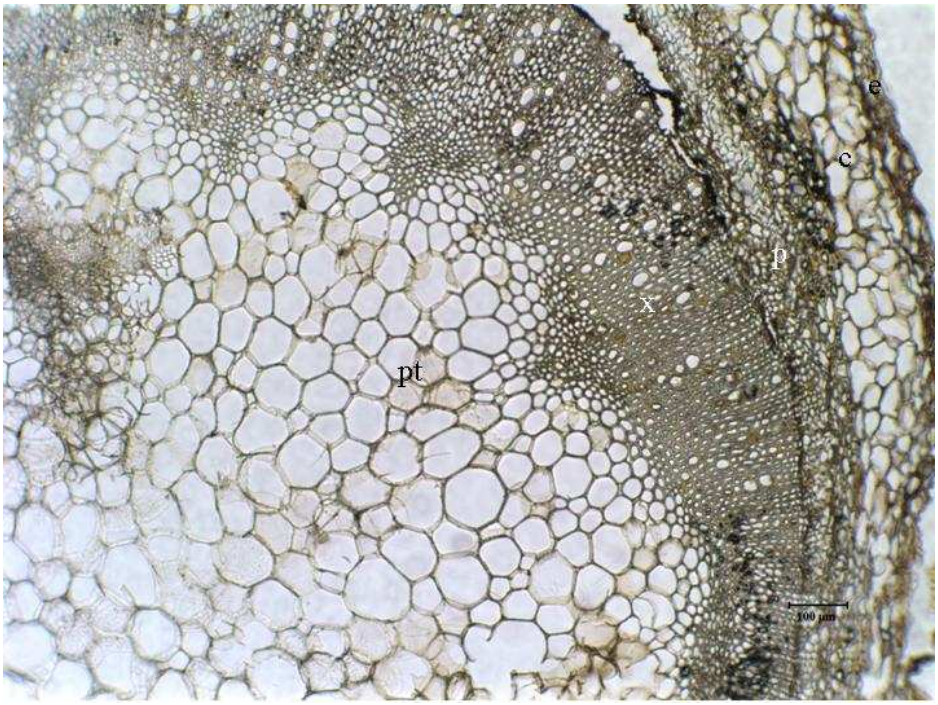

Figure 1. Cross-section of the root of *Peganum harmala* L. (Key: **e:** epidermis, **c:** cortex, **x:** xylem, **p:** phloem, **pt:** pith.)

3.1.2. Stem

Going past the epiderma tissue that had taken root on the very outer surface, we find the 5-6 cells that make up the parenchymatic cortex. After the cortex, the ring-shaped schlerancymatic cells are situated in patches. The space covered between the schlerancymatic cells and the xylem is very small, and there is hardly any selective phloem. Following this, there is a wide space covered by trache, tracheid, and xylem parenchyma and between these, in the insular space, we can uncover xylem scleranchyma. Located in the pith, and covering a wide space, is the stem parenchymatic (Figure 2).

3.1.3. Leaf

The leaf is the most outward thick cuticle; a range of epidermis can be found on the underside (Figure 3). Epidermis cells can be observed at different sizes. The contour of the chloranchyma cells cannot be easily distinguished in the mesophyll tissue. The leaf is amphistomatic. The plant has an amaryllis, a mesomorph and anisocytic type stomata. The leaf's central vascular transporter is composed of the phloem bundle and the xylem, and is situated in a spot above the external 1-2 cell layers and the xylem (Figure 3-5). The xylem is composed from trache and tracheids and covers a wide space. After the xylem, 1-2 layers of schleranchymatic cells can be observed and we can find a division directly beneath the parenchymatic tissue. The occurrence of sheaf clusters from the transporter bundle of the parenchymatic cells is typical.

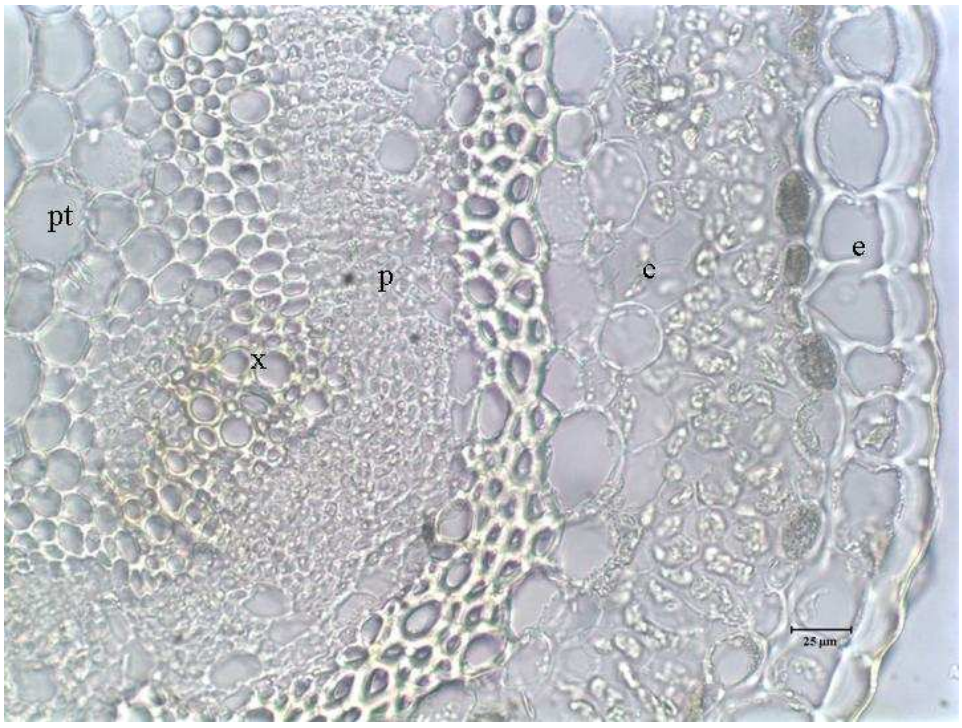

Figure 2. Cross-section of the stem of *Peganum harmala* L. Key: **e:** epidermis, **c:** cortex, **x:** xylem, **p:** phloem, **pt:** pith.

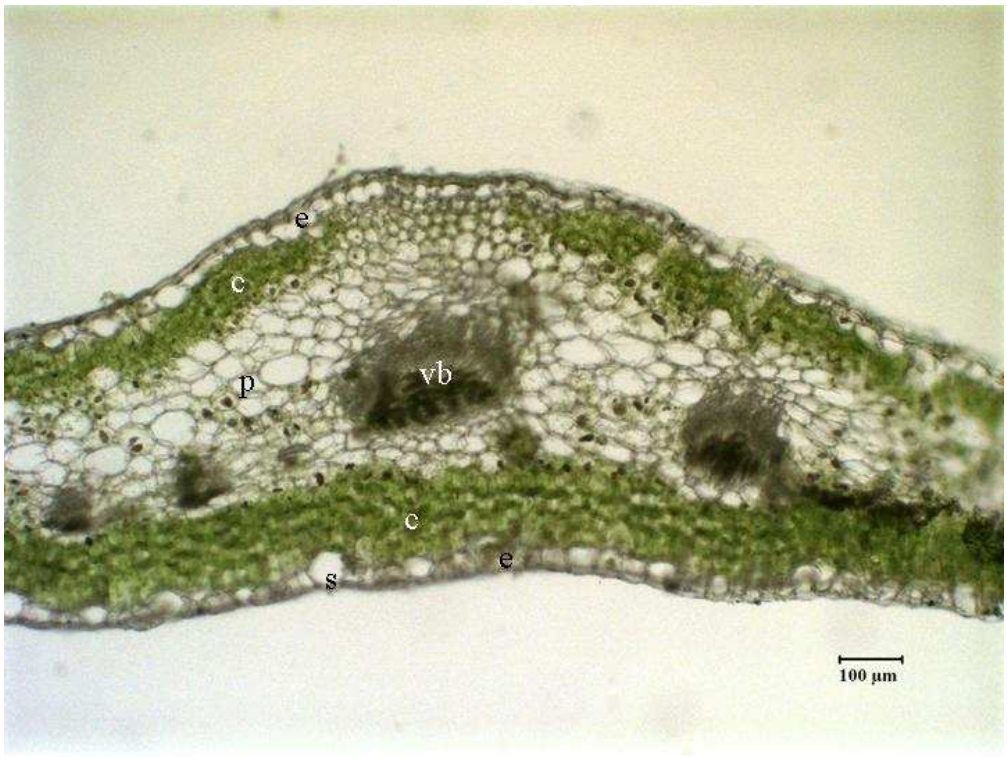

Figure 3. Cross-section of leaf of *Peganum harmala* L. (Key: **e:** epidermis, **c:** chloranchyma, **p:** parenchyma, **vb:** vascular bundle, **s:** stomata)

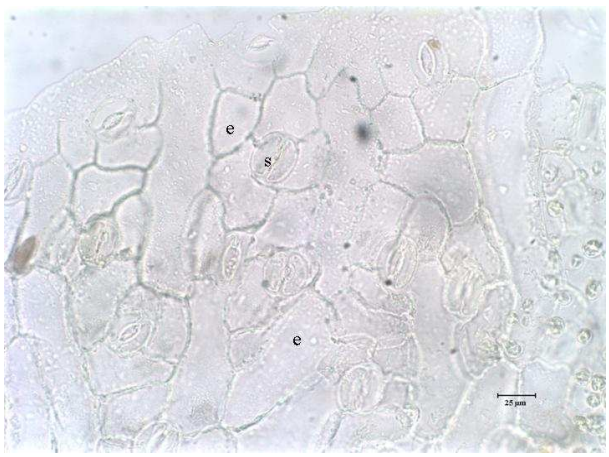

Figure 4. Upper surface section of leaf of *Peganum harmala* L. (Key: **e:** epidermis, **s:** stomata.)

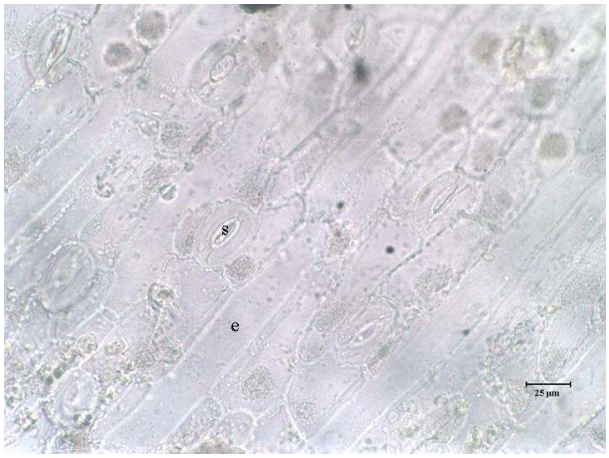

Figure 5. Lower surface section of leaf of *Peganum harmala* L. (Key: **e:** epidermis, **s:** stomata.)

3.2. Pollen Morphology Studies

Pollens of *P. harmala* L. are suboblate-subprolate and tricolporate, P/E= 1.06 (N). Ornamentation is striate-rugulate. Exine 1.2 µm (N) (Figure 6-7, Table 1).

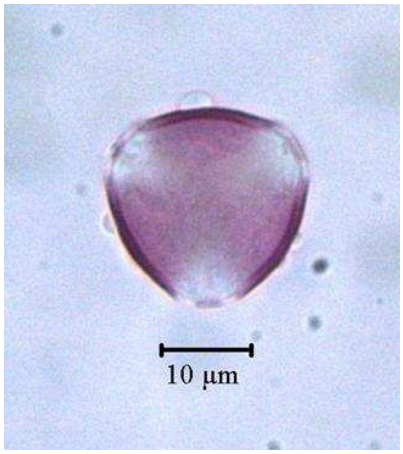

Figure 6. Pollen microphotography of *Peganum harmala* L., Polar view of a non acetolysed pollen in Light microscope.

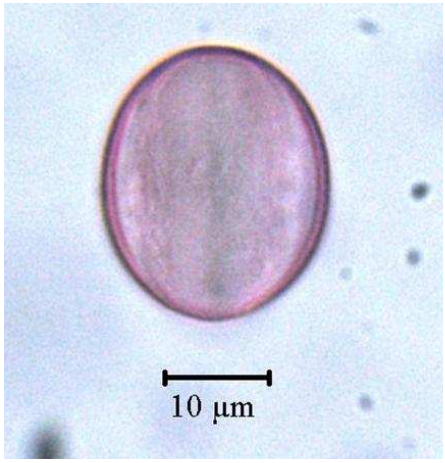

Figure 7. Pollen microphotography of *Peganum harmala* L., Equatorial view of a non acetolysed pollen in Light microscope.

Table 1. Morphometrical Parameters of *Peganum harmala* L. (N)

	P (µm)	**E** (µm)	**P/E**	**L** (µm)	**Clg** (µm)	**Clt** (µm)	**Plg** (µm)	**Plt** (µm)	**T** (µm)	**Exine** (µm)	**Intine** (µm)
M	20,56	19,28	1,06	15,12	15,92	7,04	10,66	9,09	4,04	1,2	0,78
S	1,09	0,74		0,83	1,54	1,20	2,17	1,40	0,79	0,24	0,24
Var.	22-19	20-18		16-14	18-13	9-5	13-1	11-6	5-3	1,5-1	1-0,5

Abbrevıatıons**:** N: Non acetolysed pollen (LM), P: Polar axis, E: Equatorial axis, L: Equatorial countour diameter, t: Apocolpium, clg: Length of the colpus, clt: Width of the colpus, M: Mean, S: Standard deviations, Var: Variation.

4. Discussion and conclusions

In transverse cross-sections of the upper root, it can be seen from the composition of the cortex structure and periderm during anatomic observations that secondary growth is a result of the plant's long-existence. This point has been stressed in literature related to the subject (Metcalfe & Chalk, 1972; Esau, 1967; Fahn, 1967; Özörgücü, ve ark. 1991; Özörgücü, 1993; Yentür, 1995). The plant profits in terms of protection, durability, and resistance against external effects in the ring-shaped vascular bundles of the surrounding scleranchyma (Özörgücü ve ark. 1991).

It is important for the plant that there is the existence of chloranchyma as a typical response to the photosynthetic property of the stem. In this way, the effect of photosynthesis on the leaf as well as the stem is increased (Fahn, 1967). Past the chloranchyma are situated a layer of bulk cells and ring shaped starch sheaf. Beneath the starch sheaf, schleranchymatic cell clusters can be seen in patches. The stems of the studied species yielded information on the

plant's resistance and support against external effects. Hardly any selective phloem can be found between the schlerancymatic cell clusters and the xylem. Forming the Xylem are trache, tracheids, and the parenchyma and between these, in the insular space, lie scattered scleranchyma clusters. Here in the scleranchyma, support against external effects to the stem is supplied. The parenchymatic pith, covering a wide area, is situated at the very center. The existence of the stem's parenchymatic is observable only in the stems of *Peganum harmala.* In the leaf anatomy, there is an external dense cuticle, and on the underside of this is a layer of epidermis. The dense cuticle of the epidermis, along with the position of the plant's water loss management, indicates a condition which is a characteristic peculiar to xerophytic plants (Yentür, 1995). Epidermis cells can be observed at different sizes with larger epidermis cells occurring on the underside. The contour of the chloranchyma cells cannot easily be distinguished in the mesophyll tissue. The leaf is amphistomatic. The plant has an amaryllis, a mesomorph and anisocytic type stomata. Both the leaf's upper face and underside is covered by copious amounts of blanketing and secreting down. There are amaryllis and anisosytic type stomas on both surfaces of the leaf. Thus, the leaf is amphystomatic. In the transportation bunch of the central vein, 1-2 cell levels occur on the outside of the phloem above the xylem, and the interior of the xylem covers a wider space. 1-2 layers of schlerancymatic cells can be seen in the xylem, as well as under the parencymatic cells. The transporter bunch encloses a control from the parenchymatic cells. The results of the light microscope investigation revealed a tricolporate in the pollen of *P. harmala* taxon. Upon close investigation of the exine, it was also determined that *P. harmala* is striate-rugulate. The essential criteria for the determination of the philogenetic relationship of the characteristics of the aperture and exine function of this species have been previously documented in the Literature (Kuprinova, 1967; Cronquist, 1968; Walker, 1974a-b; Takhtajan, 1980). In our analysis of this taxon, we observed that there were differences in the measurements obtained from established genetic distinctions, raising objections to the possession of a morphological characteristic passing to the pollen structure of this species (Cronquist, 1968).

We believe that we may have distinguished a criterion in the pollen morphology of *Peganum harmala* taxon' systematic characteristics ancillary sequence. At the same time, this study has also shed light on the exposed systematic-philogenetic relationship of the investigated taxon.

Establishing of the taxon' pollen morphological structure in the results has led us to think better of the usefulness of pollen studies in distinguishing the characteristics possessed by the taxon.

We believe that important discoveries unearthed during the study of anatomy and pollen morphology will lead to a better understanding of the species, and provide a contribution to any future studies.

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