

**MORPHOLOGICAL, ANATOMICAL AND PALYNOLOGICAL STUDY
OF *CENTAUREA CALCITRAPA* L. SSP. *CILICICA* (BOISS. & BAL.)
WAGENITZ AND *CENTAUREA SOLSTITIALIS* L. SSP. *CARNEOLA*
(BOISS.) WAGENITZ ENDEMIC FOR TURKEY**

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Abstract

In this study, morphological, anatomical and palynological properties of *Centaurea calcitrapa* L. ssp. *cilicica* (Boiss. & Bal.) Wagenitz and *C. solstitialis* L. ssp. *carneola* (Boiss.) Wagenitz which are endemic for Turkey were investigated. In the morphological studies, the properties of these endemic subspecies were determined and measurements made on the materials and compared with floristic-systematical references. The morphology of capitula, involucra, involucral leaves (phyllaries) and achene have been investigated. In the anatomical studies; the transverse sections were taken from the root, stem, phyllaries and leaves of the subspecies. In the palynological researches Polar diameter, equatorial length and apertures were measured in detailed. Microphotographs of pollen and anatomical structures are also presented.

Introduction

The investigations about Turkey's flora have been developed since 1948. It was published as taxonomically in the Flora of Turkey and the East Aegean Islands that collected by P.H. Davis and their colleagues. The examined subspecies were belong to *Compositae* (*Asteraceae*) family. *Centaurea* L., is an important genus which represented in Turkey by 178 species, 33 subspecies and 22 varieties. 109 of which are endemics. Endemism ratio is 61,6% (Davis 1975; Güner *et al.*, 2000, Wagenitz 1960). Anatomical characteristics of some *Centaurea* species were examined by Metcalfe & Chalk (1950); Esau (1977); Bhattacharya & Johri (1998); Kaya (1987); Yaman (1997); Wagenitz & İnceoğlu (1973); Kaya *et al.*, (2000); Uysal *et al.*, (2005); Çelik *et al.*, (2005; 2008).

Material and Methods

C. calcitrapa L. ssp. *cilicica* (Boiss. & Bal.) Wagenitz samples were collected C5 Mersin: Gözne-Soğucak road side, 900m on 06.07.1999. *C. solstitialis* L. ssp. *carneola* (Boiss.) Wagenitz samples were collected from C5 Mersin: Mersin University Campus, 150 m on 10.07.1999. Voucher specimens are deposited in the Z.K.Ü, the Forestry Faculty, Bartın. The subspecies in 70% alcohol were used for anatomical studies. Sections were taken by hand and permanently mounted by dying with sartur, sudan III and safranin for distinguishing of tissues (Algan, 1981; Yentür, 1995). The microphotographs have been taken by Olympus BX40 research microscope. For palynological studies, the Wodehouse method has been used in preparation of pollen (Wodehouse 1959; Aytuğ 1967). In the measurements; 1 ocular division has been equal to 1.02 µm.

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Morphological results

***C. calcitrapa* ssp. *cilicica*:** Annual, stem erect, 25-40 cm, repeatedly branched from near base. Plants more slender. Involucre 16-20 X 4-5 mm, cup-shaped. Appendage straw-coloured, spine length 6-13 mm, with 1-3 mm, pairs on each side. Flowers pale pink. Achenes 2-3 mm. Pappus absent. Fl: 6-7. East Medit. Element.

***C. solstitialis* ssp. *carneola* (Boiss.) Wagenitz:** Annual, 15-20 cm, adpressed-tomentose. Basal and lower leaves are mostly withered at flowering time. Median and upper leaves are lanceolate to linear lanceolate. Involucre 10-13X8-10 mm, cup-shaped. Appendages straw-coloured, spine length 5-11 mm, with spinules (1-2 mm) on each side at base. Flowers pink, marginal not radiant. Achenes 2-3 mm, dimorphic, with white pappus 3-4 mm. Fl. 6-7, East Medit. Element.

Anatomical properties

Root: At the outside on both subspecies; usually broken periderm, parenchymatic cortex and vascular cylinder take place below it. In *C. solstitialis* ssp. *carneola*, xylem has 5-15 rays which 1-2 cells wide, gland cells occur in phloem and below line of periderm. Periderm cells 43.2X20.4 µm. The pith consist of tracheidal cells. In *C. solstitialis* ssp. *carneola*; there is sclerenchyma groups on phloem. Periderm cells 27.8 µmX48.5 µm. Xylem takes up wide area. Xylem has rays with 4 or more number and 3-10 cells wide. Gland cells is in phloem and along 2-layered endodermis. Pith consist of parenchymatic cells (Fig. 1).

Stem: In cross sections of both subspecies, stem has recessed structure, the outer one-layered epidermis sparsely contains stomata. Stomatas are raised above epidermis level. The gland hairs and multicellular hairs with long whip-like terminal cell were seen. Collenchyma and chlorenchyma take place of alternating segments below epidermis. The number of collateral bundles have different size are 15-20. In *C. solstitialis* ssp. *carneola*; rather small bundles in cortex (=cortical vascular bundles) are seen in addition to central vascular bundles (medullary bundles). At *C. solstitialis* ssp. *carneola* stem has leaf-like wings which have leaf anatomy and some of these gland cells were seen. One-layered endodermis is between sclerenchyma and collenchyma. Cambium with 2-3 layers is located between phloem and xylem. Parenchymatic pith on both subspecies are located on wide area. Pith in *C. solstitialis* ssp. *carneola* contains cubical and circular crystals while *C. calcitrapa* ssp. *cilicica* contains prismatical crystals.(Fig. 2).

Leaves: In cross sections both subspecies are isolateral (=dorsiventral) type. Mesophyll is composed of 2-3 layered palisade and 1-2 layered spongy parenchyma. Gland hairs and multicellular hairs with long whip-like terminal cells are on epidermis. In vascular bundles, xylem is near upper and phloem is near lower surface. Stomatas are raised above epidermis level and parenchymatic bundle sheath is seen. Bundle sheath extensions and sclerenchyma groups are upper and below of vascular bundles, the below ones have wider area. Besides, at midvein region plate collenchyma with 2-4 layered takes place under the upper or below epidermis (Fig. 3).

Table 1. The measurements of involuclral leaves.

	All length	Spine length
Upper involuclral leaves	10-12 mm	1-2 mm
Medium involuclral leaves	12-23 mm	8-18 mm
Lower involuclral leaves	4-7 mm	2-4 mm

Table 2. The measurements of involuclral leaves.

	All length	Spine length
Upper involuclral leaves	8-10 mm	1-1.5 mm
Medium involuclral leaves	11-13 mm	7-9 mm
Lower involuclral leaves	8-10 mm	3-5 mm

Table 3. Diameter of epidermis lumen and cuticula thickness.

	Cuticula thickness	Diameter of epidermis lumen
<i>C. calcitrapa</i> ssp. <i>cilicica</i>	5 µm (on coll.) 2.5µm (on chlor.)	15.1 µm X 7.6 µm
<i>C. solstitialis</i> ssp. <i>carneola</i>	5.5µm (on coll) 5 µm (on chlor.)	8.4 µm X 4.8 µm

Table 4. The dimensions of epidermis cells and leaf thicknees.

	<i>C. calcitrapa</i> ssp. <i>cilicica</i>	<i>C. solstitialis</i> ssp. <i>carneola</i>
Lower epidermis dimensions	27.3 µm X 13.6 µm	32.3 µm X 14.1 µm
Upper epidermis dimensions	30.1 µm X 14.9 µm	31.8 µm X 16.4 µm
Leaf thickness	7.5 mm	5 mm

Table 5. The dimension of stoma cells

	<i>C. calcitrapa</i> ssp. <i>cilicica</i>	<i>C. solstitialis</i> ssp. <i>carneola</i>
Stomata cells in upper surfaces	15.3 µm X 4.9 µm	15.3 µm X 4.3 µm
Stomata cells in lower surfaces	16 µm X 5.2 µm	17.4 µm X 4.7 µm

At surface sections on both subspecies, leaf is amphistomatic and anomocytic type. Stomatas are surrounded by 3-5 epidermis cells. Stomatas and hairs on upper side outnumber those on the lower side.(Fig. 4).

Epidermis membranes at *C. solstitialis* ssp. *carneola* in more straight on lower surfaces; while at *C. calcitrapa* ssp. *cilicica* is more straight on upper surfaces. Gland cells are surrounded by 8-10 cells and hair base cells are surrounded by 5-10 cells. (Fig. 5).

Phyllary: In cross sections of both subspecies, Sclerenchyma layer which in upper and lower epidermis takes place wider space on upper region. Upper epidermis has stomatas. Vascular bundles in collaterally type are 8-10 number. Gland canals surrounded by 5 cells are on vascular bundles. Bundles are surrounded by chlorenchyma cells, at below this region there are parenchyma cells that have wide cavity. At surfaces sections, on upper surfaces, stomatas are surrounded by 4-5 cells and cristas lines are seen between sclerenchyma and epidermis lower surfaces sections, stone cells are found as sparsely groups or single.

Palynological results: The pollen grains of *C. calcitrapa* ssp. *cilicica* and *C. solstitialis* ssp. *carneola* have three apertures. The palynological characteristics of the examined specimens are given in Table 6.

Table 6. Comparison of palynological characteristics of the examined specimens

	<i>C. calcitrapa</i> ssp. <i>cilicica</i>	<i>C. solstitialis</i> ssp. <i>carneola</i>
Pollen type	Tricolporatae	Tricolporatae
Pollen shape	Sphaeroide, P/E= 1.00	Sphaeroide, P/E= 1.00
Structure	Tectatae, Infrastructurae, ect/end $\cong$ 3/1	Tectatae, Infrastructurae, ect/end $\cong$ 2/1
Sculpture	Scabratae, spinules lenght are less than 1 μ m the lenght between two spine is less than 1 μ m.	Scabratae, spinules lenght are less than 1 μ m, the lenght between two spine approximately is 2.04 μ m.
P(μ m)	27.81 $\pm$ 1.08	32.02 $\pm$ 1.69
E(μ m)	27.70 $\pm$ 0.67	31.82 $\pm$ 1.83
plg(μ m)	4.79 $\pm$ 1.04	6.63 $\pm$ 1.03
plt(μ m)	5.61 $\pm$ 1.05	7.68 $\pm$ 1.01
clg(μ m)	22.84 $\pm$ 0.63	27.02 $\pm$ 0.15
clt(μ m)	7.61 $\pm$ 1.05	9.68 $\pm$ 1.01
t(μ m)	7.34 $\pm$ 0.72	8.87 $\pm$ 1.04
Ex(μ m)	2.19 $\pm$ 0.27	2.24 $\pm$ 0.31
i(μ m)	Thin, Ex/int = 4/1	Thin, ex/int=3/1
dh(μ m)	-	0.85 $\pm$ 0.06
dt(μ m)	-	2.24 $\pm$ 0.31
Apertures	Colpi long and wide, definite margines, colpi tips acute, pori definite, Pori shape plg/plt = 0.85, the polar triangle is medium large and regular	Colpi long and wide, definite margines, colpi tips acute, pori definite, pori shape plg/plt = 0.86, the polar triangle is medium large and regular

Abbreviations: P: polar axis, E: equatorial axis, P/E: polar to equatorial diameter ratio, W: Wodehouse method, p: pores (pori, porus), plg: pori wideness, plt: pori length, clg: colpi wideness, clt: colpi length, ex: exine, i: intine, M: avaage, σ : standart deviation, dh: length of the spina, dt: width of the spina, t: length of the one side of the triangular polar area (Fig. 6).

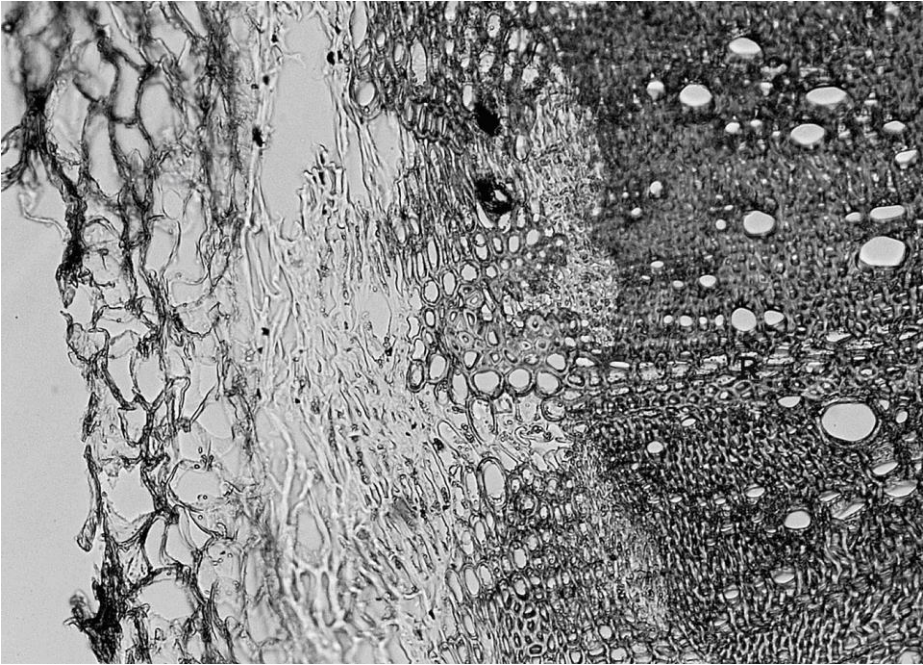
Discussion

In this study, *C. calcitrapa* ssp. *cilicica* and *C. solstitialis* ssp. *carneola* which are endemic subspecies belong to *Compositae* (*Asteraceae*) family were examined morphological, anatomical and palynological.

1. The morphological and systematical results; both subspecies were collected from Mersin region. Morphological results generally agree with the description in *Flora of Turkey*.
2. Although our anatomical results are similar to Metcalfe & Chalk (1950) and Kaya *et al.*, (2000), but some differences have been also identified with Kaya (1987), and Yaman (1997). These are
 - a. At stem structure, there is collenchyma and chlorenchyma segments below epidermis,
 - b. Having bundle-sheath extensions in cross sections of leaves,
 - c. Stone cells and sclereids with crystals at sections of upper epidermis of phyllary,
 - d. The pith in stem has some crystall cells,
 - e. The stem of *C. solstitialis* ssp. *carneola* has leaf-like wings,
 - f. Stem has medullar and cortical bundles.

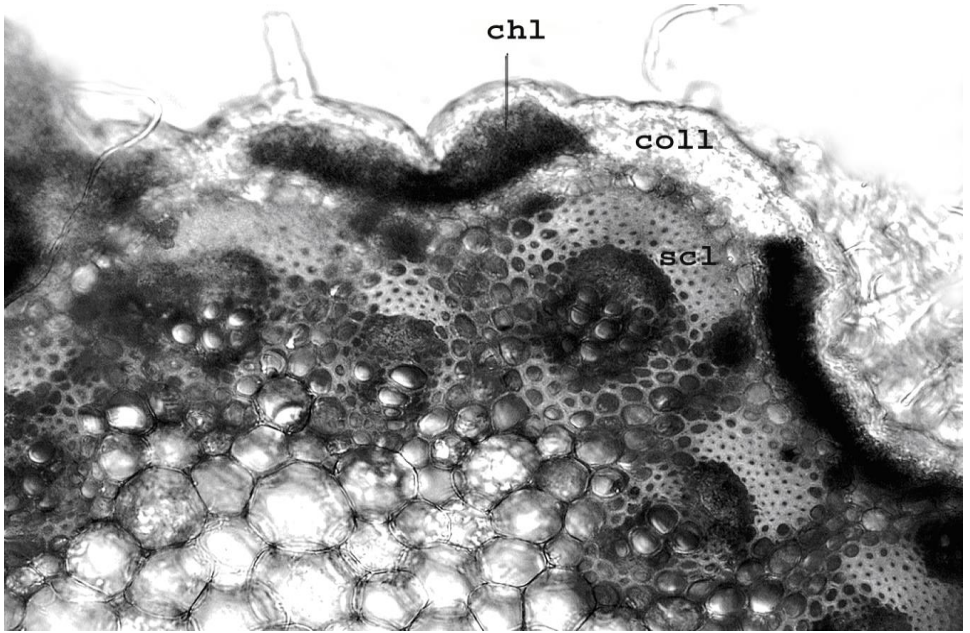


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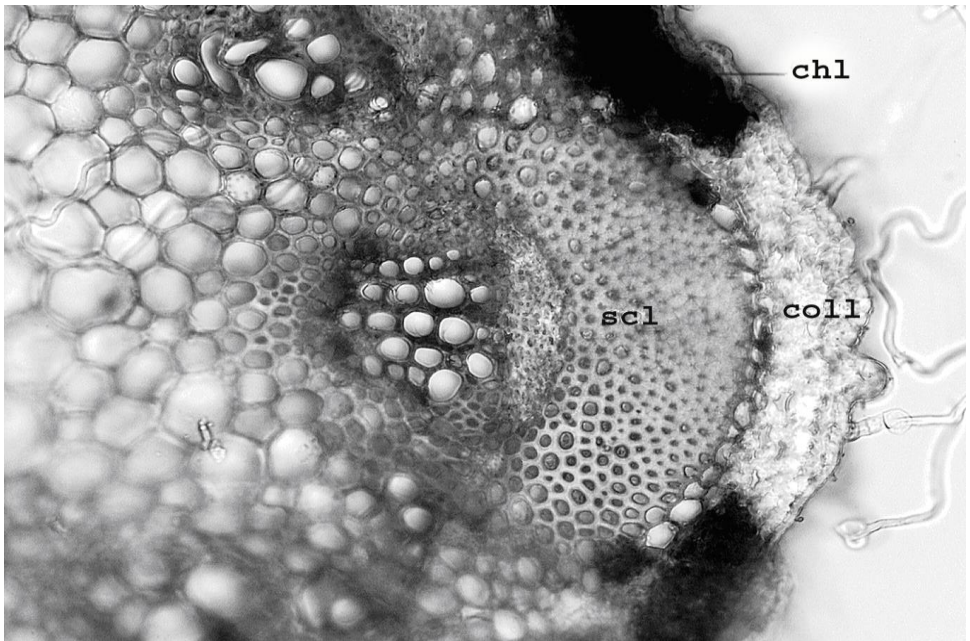


II

Fig. 1. The root. I. *C. solstitialis* ssp. *carneola* x10, II. *C. calcitrapa* ssp. *cilicica* x10, r: ray, gc: gland cell.

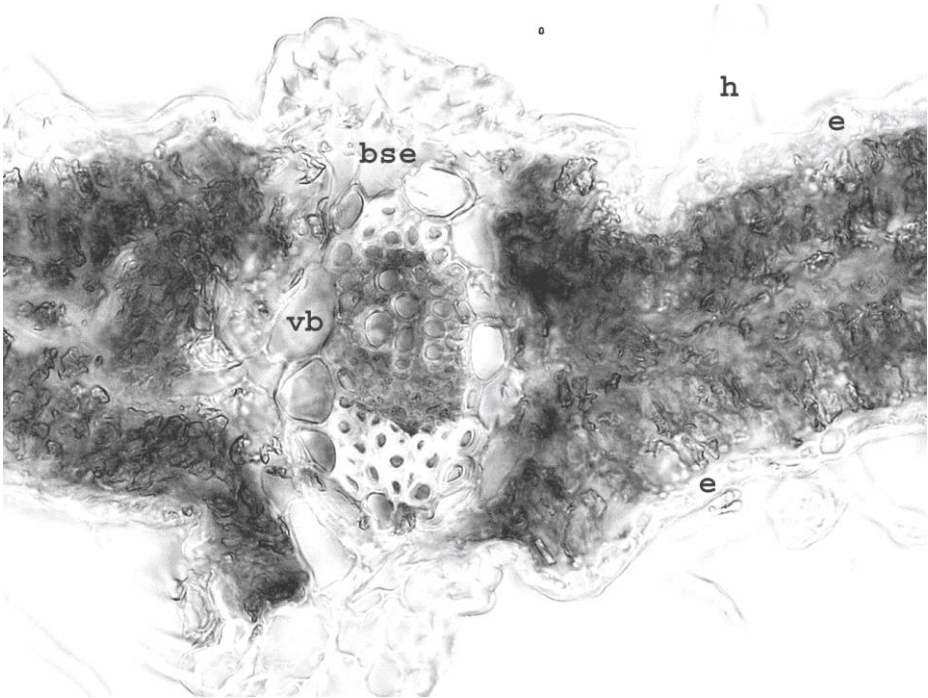


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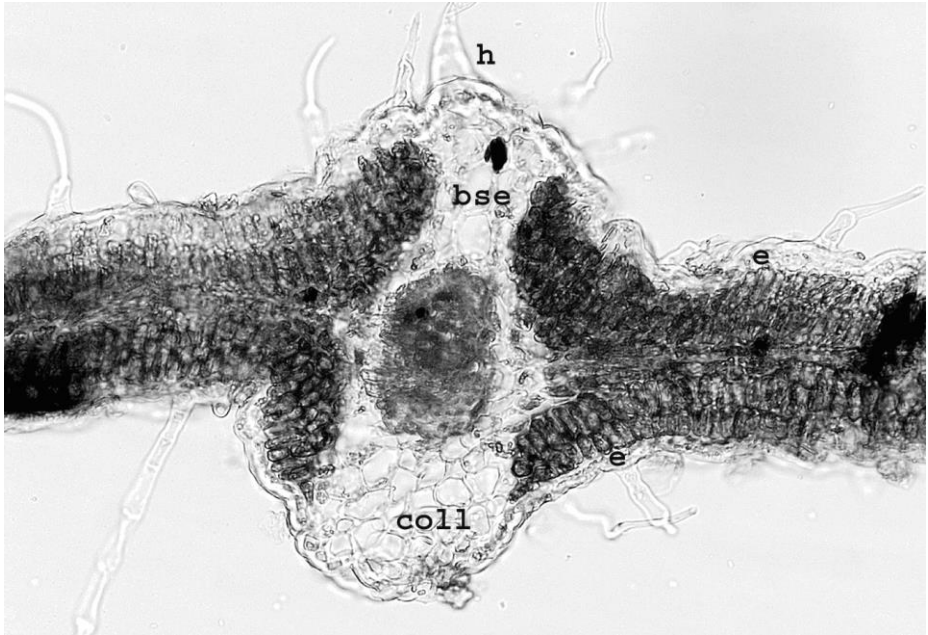


II

Fig. 2. The stem. I. *C. solstitialis* ssp. *carneola* x10, II. *C. calcitrapa* ssp. *cilicica* x10, chl: chlorenchyma, coll: collenchyma, scl: sclerenchyma.



I

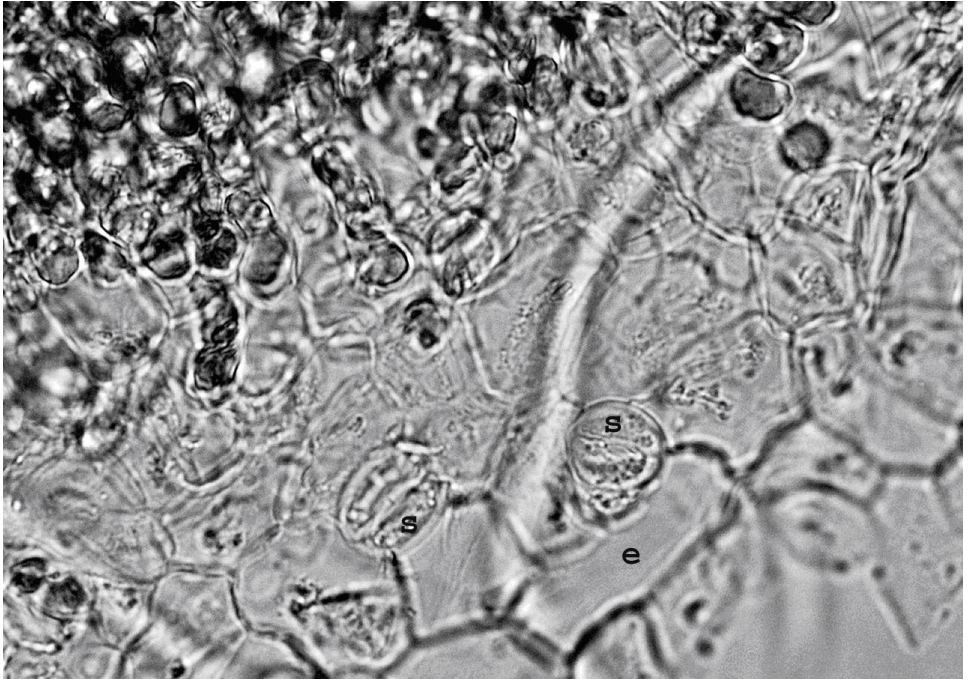


II

Fig. 3. Transverse sections of leaves. I. *C. solstitialis* ssp. *carneola* x20, II. *C. calcitrapa* ssp. *cilicica* x10, e: epidermis, vb: vascular bundle, bse: bundle sheath extension, coll: collenchyma.

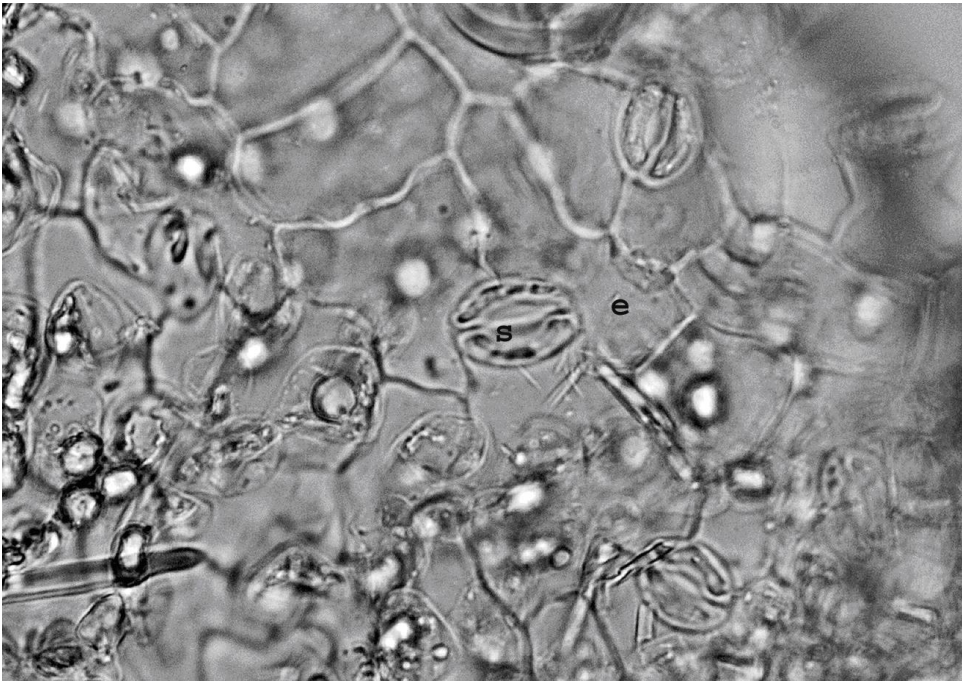


I



II

Fig. 4. Upper side of the leaves. I. *C. solstitialis* ssp. *carneola* x40, II. *C. calcitrapa* ssp. *cilicica* x40, e: epidermis, s: stomata.



I



II

Fig. 5. Below side of the leaves. I. *C. solstitialis* ssp. *carneola* x40, II. *C. calcitrapa* ssp. *cilicica* x40,e: epidermis, s: stomata.

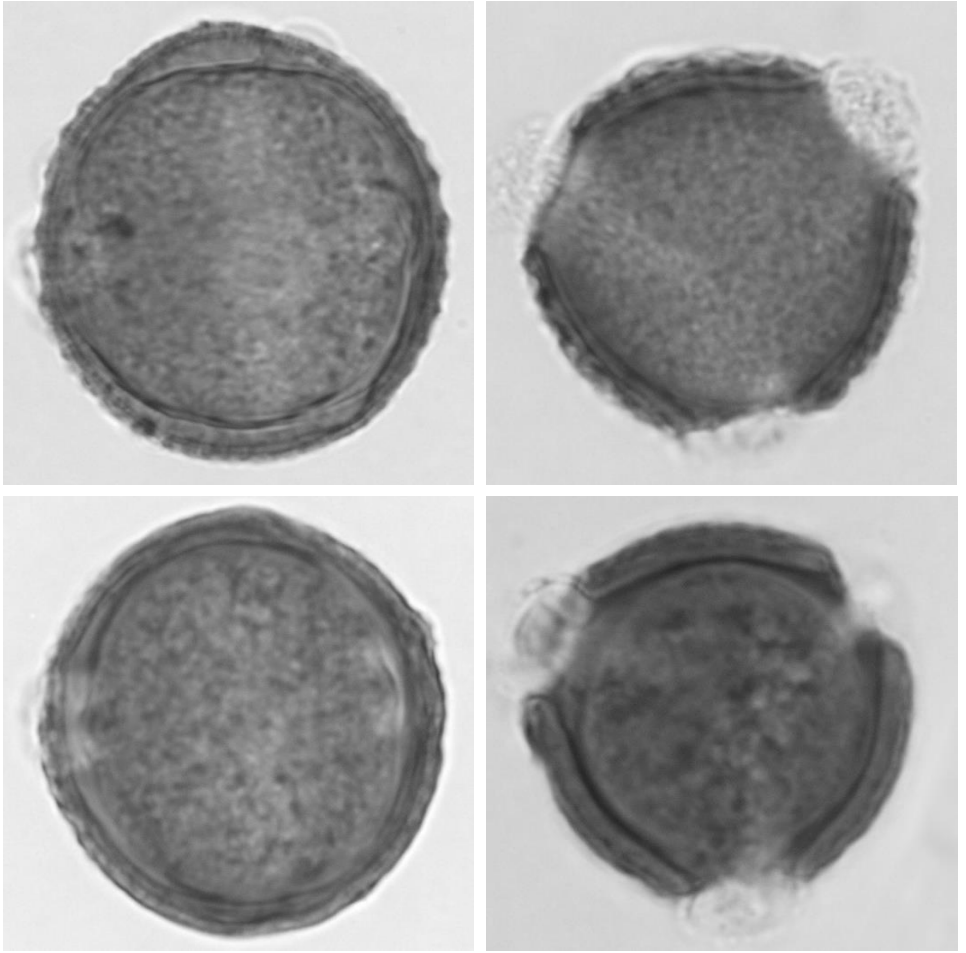


Fig. 6. Pollen. *C. solstitialis* ssp. *carneola* (a,b); *C. calcitrapa* ssp. *cilicica* (c,d), (x1000).

There are many secretory cells in surface sections of leaves. Kaya (1987) mentioned that *Centaurea* has schizogen secretory canals. Some structures was seen at *C. squarrosa* by İnceoğlu (1973) was explained that leaves have gland cells and multicellular hairs with long whip-like terminal cell were seen. According to Metcalfe & Chalk (1950) the occurrence of medullary and cortical bundles are of considerable taxonomic value; the petiole provided with leaf-like wings in *C. calcitrapa* ssp. *cilicica* subspecies. *Grindelia* genus has acicular and prismatic crystals in the stem pith, medullar and vascular bundles present in species of *Centaurea* as our subspecies. In *Senecio* stem, the outer part of the vascular bundles, broad fleshy cortex consist of palisade chlorenchyma as our subspecies.

3. The result of palynological studies showed that the pollen grains of both specimens have been identified as tricolporatae and the pollen shapes of both specimens are spheroide. The exine of *C. calcitrapa* ssp. *cilicica* is thinner than other subspecies. The pollen lenght of *C. solstitialis* ssp. *carneola* is longer than other subspecies. Besides, the spinules on exzine of *C. solstitialis* ssp. *carneola* are longer than other subspecies other characteristics of pollen grains are given in the Table 6.

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