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Micromorphological, Anatomical and Pollen Ornamentation Study on Four Desert Species of *Salvia* in Center of Iran

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Abstract: The present study tends to investigate the micromorphology, anatomy and palynology of four desert species of *Salvia* in center of Iran. For comparative micromorphology investigation, the shape of leaf, bracts and calyx were studied with SEM. To conduct the comparative study of anatomy characters, sections from stem were prepared using microtome and differential staining. In this part of investigation, arrangement of vessel and arrangement of tissues in stem were studied. For the palynology study, too, a comparative investigation on the species showed, the pollen was prolate spheroidal, hexacolpate, bireticate and semi- tectate or tectate. Finally, micromorphology study, is useful for identification of studied species.

Key words: Anatomy, Lamiaceae, palynology, pollen, *Salvia*, Iran, micromorphology

INTRODUCTION

Salvia L. from Lamiaceae Family, Stachyioideae subfamily, Salviae tribe having numerous variety of species, it is expanded from Italy to Iran, Iraq, Pakistan, Afghanistan (Parsa, 1949; Tutin, 1972; Boissier, 1975; Davis, 1982; Hedge, 1982, 1990). *Salvia* has 56 species in Iran about four of which are found in the deserts of center of Iran and 14 of them are endemic for Iran (Hedge, 1982). This genus has aromatic essential oils and antimicrobial effects. We collected *S. eremophila*, *S. macilenta*, *S. tebessana*, *S. santolonifolia* from their localities in Kerman and Yazd provinces. *S. eremophila* was endemic for Iran. Then micromorphologically, we studied shape of bracts, calyx and density of hairs. Also we prepared some cross section of stem of them. In terms of anatomic studies on *Salvia*, there has been a report about *S. sclerea*, *S. trichoclada* and *S. napiflora* (Ozdemir and Senel, 1999; Baran and Ozdemir, 2006). Another report for anatomy study of *Salvia* explained by Metcalf and Chalk (1983). In palynological studies, the comparison were made among the pollen grains of *Salvia*. In this part of study, pollen grains were extracted and acetolysed, to study shape and ornamentation of the pollen through SEM and LM. The previous palynology investigation had been done in *Salvia*, *Origanum* and *Lycopus* (Moon and Hong, 2003; Akyalcin, 2003). But, comparative palynology of studied species carried out for first time in Iran. The purpose of present study was to investigate variation of internal structure and identification of *Salvia* species on the basis of micromorphology because morphologically identification of this species is difficult.

MATERIALS AND METHODS

For micromorphological study, bracts, calyx and stem observed with SEM. As for the anatomic study, the examined species were collected from the localities in desert of center of Iran (Kerman and Yazd provinces) during May-June 2005-2006 (Table 1). For preparing of cross section of stem, the base of stem were selected from 8-9 specimens. The fresh specimens were fixed in FAA then, dehydrated with ethanol and later, some slices prepared with microtome. The section- 12 micron thick-stained with Safranine and Fast-green (Johnson, 1940; Chamberlain, 1990).

In the palynological study, the pollen was extracted from the anther and dehydrated by glacial acetic acid, then acetolysed, coated with sputter finally studied by LM Olympus and SEM LEO1450VP (Erdtman, 1971;

Table 1: The localities of studied *Salvia* species

Species	Localities
<i>S. santolonifolia</i>	Kerman, Jiroft to esfandegheh, 1000 m, Saber and Ghonchehee, 355; Jiroft, Delfard, 1600 m, Nikian and Amandadi, 27181; Zahedan to Bam road, 470 m, Khodashenas and Saber, 5835.
<i>S. macilenta</i>	Bam to Jiroft, Jabalbaz mountain, Khodashenas and Saber, 3696; Tabas, Niestan, 1100 m, Zangooi and Joharchi, 24862.
<i>S. eremophila</i>	Kahnuj, Deghmil, 650 m, Saber and Khodashenas, 731; Tabas, Naibandan, between Aliabad and Ab Band, 1519 m, Joharchi and Zangooi, 35273;
<i>S. tebessana</i>	Tabas, Niestan, 1100 m, Joharchi and Zangooi, 4020; Tabas, Naibandan, 1510 m, Joharchi and Zangooi, 26460; Tabas, Ali abd, 1519 m, Joharchi and Zangooi, 29846; Tabas, Zoghal sang to Parvadeh, 1500 m, Joharchi and Zangooi, 24860.

Moore *et al.*, 1991). The pollen terminology was adapted from Punt *et al.* (1994).

RESULTS AND DISCUSSION

Micromorphological study: The results of stems hairs showed dense, long hairs (e.g., *S. eremophila* and *S. santolinifolia*) and lax, short hairs (e.g., *S. macilenta* and *S. tebessana*) (Fig. 1a, b). The shape of leaves were obovate-oblong (e.g., *S. santolinifolia*), obovate (e.g., *S. macilenta*), triangular (e.g., *S. tebessana*) and obovate-oblong and retuse (*S. eremophila*) (Fig. 2a-d). The stems hairs of *S. eremophila* was dichotomous (Fig. 3). The shape of bracts were lanceolate- oblong with dense and long hairs (e.g., *S. tebessana*), rhomboid- obovate with and lax, short hairs (e.g., *S. santolinifolia*), rhomboid with long hairs (e.g., *S. eremophila*) and obovate, retuse with lax, short hairs (*S. macilenta*).

(Fig. 4a-d). The shape of calyx was tubular- campanulate except *S. eremophila* which was campanulate (Fig. 5a, b, Table 2).

Anatomic results: The results from the anatomic studies demonstrated the stem with following tissues:

- Epidermis layers with rectangular and ellipsoid cells.
- Three to five layers of spongy parenchymatous under the epiderm
- Some angular collenchymatous layers
- Some sclerenchymatous layers between vascular bundles

The arrangement of vessel was variable for example solitary in *S. eremophila* and *S. tebessana*, radial chain pore in *S. santolinifolia* and *S. macilenta*. Reticulate and pitted thickening vessel were observed in *S. tebessana* and *S. eremophila* and spiral in *S. santolinifolia* (Fig. 6, 7a, b).

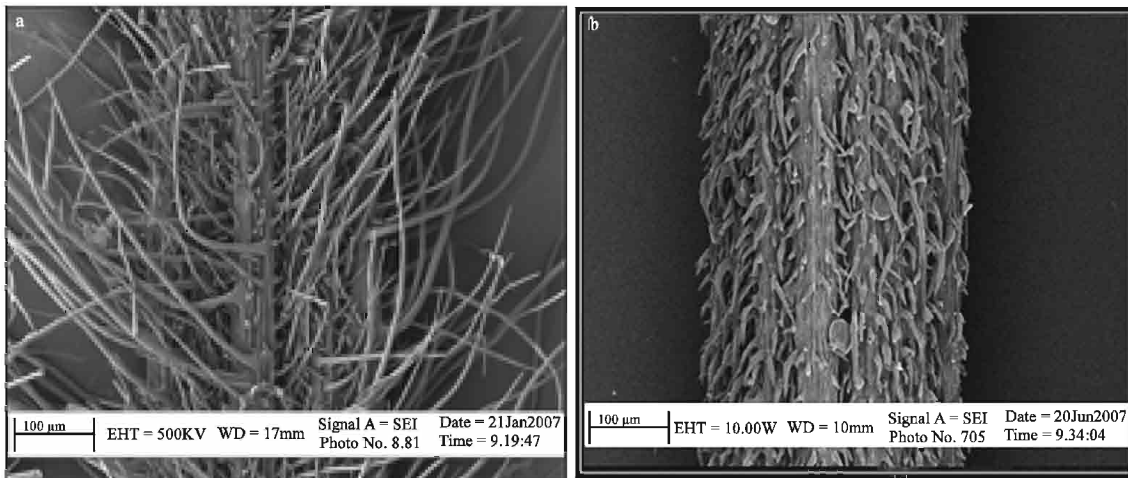


Fig. 1: Electromicrograph of stems hairs (a) *S. eremophila* and (b) *S. macilenta*

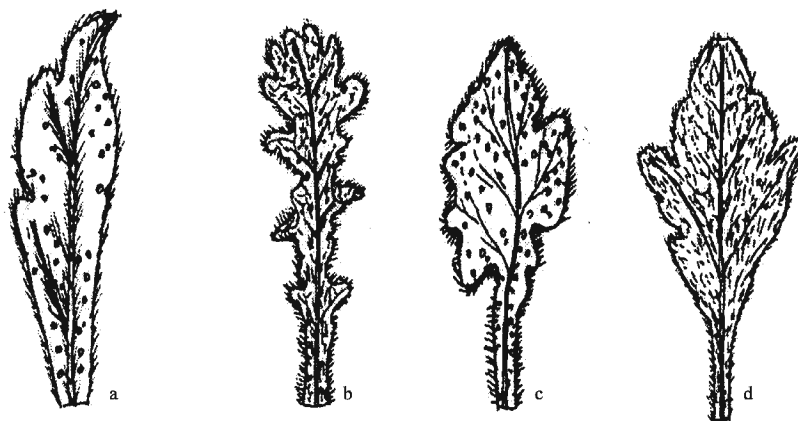


Fig. 2: Leaf shape (a), *S. macilenta*, (b) *S. santolinifolia*, (c) *S. eremophila* and (d) *S. tebessana*

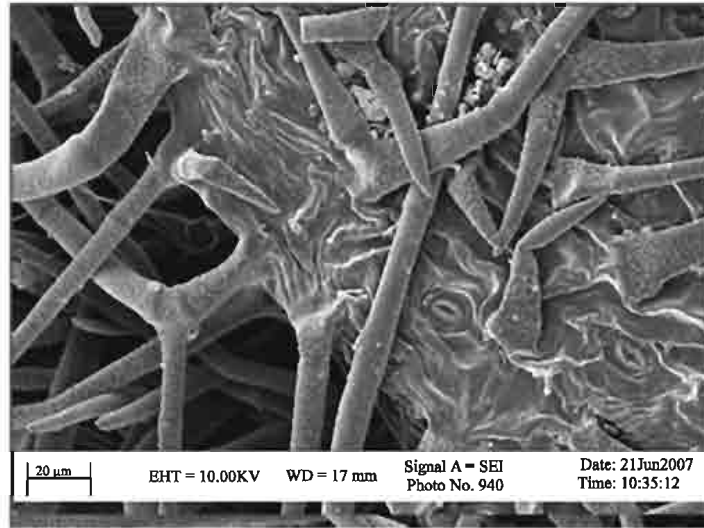


Fig. 3: Dichotomous hairs on the leaf of *S. macilenta*

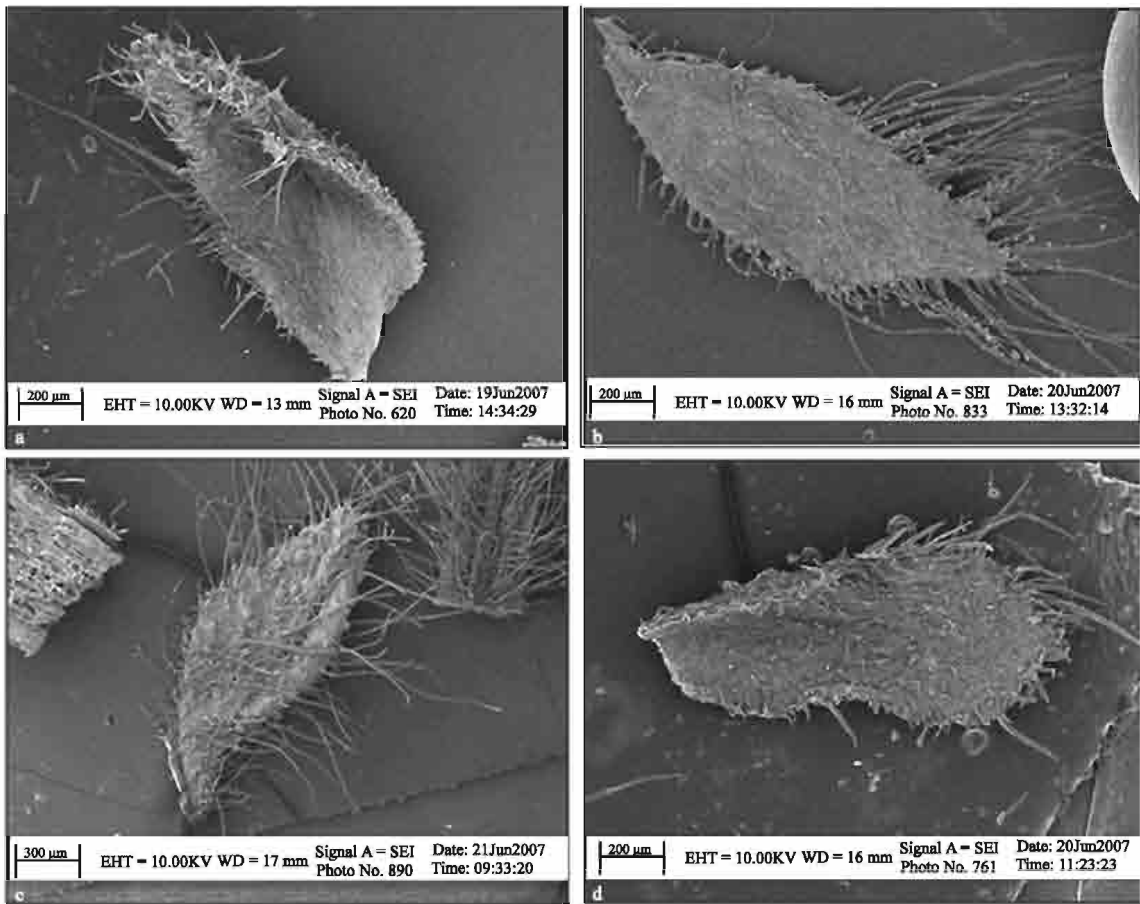


Fig. 4: Electromicrograph of bracts shape (a) *S. macilenta*, (b) *S. santolinifolia*, (c) *S. eremophila* and (d) *S. tebossana*

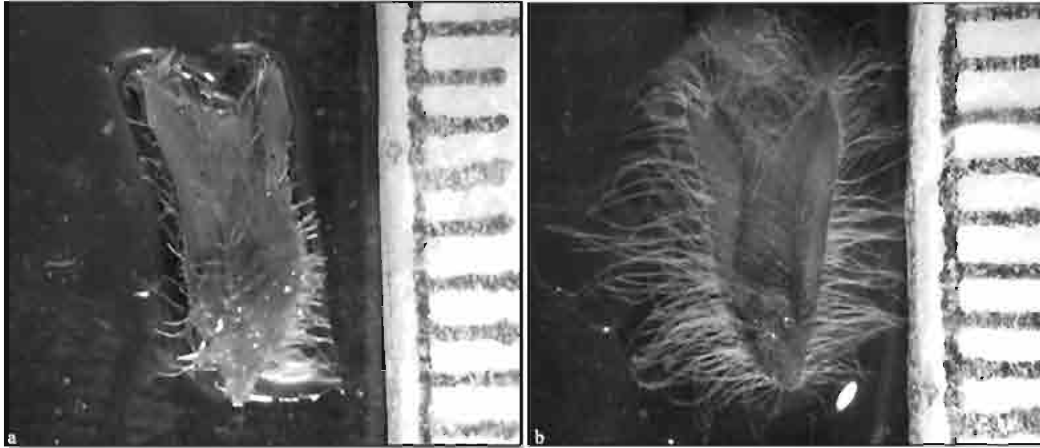


Fig. 5: Calyx shape (a) campanulate in *S. eremophila* and (b) tubular-campanulate in *S. tebessana*

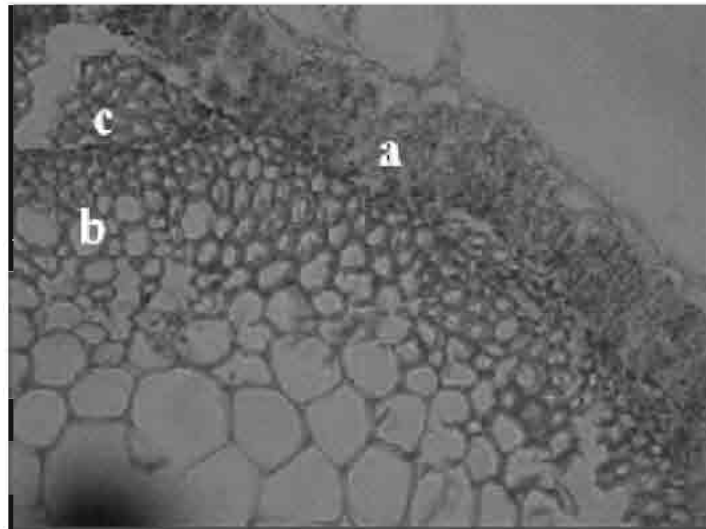


Fig. 6: Cross section of *S. santolinifolia* stem (a) spongy parenchyma, (b) radial chain pore vessel and (c) angular collenchyma

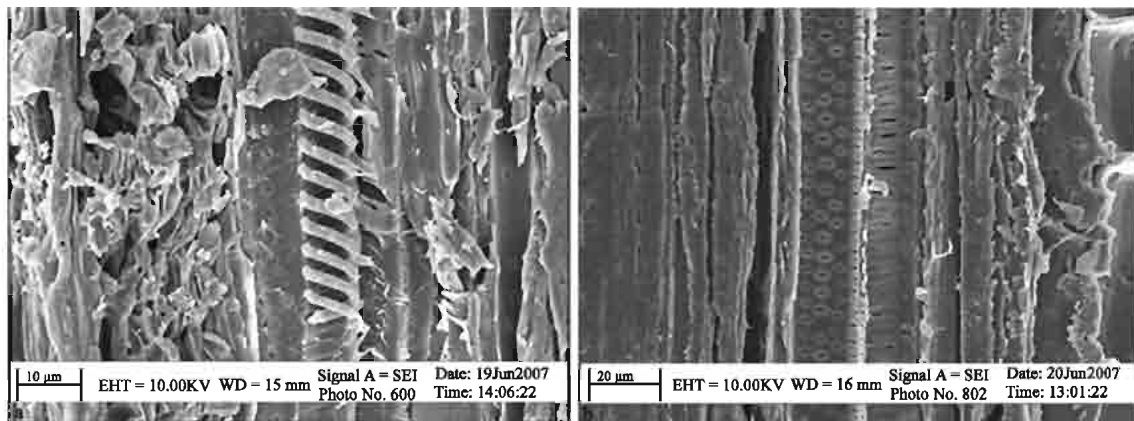


Fig. 7: Electromicrograph of (a) spiral vessel in *S. santolinifolia* pitted and (b) reticulate vessel in *S. eremophiolo*

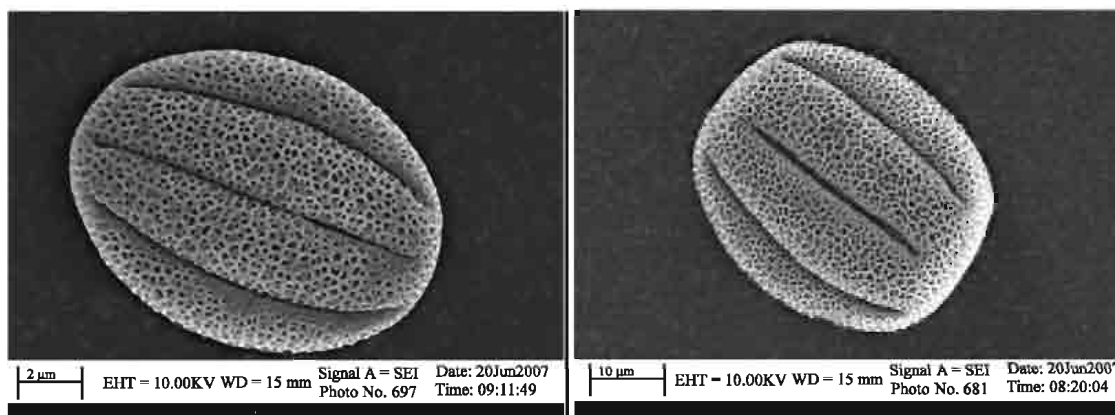


Fig. 8: Electromicrograph of pollen (a) *S. eremophila* and (b) *S. macilenta*

Table 2: The morphology and micromorphology characters of studied *Salvia* species

Species	The arrangement of vessel	Stems hairs	Leaf shape	Calyx shape	Pollen shape
<i>S. macilenta</i>	Radial chain pore	Dense, long	Broad obovate, acute with lax hairs	Campanulate- tubular	Rectangular
<i>S. santolinifolia</i>	Radial chain pore	Dense, long	Lanceolate- obovate with dense hairs	Campanulate- tubular	Ellipsodic
<i>S. eremophila</i>	Solitary	Lax, short	Rhomboid with dense hairs	Campanulate	Ellipsodic
<i>S. tebessana</i>	Solitary	Lax, short	Oblong- obovate, obtuse with lax hairs	Campanulate- tubular	Ellipsodic

Palynology analysis: The pollen was prolate, elipsodic, bireticolate, hexacolpate and semi- tectate. Only *S. macilenta* had rectangular pollen (Fig. 8a, b, Table 2).

In present research to identifying species of *Salvia* used floral segments micromorphology study for first time. The studied species were similar as anatomy structure. Metcalf and Chalk (1983) carried out Labiatae anatomy structure. Also, anatomical study on *S. sclerea*, *S. trichoclada* and *S. napiflora* showed sclerenchymatous tissue above the phloem and around the vascular bundles (Ozdemir and Senel, 1999; Baran and Ozdemir, 2006). The results of palynology study on studied species showed similar structure. The other reports about *Salvia* sect. *Audiertia*, *Hymenocrater* and *Lycopus* showed similar pollen shape with studied species (Emboden, 1965; Moon and Hong, 2003). Finally we recommend using micromorphological study of floral and leaves segments help to identify exactly.

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