

RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

THE FIRST OBSERVATIONS ON THE POLLEN MORPHOLOGY OF *VITEX AGNUS-CASTUS* L. (VERBENACEAE) (HAYIT)

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ABSTRACT

The small shrub *Vitex agnus-castus* L. (Verbenaceae) (Hayıt) is commonly found in Turkey and some Mediterranean countries and has been used amongst the publics for many years as a medicinal plant treating a variety of illnesses. The fruits, flowers and leaves of plant contain flavonoids, iridoid glycosides, volatile and stable oil. In recent years a drug has been prepared from the fruit for the treatment of some pre and post menopause complaints, with recent renewed interest in the plant.

In this study, the detailed morphological structure of the pollens of *Vitex agnus-castus* is reported for the first time. The samples were gathered from the Bergama Cocoon Plateaus were investigated with a light and a scanning electron microscope (SEM). The subprolate and tricolpate characteristics of the pollen of *Vitex agnus-castus* and semitectate-reticulate structure of the exine were determined.

Key Words: *Vitex agnus-castus*, Hayıt, Pollen, Light Microscope, SEM.

***VITEX AGNUS-CASTUS* L. (VERBENACEAE) (HAYIT)' IN POLEN MORFOLOJİSİ ÜZERİNDE İLK GÖZLEMLER**

ÖZ

Türkiye' de ve bazı Akdeniz ülkelerinde yaygın olarak yetişen küçük çalı formundaki *Vitex agnus-castus* L. (Verbenaceae) (Hayıt) halk arasında yıllardır çeşitli hastalıkların tedavisinde tıbbi bitki olarak kullanılmaktadır. Bitkinin meyva, çiçek ve yaprakları flavonoidler, iridoid glikozitler, uçucu ve sabit yağ taşımaktadır. Son yıllarda meyvalarından hazırlanan preparatlarının menapoz öncesi ve sonrası görülen bazı rahatsızlıklarda etkin olması ile tekrar ilgi odağı olmuştur.

Bu çalışmada Bergama Kozak Yaylasından toplanan *Vitex agnus-castus* örneklerinden elde edilen polenlerin ayrıntılı morfolojik yapısı Işık ve Tarama elektron mikroskobu (SEM) kullanılarak ilk kez rapor edilmiştir. *Vitex agnus-castus* polenlerinin subprolat ve trikolpat özellikte ve ekzin yapısının semitektat-retikülat olduğu saptanmıştır.

Anahtar kelimeler: *Vitex agnus-castus*, Hayıt, Polen, Işık Mikroskobu, SEM.

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1. INTRODUCTION

Vitex agnus-castus L. is a member of the Verbenaceae family (and shown widespread) is an element of the Mediterranean. The genus *Vitex* has been shown to have 2 taxa naturally distributed in Turkey (Davis, 1982-1988).

Systematic studies have been conducted into the *V. agnus-castus* species in Turkey by Davis (1982-1988). However, no study has been observed into the pollen morphology of the *V. agnus-castus* species in Turkey. In this study, individual forms of the detailed morphological structure of the pollen of *Vitex agnus-castus*, a member of the *Verbenaceae* family, were observed through light and scanning electron microscopes (SEM) for the first time.

The plant presently under study is used from gene sources for medical purposes in our country. Due to the plant's having an abundant yield from nature and its resulting high crop, as well as the lack of any dangerous effects being encountered, the small shrub *Vitex agnus-castus* L. (Verbenaceae) (Hayıt) has been used amongst the publics for many years as a medicinal plant for treating a variety of illnesses. The fruits, flowers and leaves of plant contain flavonoids, iridoid glycosides, volatile and stable oil. In recent years a drug has been prepared from the fruit for the treatment of some pre and post menopause complaints, with recent renewed interest in the plant. In order to be able to more advantageously establish the evolutionary and systematic relationships in the plant's biological characteristics, I conducted this study towards supporting the concept of study into pollen morphology. Within this context, SEM and light microscopes were used to determine the palynological characteristics of the naturally spreading *V. agnus-castus* seen in the Bergama Cocoon Plateaus.

It was also the aim of the study to simultaneously contribute taxonomic and evolutionary problems.

2. MATERIALS AND METHODS

The plant *V. agnus-castus* was gathered from the Bergama Cocoon (Kozak) Plateaus collected on the 1st of July 2005. In order to ensure a systematic study of the material obtained, herbarium samples were prepared and these samples were accepted as herbarium samples at the Eskişehir Osmangazi University Herbarium (OUFE 13001). A part of the species' detailed morphological characteristics were established and pollen preparation arrangements were applied.

The pollen samples were obtained from dried plants, found at the Herbarium of Osmangazi University Faculty of Science and Arts, Biology Department (Table 1).

The pollen morphology of the taxa in the study was investigated through light microscope and scanning electron microscope. Faegri and Iversen's terminology for the names of the exine layers was used.

In the light microscope investigations, the pollen obtained from the samples were arranged according to the method of preparation described by Wodehouse (1935) and Erdtman (1969).

The Prior marker of the pollen investigation was performed by light microscope with apochromatic oil immersion objective (x100) and micrometric ocular (x10). One space on the micrometric rule used was calculated to be 1 μ . Pollen measurements of the taxon for P and E were conducted until the Gaussian curve was obtained.

When preparing according to the method described by Wodehouse (1935) and Erdtman (1969), the exine and intine thickness pertaining to each pollen taxa is to be measured a minimum of 20 and a maximum of 50 times, and from these obtained measurements a natural mathematical mean is obtained.

The pollen count was acquired with an x 10 ocular, as well as x 10 and x 40 plan objectives; while for the purpose of identification an x 100 plan oil-immersion objective was used. The spacing between each ocular micrometer was 0.98 μ m. Microphotographs were taken at the Osmangazi University Faculty of Science and Arts, Biology Department with Spot In-SIGHT Colour Digital camera and an Olympus type microscope. The photograph dimensions were 10 μ m, 25 μ m and 100 μ m.

The method employed for the Scanning Electron Microscope (SEM) was as follows: for the pollen taken from the herbarium material of each type of species found on double-sided adhesive tape, metal pollen regulations (stap) were pre-stressed under binocular microscope and coated in order to procure the appearance of the pollen under electron microscope. The appearance of the pollen and the ornamentation was taken in the Electron Microscope Centre of Osmangazi University by Jeol 5600 LV microscope. In the dimensions given in scale photographs, microphotographs determined the aperture characteristics and exine ornamentation.

A variety of foundation palynological books and conducted studies were used as sources for identification of the pollens (Wodehouse, 1935; Erdtman et al., 1954; Pokrovskaja, 1958; Kuprianova, 1965; Erdtman, 1966; Erdtman, 1969; Aytuğ et al., 1971; Charpin et al., 1974; Faegri and Iversen, 1975; Moore et al., 1991; Pehlivan, 1995; Kapp, 1968; Walker, 1974a-b).

Table 1. Morphometrical parameters of *Vitex agnus-castus*

Taxon	P			E			PE	L			clg			clt			t			exine			intine		
	M	S	Var.	M	S	Var.		M	S	Var.	M	S	Var.	M	S	Var.	M	S	Var.	M	S	Var.	M	S	Var.
<i>Vitex agnus-castus</i> L(N)	22.81	0.37	19-30	26.58	1.32	17-31	0.85	23.41	0.22	19-28	17.05	0.28	10-23	9.71	0.29	4-17	8.2	0.21	5-15	1.2	0.03	0.5-2	0.59	0.19	0.25-1
<i>Vitex agnus-castus</i> L(A)	21.29	0.25	15-27	23.58	0.24	17-29	0.90	22.53	0.24	15-27	15.51	0.28	10-23	10.52	0.29	5-20	8.11	0.18	5-12	1.29	0.03	0.75-2	-	-	-

Abbreviations: N: Non acetolysed pollen (LM), A: 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.

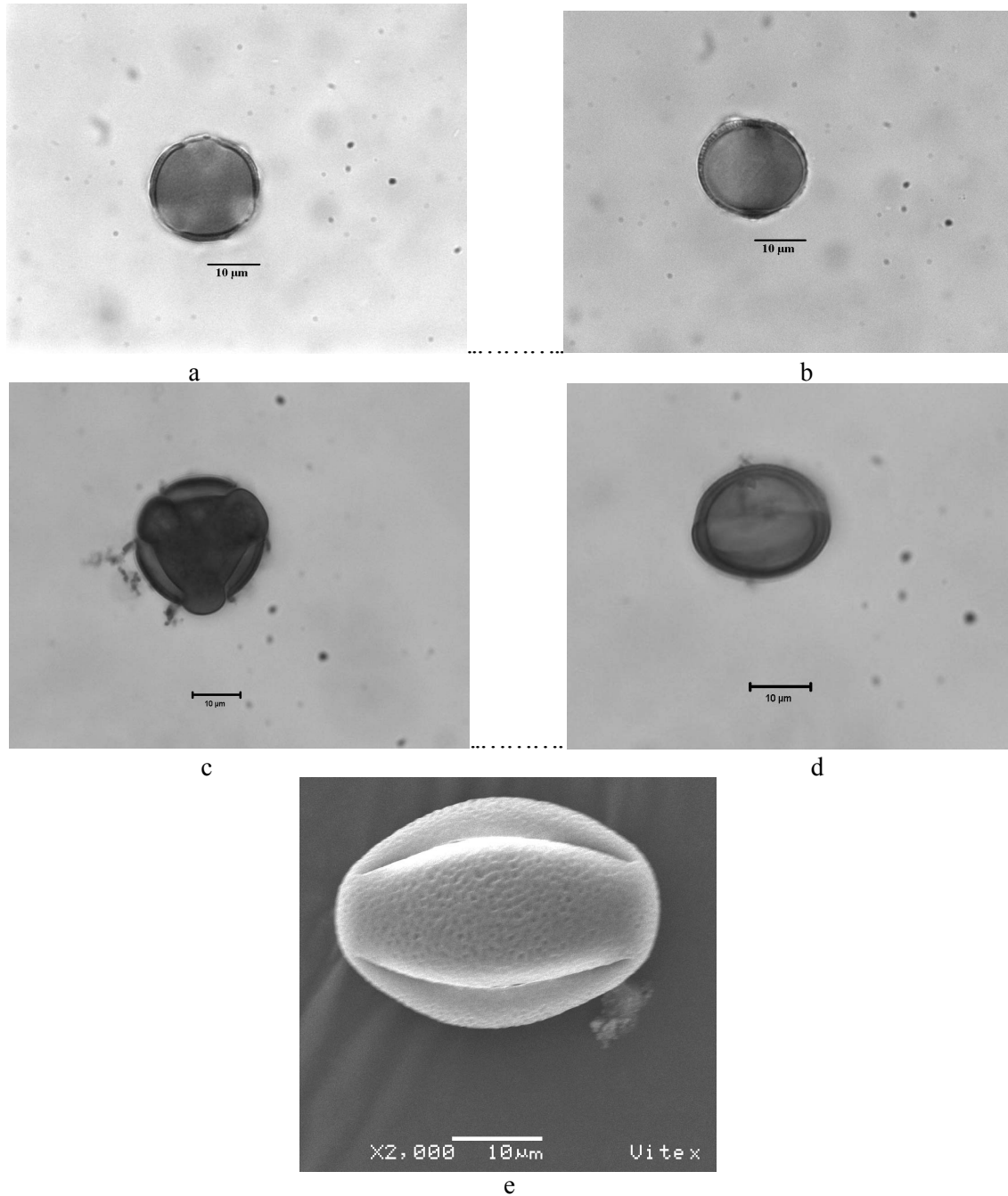


Figure 1 a-e. Pollen microphotography of *Vitex agnus-castus*, a) Polar view of a non acetolysed pollen in Light microscope, b) Equatorial view of a non acetolysed pollen in Light microscope, c) Polar view of an acetolysed pollen in Light microscope, d) Equatorial view of an acetolysed pollen in Light microscope, e) Equatorial view of a non acetolysed pollen in SEM.

3. RESULTS AND DISCUSSION

Vitex agnus-castus L. pollen subprolate and tricolpate. P/E= 0.85 (W), 0.90 (E). Ornamentation semitectate-reticulate. Exine 1.2 μ (W), 1.29 μ (E). Semitectum reticulate (Figure 1 a-e, Table 1).

The pollen subprolate and tricolpate for the species *Vitex agnus-castus* were determined during this study. In addition, the semitectate-reticulate in the exine structure was established. There have been some reports in the literature concerning essential criteria among the determined phylogenetic relationships of *Vitex* in terms of aperture characteristics and exine structure (Kuprinova 1967, Cronquist 1968, Walker 1974a-b, Takhtajan 1980). In analysis of the species, I observed that determined genetical distinctions covered differences in the measurements determined, raising objections to the possession of a morphological characteristic passing to the pollen structure of these species. (Cronquist, 1968).

I believe that I might have distinguished a criterion in the pollen morphology of *Vitex*'s systematical characteristics ancillary sequence. This study at the same time has also shed light on the uncovered systematical-phylogenetical relationship of the investigated taxon.

Determination of the pollen morphological structure of the pollen in the results has prompted me to reconsider the usefulness of pollen studies in distinguishing the characteristics possessed by the taxon.

I believe that the important studies made from the study conducted into pollen morphology will lead to a better understanding of the species and provide a contribution to any future studies.

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