Research Article

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Cuscuta campestris Yunck. Morphology, Anatomy and Traditional Use in Turkey

Cuscuta campestris Yunck.: Morfoloji, Anatomi ve Türkiye'de Geleneksel Kullanımı

Golshan ZARE[1*]
Ali. A. DÖNMEZ[2]

[1]Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Ankara, Turkey

[2]Hacettepe, University, Faculty of Science, Department of Biology, Ankara, Turkey

Corresponding author:
Golshan ZARE
Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Ankara, Turkey
E-mail: golshanzare@gmail.com
Tel: +90 (312) 310 35 45/1089-2197

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ABSTRACT

Cuscuta (dodder) are achlorophyllous plants which parasite their host stems via haustoria and obtain their nutrients from them. *Cuscuta* seed and aerial part have extensive use in traditional medicine of Asian countries, especially in China. This genus generally called with various vernacular names and used for liver disease, wounds, sciatica pain, impotence, gall bladder treatment and as laxative in Turkey. In this study, we clarify general distribution, morphology, anatomy, seed and pollen micromorphology of *Cuscuta campestris* in detail. Anatomical study of reduced vegetative structure did not provide any diagnostic character while, fruit and flower morphology provided valuable evidence to identification of this species.

Keywords: *Cuscuta campestris*, holoparasite, dodder, anatomyt

ÖZET

Cuscuta (cinsaçı), bitkilerin gövdesi üzerinde asalak olarak yerleşerek, emeçler yoluyla ihtiyacı olan besini onlardan elde eden, klorofilsiz bitkilerdir. *Cuscuta* bitkilerinin tohumları ve toprak üstü kısımları, Asya ülkeleri, özellikle Çin geleneksel tıbbında yaygın olarak kullanılmaktadır. Bu cins Türkiye'de halk arasında farklı isimlerle adlandırılır ve karaciğer rahatsızlıkları, yaralar, siyatik ağrısı, iktidarsızlık, safra kesesi tedavisi için ve müshil olarak kullanılır. Bu çalışmada, *Cuscuta campestris*'in genel dağılımı, morfolojisi, anatomisi, tohum ve polen mikromorfolojisi ayrıntılı olarak çalışılmıştır. İndirgenmiş vejetatif yapısının anatomik olarak araştırılması herhangi bir tanısal özellik sunmazken, çiçek morfolojisi bu türlerin tanımlanması için değerli kanıtlar sağlamıştır.

Anahtar Kelimeler: *Cuscuta campestris*, tamparazit, Cinsaçı, anatomi

1. Introduction

While the genus *Cuscuta* L. (dodder, field dodder, golden dodder) has been placed in Cuscutaceae family for a long time, this genus now is classified in Convolvulaceae. family according to recent molecular studies and phylogenetic evidence [1, 2]. This genus with ca. 200 species is a subcosmopolitan which grow throughout the temperate and tropical regions of the world [3].

Cuscuta are obligate holoparasitic plants, with reduced vegetative characters which parasitise plant stems via their extensions called haustoria and obtain their nutrients from them. Majority of *Cuscuta* species are not host specific and are able to parasitise a wide variety of plants from different families. Dodders are considered as serious agricultural pests and cause yield losses in crops [4, 5]. However, decoction of the seeds or stem are used for the treatment of different types of ailments, especially for kidney, liver and reproductive problems in both males and females in China, India and other Asian countries [6-10].

Cuscuta species are rich source of diverse phytochemical components such as flavonoids, polysaccharides, alkaloids, volatile oils, lignans which are responsible for these plants' biological activity such as antioxidative and hepatoprotective activity [11–15]. Despite all, only a few species of the genus such as *C. reflexa* Roxb. and *C. chinensis* Lam. are comprehensively investigated, while traditional use and phytochemistry of other species are partially known [16, 17]. *Cuscuta* seeds mainly refer as the *C. chinensis,* which have long been used by Chinese to nourish the liver and tonify the kidney [18, 19]. According to recent researches *C. campestris* have high phenolic content especially flavonols (quercetin, kaempferol, isorhamnetin), which reveal potent antioxidant activities of this species [20]. Also, Pharmacological analysis of *C. campestris* unveiled their different extracts possess analgesic properties [21], antifungal and antibacterial effect [22, 23], antipyretic [24], anti-inflammatory [24], anticancer [20, 25, 26, 27] and hepatoprotective [15] activities. Presence of rich bioactive constituent profile, wide variety of pharmacological activities and remedial value of these plants in folk medicine suggested these taxa as a valuable source to new pharmacological research.

Despite the wide distribution of some *Cuscuta* species such as *C. campestris* Yunck., *C. approximata* Babington, *C. europaea* L. and *C. epithymum* (L.) L. in Turkey there is not sufficient research on phytochemisty and biological activity of these taxa [28 , 29]. We could only access a few researches, mentioned medicinal use of this plant by local people [30–38].

The traditional use of *C. campestris* has been evaluated according to the information collected from screening of 204 ethnobotanical investigation from 1985 up to 2019. According to Akgül et al. (2016) *Cuscuta* sp. used for wounds and as laxative in Nevşehir [31] and Şekeroğlu et al. (2012), indicated that the *Cuscuta* species under the name of İkşut are used extensively for liver problems and knee pain as infusion or maceration of aerial parts in Mardin [15, 32]. But none of these researches indicate which species are used [31-33]. *C. campestris* called as Kafirsaçı in Samsun and seeds and aerial part are used for sciatica pain and impotence [34], decoction of aerial parts is used for hepatitis and gall bladder in Manisa [35, 36]. In addition, *C. europaea* is used as herbal tea in Karaman province [37].

Although local people are aware of medicinal properties of these taxa and use them for the treatment of different diseases due to their handiness and low cost, these taxa do not seem to be getting enough attention of researchers.

Baytop (1997) reported many different local names such as, Canavarotu, Bağbozan, Cinsaçı, Eftimon, Gelinsaçı, Kızıl Sarmaşık, Küşüt and Şeytansaçı, which are generally used for the dodder plants in Turkey [38]. In literature the plants in this genus are also reported as İlembeç, Verem Otu (Amasya) [24] Bağboğan, Boğmaca, Ürün (Manisa) [36].

But there is not any detailed work about the scientific names refer to the used traditional plants while some of the species such as *C. campestris, C. europaea* and *C. palaestina* have large overlapped distributions and also some of these names are also used for other genera of parasitic plants such as *Orobanche* (Canavarotu and Bağbozan) species.

In addition to all mentioned above, the lack of significant diagnostic vegetative characters such as root, stem and leaves causes confusion and difficulty in scientific identification of these taxa as the first step in medicinal plant research.

In this study, we investigated morphology, anatomy, seed and pollen micromorphology of *C. campestris* as one of the widely distributed taxa in Turkey. We

tried to find reliable characters for identification of this plant both as mature fresh plant and dried powdered material which will helps to build future research on a reliable data.

2. Materials and Methods

Plant materials belong to 8 different populations of *C. campestris* were collected from different regions of Turkey. The data of all examined specimens are listed in Table 1. Plant material was collected from the field between 2014 and 2018. The voucher specimens have been deposited in Herbarium of Hacettepe University, Faculty of Science (HUB) and Herbarium of Faculty of Pharmacy (HUEF), Ankara, Turkey. Morphological investigation was conducted on the collected fresh material and herbarium specimens of 6 different herbaria in Turkey: including Ankara University Faculty of Science Herbarium (ANK), Ege University Herbarium (EGE), Hacettepe University Faculty of Science Herbarium (HUB), Hacettepe University Faculty of Pharmacy Herbarium (HUEF), Inonu University Faculty of Science Herbarium (INU) and Van Yüzüncü Yıl University Faculty of Science Herbarium (VANF). The distribution records were plotted on a map, according to the examined herbarium specimens, Flora of Turkey and The East Aegean Islands and collected materials in our field work. For morphological characters terminology and descriptions order the Flora of Turkey and the East Aegean Islands were followed [28].

Data on the measurement of microscopic characters were collected by means of direct observation under Leica Stereo Microscopes (Model MZ 16) and photographed with a DFC 320 camera. Seed and pollen measurement were based on 30 samples using the digital measurement tool of Leica.

In the anatomical analysis, free hand sections were prepared using razor blades from 3 samples (GZ1120, GZ1187, GZ1315). All sections are stained by double stain, containing astra blue and safranin. Anatomy and pollen slides were observed with an Olympus CX41 microscope light microscope and photographed.

For scanning electron microscopy (SEM), the pollen grains and seeds (GZ1120, GZ1315) were mounted on stubs and sputter-coated with gold–palladium. SEM examination was carried out using a JEOLJSM-6490 LV microscope.

3. Results and Discussion

1-Morphology

Cuscuta campestris Yunck.

Kâfirsaçı [39]

Type: Texas, Lindheimer 126 (holo. MO).

Stem thin, 0.3-0.6 mm in diameter glabrous, twining, filiform, white to yellow or orange. Leaves reduced to minute scales (Figure 1A-D). Inflorescence globose, 4-18 flowered often many-flowered and dense, rarely few-flowered and loose, 3-12 (-14) mm diam

Table 1. The data about the voucher specimens of *C. campestris* from Turkey.

Species	Collecting No.	Locality
C. campestris	GZ916 (HUB)	C1 Muğla: Ören.
	GZ1014 (HUB)	A9 Erzurum: from Oltu to Narman.
	GZ1120 (HUEF 20008)	B1 İzmir: Ödemiş, Gölcük.
	GZ1187 (HUEF 20009)	A4 Ankara: Ankara to Sivrihisar road, limestone area.
	GZ1216 (HUEF 20010)	B4 Aksaray: Aksaray, from Yeşilova to Yenikent.
	GZ1243 (HUEF 20011)	B7 Malatya: Darende, from Hekimhan to Kangal.
	GZ1248 (HUEF 20012)	A8 Rize: Ikizdere, Ballıkoy village.
	GZ1315 (HUEF 20013)	A4 Ankara: Hacettepe University, Beytepe campus.

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(Figure 1E-F). Flower sessile or shortly pedicellate, ca. 1 mm. Flowers small, 2-3 (-4) mm, white, cream or reddish, (4-) 5-merous (Figure 1G-I). Calyx ca. 1.5-2 mm gamosepalous, ± deeply lobed, sepals 5, usually as same length as the corolla tube, lobes ± imbricate, large ovoid to orbicular, generally obtuse, sometime acute; tube campanulate, usually the same length as the lobes (Figure 1H). Corolla sympetalous, infundibular, lobes shorter than to ± as long as campanulate tube, suberect then patent to recurved, widely triangular, acute or obtuse, often inflexed at apex. Stamens generally a little shorter than corolla lobes; anthers shorter than to almost as long as subulate filaments, scales ca. as long as corolla tube, reaching stamens, ovate or oblong-ovate, entire, deeply fimbriate (Figure 1I). Ovary globose, styles 2, 1/2 as long as capsule, slender, suberect to divaricate, stigmas capitate or subcapitate, together almost as long as ovary (Figure 1J). Capsule (1.4-) 3 mm diameter, with withered corolla at base, depressed-globose, dehiscing irregularly (Figure 1K). Seeds 1-1.5 mm, 2-4 per capsule often 3, dull yellow or alight brown (Figure 1L). The seeds are endospermous.

Flowering time: (May) June – October.

Altitude: 0-1500 m.

Distribution in the world: Native to N America, but now cosmopolite.

Distribution in Turkey: Wide distribution (Figure 2).

Examined specimens: Al Tekirdağ: Şarköy to Mürefte, *A. Baytop* (ISTE 13511); A2 İstanbul: nr. Djendere, 17 vii 1898, *Aznavour*.; A2 Bilecik: Osmaneli, 100 m, *P.H.Davis* 36336; A3 Düzce: Akçakoca, around Yalılar, seashore, sandy places, 0-2 m, 9.12.2002, *Aslı Doğru Koca* 1973 (HUB!); A4 Ankara: *R.Richter* 1235 (ANK!); A4 Ankara: Kızılcahamam, Soğuksu National Park, stream bed, 13.11.1966, *Leblebici & Ersoy* 6171 (EGE!); A4 Kırıkkale: 500 m E. of Irmak, 700 m, 23.06.1990, *Ali A. Dönmez* 22492 (HUB!); A5 Kastamonu: Taşköprü, arrival to Hamzaoğlu village, 536 m, 15.06.2008, *Ali A. Dönmez* 14432 (HUB!); A5 Kastamonu: Taşköprü, Bayamca village, 20.08.2010, *Ali A. Dönmez* 17432 (HUB!); A8 Trabzon: 30 km E. of Trabzon, *M. & D. Zohary* 25352; A8 Rize: Rize, Çay Enstitüsü nursery. 200 m, 18.07.1986, *A. Güner, M. Vural, H. Duman*-7057 (HUB!); B1 İzmir: Mersinli, 20.06.1957, *M. Orhan Özalp* (ANK!); B1 İzmir: Bornova, 5.06.1957, *M. Orhan Özalp* (ANK!); B1 İzmir: Karşıyaka, 22.07.1957, *M. Orhan Özalp* (ANK!); B1 İzmir: Şaraphane, around Melez stream, 21.10.1982, *Y. Gemici* 25119 (EGE!); B1 İzmir: Karaburun, Kösedere village, ca. 350 m, *L. Bekat* (178) *Y. Gemici* (EGE!); B2 Manisa: Vuriye village, 19.08.1957, *M. Orhan Özalp* (ANK!); B2 Denizli: Çivril, *Y. Gemici* 2881 (EGE!); B4 Ankara: Beytepe campuse, steppe, 900 m, 8.09.1983, *S. Erik* 1528 (HUB!); B4 Ankara: Şereflikoçhisar, on the Adana road, 130. km. around Tuz Gölü, 900-1000 m, 5.10.1984, *N. Demirkuş* 2783 (HUB!); B4 Ankara: Gölbaşı, İncek, around Tek Yapı Koop., roodside, 1170 m, 5.09.2008, *S. Arabacı, Z.C. Arıtuluk* 08078 (HUEF!); B5 Kayseri: from Kayseri to İncesu, 1200 m, D. 32759; B5 Kayseri: Yemliho, below Aks, irrigarion channel, 22.08.1999, *S. Erik* 6244 (HUB!); B6 Yozgat: Akdağmadeni, Yavu-Falaş valley. Ca. 1350 m, 20.07.1980, *T. Ekim* 4721 (ANK!); B7 Elazığ: between Kömürhan-Haroğlu Mt., c. 1200 m., 17.06.1981, *Y. Ahan & H. Evren* (ANK!); B7 Malatya: Konak, 2.07.1996, *B. Yıldız* 13632 (INU!); B7 Malatya: Meyve Araştırma Enstitüsü. hedge, 950 m, 23.06.1995, *B. Yıldız* 12905 (INU!); B9 Kars: 10 km from Kars to Iğdır, *Hewit* 1970:308; B9 Muş: Malazgirt, Aktuzla, 1600 m, *L. Behçet* 7084 (VANF!); B9 Van: 100. Yıl Üniversity campuse, 1700 m, *L. Behçet* 6402 (VANF!); B9 Van: Muradiye, Görçek village 1800 m, 12.10.2002, *O. Karabacak* 3625 (VANF!); B9 Bitlis: Hizan Soğanlı, 2000 m, 2.05.1989, *Y. Altan- L. Behçet* 2785 (VANF!); B9 Van: Gürpınar, Cevitatan village, 2180 m, 20.08.2008, *İbrahim Demir* 1709 (VANF!); B9 Van: Erciş, 1800 m, 12.09.2005, *O. Karabacak* 4183 (VANF!); B9 Bitlis: Sarıkonak village, 1650 m, *A. Altıok* 2830 (VANF!); B10 Kars: d. Aralık, S.E. of Iğdır D.Ü.Ç., 800 m, *P.H.Davis* 47007; C1 Aydın: Kuyucak, *M. Orhan Özalp* (ANK!); C2: Antalya, Yuva, Karagöl, ca. 1000 m, *P.H.Davis* 13918 (ANK!); C2 Muğla: Ortaca, between Dalyan-Tepe, 10-20 m, 18.06.1991, *A. Güner, M. Vural, H. Şağban* (9500) (HUB!); C2 Burdur: Tefenni, Korkuteli-Tefeni road, 3. km, field, 1365 m, 20.07.2009, *ZC Arıtuluk* 091089 (HUEF!); C2 Antalya: Yuva, Karagöl, 1000 m, *P.H.Davis* 13918; C1 Muğla: 1 km N. of Fethiye, *Lambert & Thorp* 545; C4 Konya: from Konya to Çumra, Küçükköy, 980 m, *Helbaek* 2717. C8 Diyarbakır: Bismil-Savur road, corn field, 7.07.2019, *Deniz Dalgın* 19067 (HUEF)!

Host: This species is not host specific and can parasitise wide range of taxa, generally herbaceous cultivated plants. Also, every individual can penetrate more than one species in same time. All recorded host plants on field and herbarium specimens are listed below:

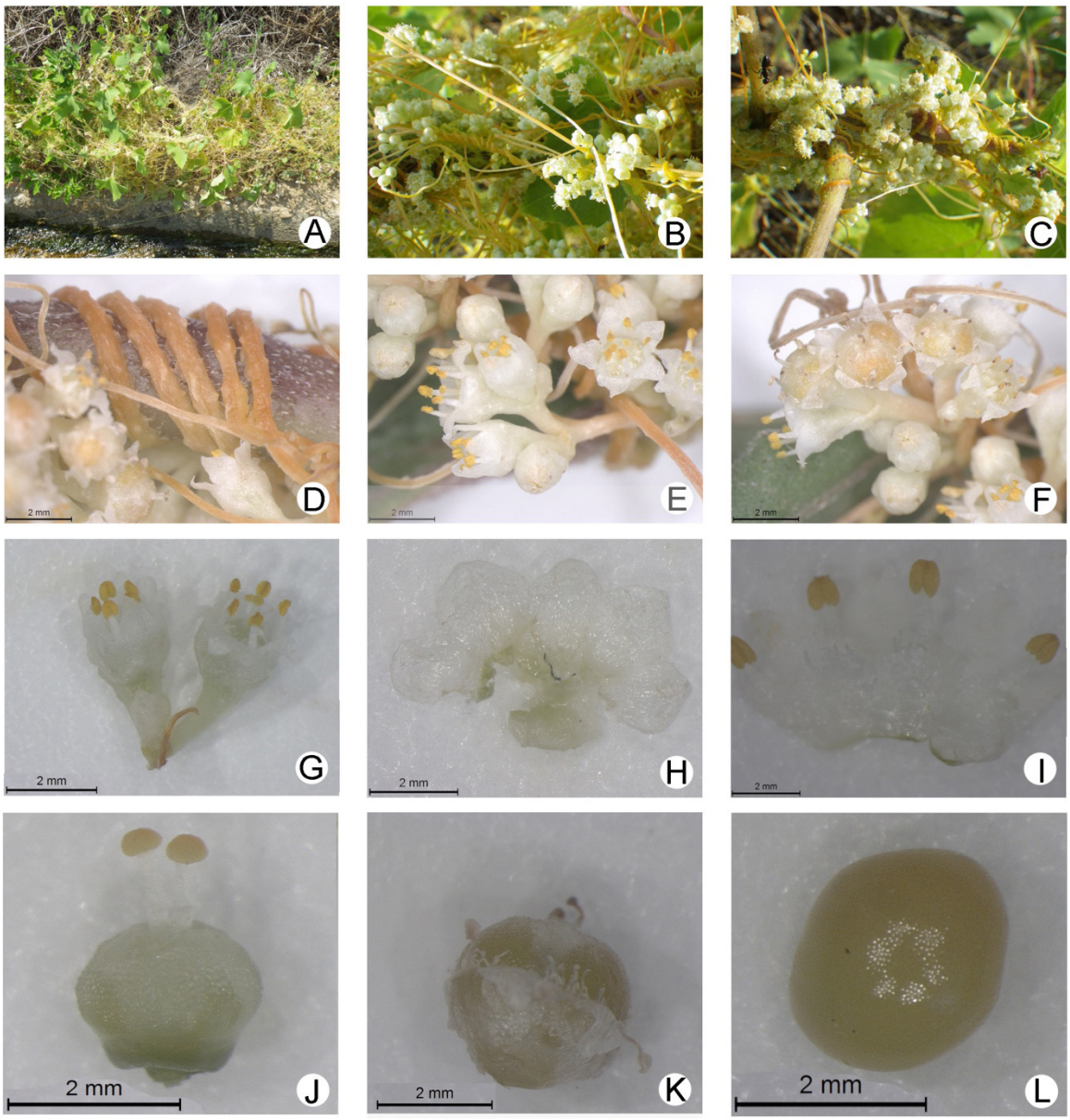

Figure 1. *C. campestris* (GZ1120): A-C habitus, D. haustoria on the stem of host plant, E. inflorescence, F, mature flower and fruits in inflorescence, G. flower, H. calyx, I. corolla, J. ovarium with two stylus and subcapitate stigmas, K. capsule with corolla remaining parts, L. seed.

Eryngium sp., *Daucus carota* L., *Pimpinella anisum* L., *Carum carvi* L. (Apiaceae); *Artemisia* sp., *Chondrilla juncea* L., *Cichorium intybus* L., *Xanthium strumarium* L., *X. spinosum* L. (Asteraceae); *Berberis* sp. (Berberidaceae); *Beta vulgaris* L., *Salsola kaki* L.(Chenopodiaceae); *Convolvolus arvensis* L. (Convolvolaceae); *Alhagi maurorum* Medik., *Astragalus* sp., *Cicer arietinum* L., *Trifolium* sp., *Medicago sativa* L., *Ononis spinosa*, *Vicia faba* L., *Vicia sativa* L. (Fabaceae); *Verbascum* sp. (Scrophulariaceae); *Antirrhinum majus* L., *Nicotiana tabacum* L., *Solanum lycopersicum* L., *Capsicum annuum* L, *Solanum melongena* L., *Solanum tuberosum* L. (Solanaceae); *Citrus* sp. (Rutaceae); *Tribulus terrestris* L. (Zygophyllaceae); *Linum usitatissimum* Lin (Linaceae); *Allium cepa* L. (Liliaceae).

Pollen micromorphology: Pollens are shed as monads, pollen grains are small, 23-25 × 17-19

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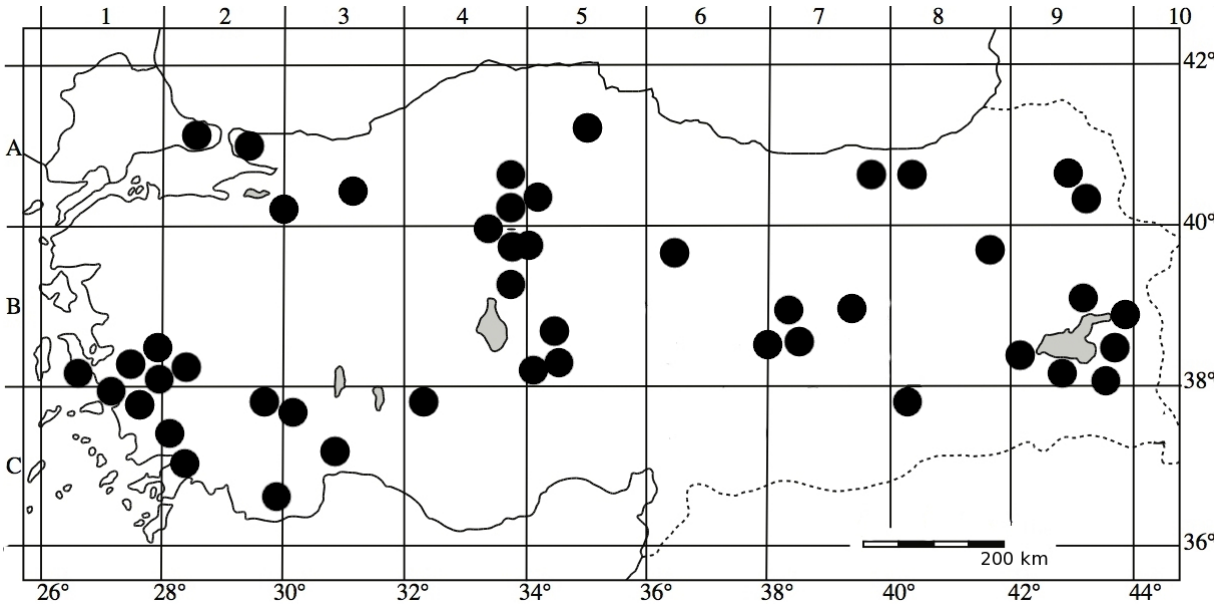

Figure 2. Distribution map of *C. campestris* in Turkey.

µm, dry pollen shape, prolate, oblate spheroidal (P/E:1.29±0.08), isopolar, radially symmetrical, AMB polar view triangular, 5.5 µm, tricolpate, aperture(s) sunken, aperture length 15-17 µm, exine ornamentation microechinate-perforate (Figure 3A-B).

Seed micromorphology: Seeds generally are 4 per capsule but every individual produce lots of seeds. Seed medium size, 800-1200 × 720-1030 µm, yellowish-brown or dark brown, seed shape irregular spherical, dorsal side convex, ventral more or less flattish, a funicular insert at the ends, seed surface consists of a characteristic thick layer and sculpture is rugulate or microreticulate. The endosperm layer containing starch and oil grain is surrounded by an embryo without cotyledons (Figure 3C-D).

2- Anatomy

The vegetative organs especially leaf reduced and haustoria developed to obtain nutrition from the host plant. Stem and haustoria have a round structure in cross-sections. The stem and haustoria cross section structure contain of a single layer epidermis with thin cells and are without hairs and cuticula (Figure 4A). The epidermal cells of the haustoria are elongated and the outer wall is thicker than the other walls. Cortex contain 5-10 layers isodiametric parenchymatic cells in different sizes. There are some free spaces between the cells for gas exchange. Central stele is distinct and in vascular bundles phloem placed in outside, and xylem elements with vessels placed inside (Figure 4A-B).

This species first surrounds the host and then penetrates its stem deeply via haustoria vascular bundles. All cross-sections taken from the suctioning part generally have the same structure and did not show any difference between species (Figure 4C-F).

Anticlinal division of epidermal cells and anticlinal and periclinal division of cortical cells constitute a meristematic cell mass which form haustoria. These cells are dense and contain starch grains abundantly and called search hyphae. As soon as this hypha accesses the host plant xylem, they perform secondary walls and change to xylem hyphae with evident vessel. In longitudinal section of the penetrated plant, these vessels distinctively seem as annular (Figure 4F). After the penetration, the tip cells of haustoria are connected to the vascular tissue of the host stem and the peripheral cells connected and pressed the host cortical cells and are combined with them. This taxon generally penetrates stem of the host instead of leaf lamina and petiole.

Cuscuta species are known as taxonomically difficult taxa. Stefanović *et al.* [40] and Austin [41] believe parasitic life in plant is associated with the extreme reduction or modification in vegetative structures, which makes difficult the identification of

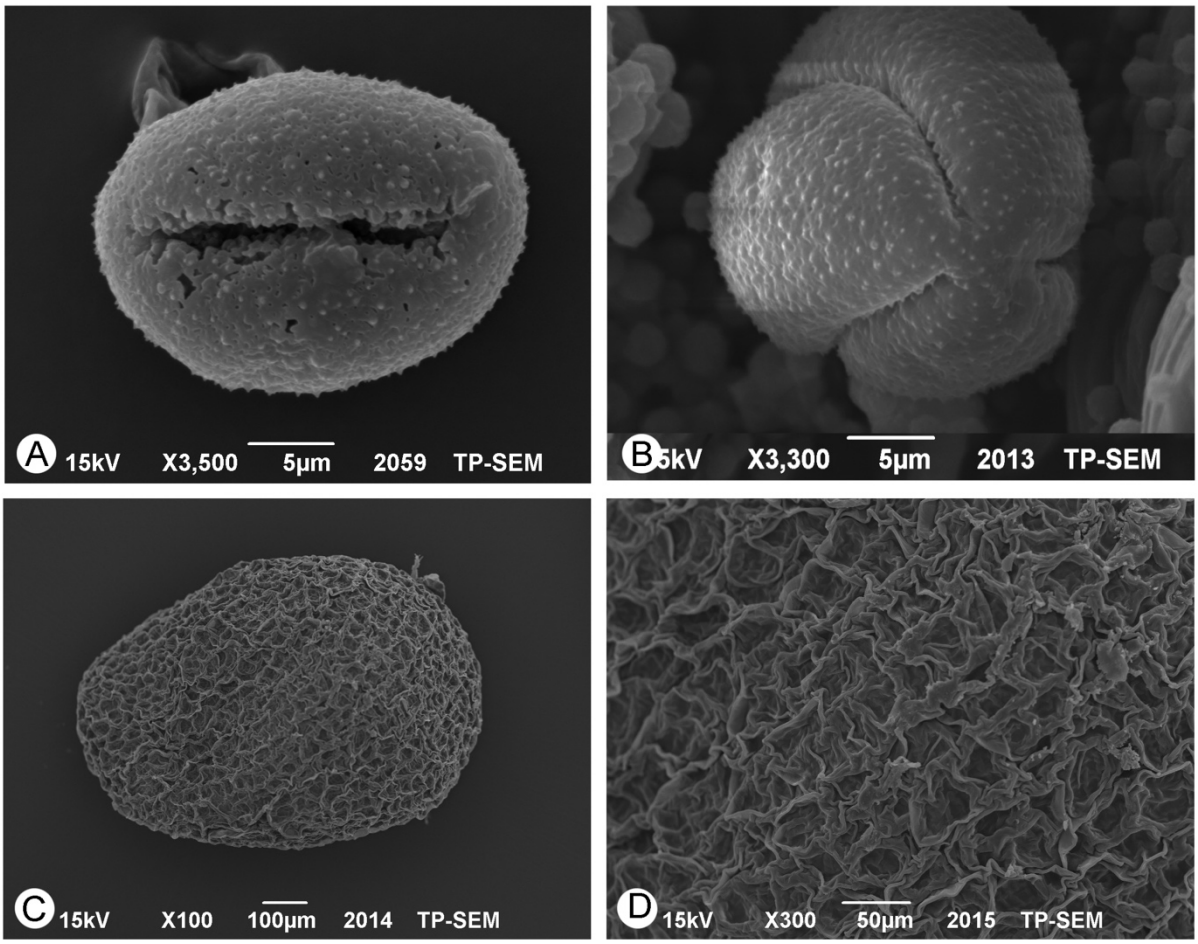

Figure 3. *C. campestris* pollen and seed micrograph by SEM (GZ1315); A. Pollen equatorial view, B. Pollen Polar view; C and D. Seed surface ornamentation.

this taxa, because the main characters used in identification of vascular plant such as stem, leaves and flower structure are completely absent or restricted to microscopic differences in the small flowers.

During this study comprehensive revision of *C. campestris* beside seed and pollen micromorphology and anatomy has been undertaken as one of the folk remedies in Turkey. Taxonomical description, distribution map and host rage were provided based on fresh material from field and herbarium samples.

Austin [41] suggested that to facilitate identification in these taxa, botanists should make ample collections with mature flowers and fruit along with the host plant. In agreement, our results indicated, morphological characters related to mature flower, such as corolla segment shape, stylus number and length, stigma shape length, scale shape, seed size and seed surface sculpture beside host range are diagnostic characters for identification of this species.

Although several taxonomic studies, reported the exclusive host plant can provided useful information to facilitates the identification of these taxa, *C. campestris* is one of the frequent *Cuscuta* species with wide host ranges [41-43]. Our observation revealed few genera of Asteraceae (specially *Xanthium*), Solanaceae and Fabaceae are among the most favourable host plant for this species and as Barath & Csiky [42] and Erdös [43] reported that annuals species are dominate hosts group.

Isopolar, tricolpate pollen with microechinate-perforated ornamentation and seed shape and micromorphological structure are common characters shared among other *Cuscuta* species too [44- 46]. Also, our finding on anatomical structure of cortex and cen-

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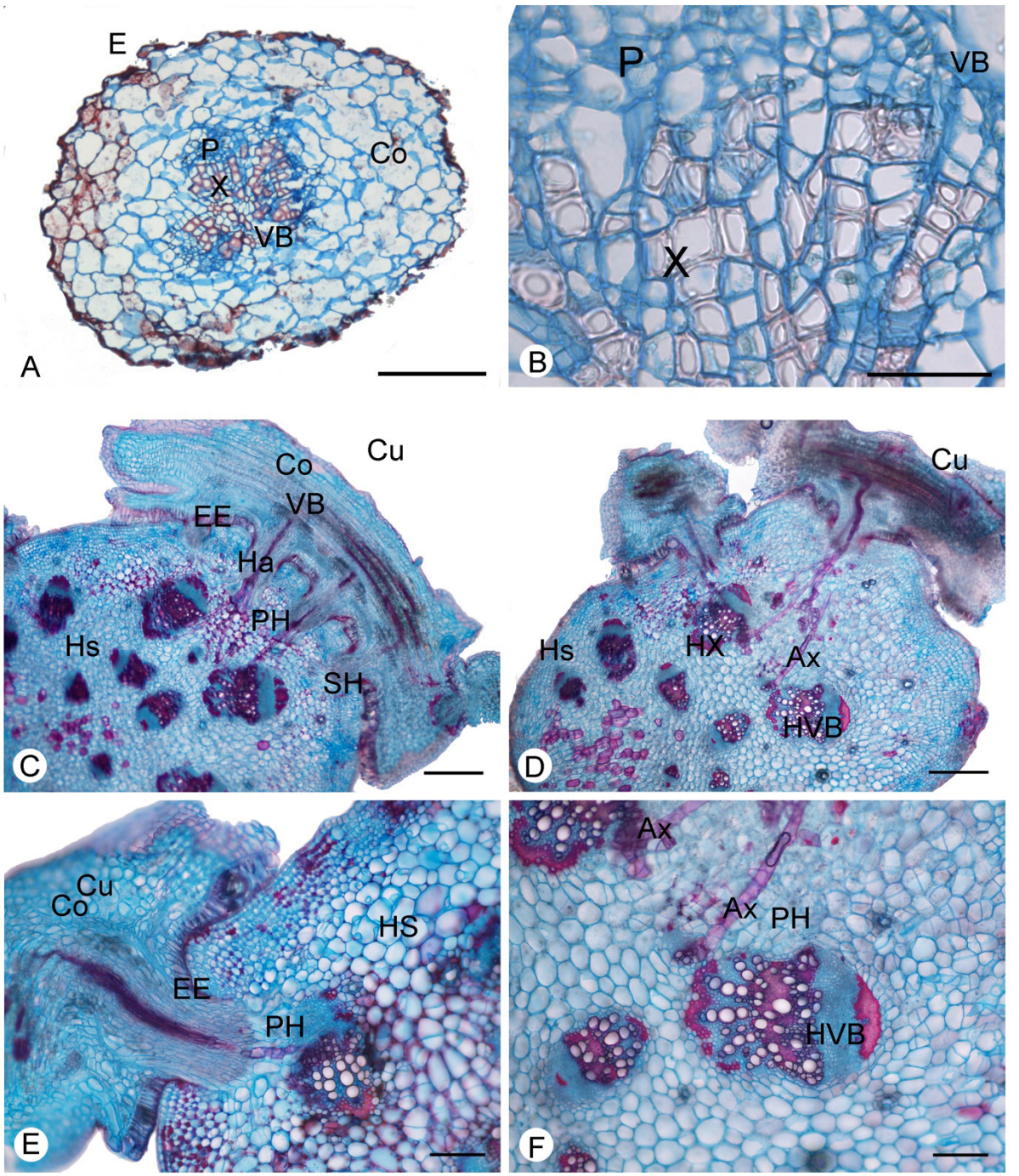

Figure 4. Light micrographs of *C. campestris* (A-B GZ1187, C-F GZ1120): A-B. stem cross section; C-E. stem cross section with host plant; F. Annular xylem vessels (Ax), vascular bundle (VB), *Cuscuta* (Cu), cortex (Co) elongated epidermal cells (EE), haustorium (Ha), host stem (HS), phloem (P), phloem hyphae (PH), host vascular bundle (HVB), search hyphae (SH), xylem (X). Scale bar (B) 50 µm, (A, F) 200 µm, (C, D, E) 500 µm.

tral vascular bundle in stem and haustoria is in accordance with the Lyshede [47] and Hong *et al*. [48] results.

4. Conclusion

In this study anatomy, morphology, seed and pollen micromorphology of *C. campestris* were investigated for first time as one of the species with wide distribution, and possible folk remedyin Turkey. While morphologiacal diagnostic characters related to mature flowers and fruit beside host information were documented for identification of this species. Seed and pollen micromorphology and anatomical study of cross-sections taken from the suction in different species did not show any difference between species.

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