



Morphology, Palynology, Nutlet and Seed micromorphology of reported *Salvia viridis* (Lamiaceae) in Libya

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Article info

Received: 10/01/2021

Revised: 28/01/2021

Accepted: 27/02/2021

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Abstract

Salvia viridis L. was first collected from (Telmayta) Libya. The species is an annual herb usually distributed at the field road sides. Morphological characteristics of leaves, calyxes, corollas and types of stamens are useful for sectional and specific delimitation in *Salvia*. In this study, micromorphological characteristics of the pollen and the nutlet of this species have been investigated using Scanning Electron Microscopy (SEM). The pollen grains are hexocolpate, radially symmetrical, isopolar and suboblate. Their exine sculpturing is bireticulate. In addition, size, shape and ornamentation of nutlets and seeds are diagnostic.

Key words: *Salvia viridis*; Lamiaceae; morphology; palynology; nutlet; seed; Libya.

Introduction

The genus *Salvia* L. from Lamiaceae is one of the largest genera in this family (Wang 2013; Cvetkovikj et al. 2015). The plant name *Salvia* (sage) comes from the Latin word salvare, which means healer (Topcu et al. 2013). The genus *Salvia* L. belongs to the Mentheae tribe within the Nepetoideae subfamily (Kharazian 2014). It includes around 1000 species that have almost cosmopolitan distribution (Walker and Sytsma, 2007; Salehi et al. 2014; Saravia et al. 2018); In Libya, it is represented by 10 species; out of which 3 are cultivated (Jafri 1985). Some of these species are annual, perennial, herbaceous, suffruticose, fruticose and subshrubby (Kharazian 2014). The main speciation centers of these taxa are considered to be the eastern Mediterranean region; the southwestern, western, eastern and central regions of Asia; Southern Africa; and Central and South America (Esra et al. 2011; Kahraman et al. 2010; Kharazian 2014). Saravia et al. 2018).

Numerous species of the *Salvia* genus are economically important since they are used as spices and flavouring agents in the field of perfumery and cosmetics (Wang, 2013); and some species of *Salvia* have been cultivated worldwide for use in folk medicines (Tohamy et al. 2012).

Salvia species are used in traditional medicines all around the world, possessing antioxidant, anti-diabetic, antibacterial, antitumor and anti-inflammatory features. Annual, with white hairs and sessile glands, sometimes scabrous or glabrescent.

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Stem erect, simple or branched from base or above. Leaves 1.2-4.2 cm in length while, it 0.5-2 cm in width, ovate to oblong, obtuse, crenate, crenate or rounded to cordate at base; petioles length of both basal and upper leaves 0.3-4 cm with short hairs; floral leaves bract-like, sessile, ovate, broad, acute, about as long as calyx or longer; terminal leaves sterile, violet, membranous, elliptic to obovate, or with spikes devoid of tuft of coloured sterile floral leaves, Verticillasters 4-6 flowered, generally remote. Calyx 6-8 mm, corolla 10-14 mm, purplish-pink, rarely white (Odeh 2014).

Annual or biennal herb, 20-50 cm tall, erect simple or branched, with short to long eglandular hairs intermixed with capitate glandular hairs. Leaves 5×2.5 cm, petiolate, ovate or oblong, with cordate or rounded base, obtuse, regularly crenate, covered with short and eglandular hairs on both sides. Verticils 4-8 flowered, with or without bracts, lowermost 1-7 cm apart; bracts linear, up to 15×0.5 mm. calyx tubular 7 mm, accrescent in fruit, up to 10 mm with 13 nerves; upper lip with two, 1.5 mm lateral and cusp like median teeth; lower lip with 2, acuminate, 3 mm teeth. Corolla pink or violet, 14-18 mm. Nutlets oblong-trigonous, 3×1.5 mm, pale orange brown (Jafri 1985).

The genus *Salvia* was studied by several investigators (Yildiz et al., 2009; Doagey et al., 2018) who reported the pollen characters of species were useful for their identification. (Celenk et al. 2008). Kahraman and Doghan (2010) and Al-Watban et al. 2015 reported that the pollen size, shape and exine ornamentation in the genus *Salvia* are important in distinguishing between the species. In general, the shape of pollen grains is specific to the taxonomic ranks, such as family, genus and species (Myoung & Yuon; 2012). Studies on nutlet micromorphology within Lamiaceae showed that nutlets features e.g. shape and surface sculpturing, were potentially useful at different taxonomic level (Moon et al., 2009; Khosroshahi & Salmaki, 2018). Seed surface micromorphology was found to have a systematic value at the generic and specific levels (Marinet al. 1996, Hedge 1970).

The studied plant reported from El Merj as *S. horminum* L. var. *viridis* (L.) Briquet by Keith(1.c.) (Jafri et al. 1985). The macro-

micromorphological and palynological properties of the *Salvia* species found in Libya have been poorly studied. Therefore, the main objectives of the present study are to provide a detailed account of the properties of for this species, using LM and SEM.

Material and Methods

Plant material were collected from one location only in Libya (Telmayta), it is distributed at the field road sides. specimens collected from Libya between February and March, 2020, located in Telmaita district. Samples were fixed in FAA and kept in alcohol 70% for morphological and palynological studies. Pollen was sampled from the flowers at the beginning of anthesis. Pollen grains were compared in terms of their morphological characters by determining their size, shape, and exine sculpture. Transverse section preparations of leaves, petioles and stems were prepared manually. Size measurements for the pollen grains were taken according to Erdtman (1971).

Results and Discussion

Annual herbs, 8.5-32.5 cm long. Stem 1-15.5 cm, erect, simple, much branched below or upper and unbranched, quadrangular, solid, glandular and eglandular hairy, retrorse (Fig.1.A&B) Leaves simple, petiolate but upper leaves sessile, exstipulate, decussate opposite, ovate or oblong or elliptic, attenuate to rounded at base, retuse to obtuse, regularly crenate, with little short eglandular hairs on both sides and oil globules on lower surface, 4-5.5×1.7-3 cm, petiole 3-4.6 cm; rosette leaves 3-4×1.4-3 cm, petiole 2.7-5 cm; sessile leaves 3-5×1.5-2.6 cm. Verticils up to 11; 2-6 flowered; Flowers are at the base of bracts. Peduncle 8-30 cm. Bracts 12-21 × 8 -15 mm, sessile to sub-petiolate, acuminate-acute, dentate-crenate, ovate at base; upper violet-lower green, with eglandular and glandular hairs on both sides and oil globules on lower surface (Fig.2. D&E) Calyx tubular, 12×3-4 mm, accrescent in fruit, 8-10×2.5-3 mm in flowering, with 13 nerves; upper lip with two, c.1 mm lateral and cusp like median teeth; lower lip with 2, acuminate, 4-4.5 mm teeth. Pedicels erect or suberect, 3-5 mm in fruit; 2.5-3 in flowering. Corolla 11-12 mm, pink or violet, with hairs and oil globules (Fig.2 D&E). Anther 2×0.25 mm; pollen yellow, longitudinal, versatile, diandrous, filament 4-4.5 mm with hairs. Style 10

mm; stigma 1.5 mm, bifurcate, pink or violet (Figure 1).

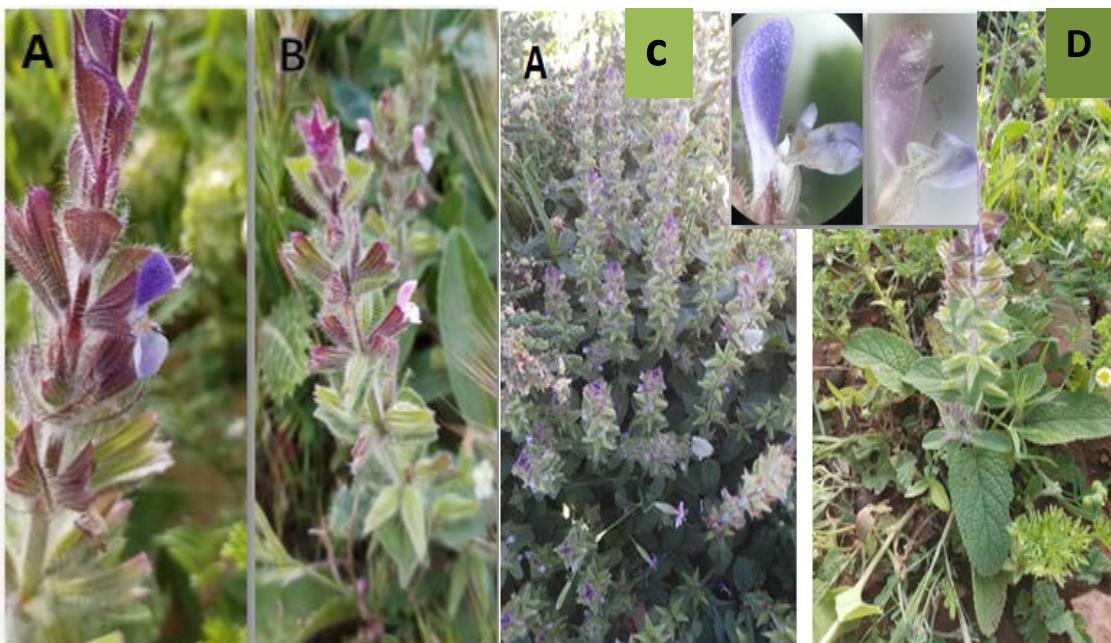


Figure 1: Whole plant of *Salvia viridis* L.: A) Branched plant; B) Unbranched plant; C) Show inflorescence with violet flowers; D) Show inflorescence with pink flowers

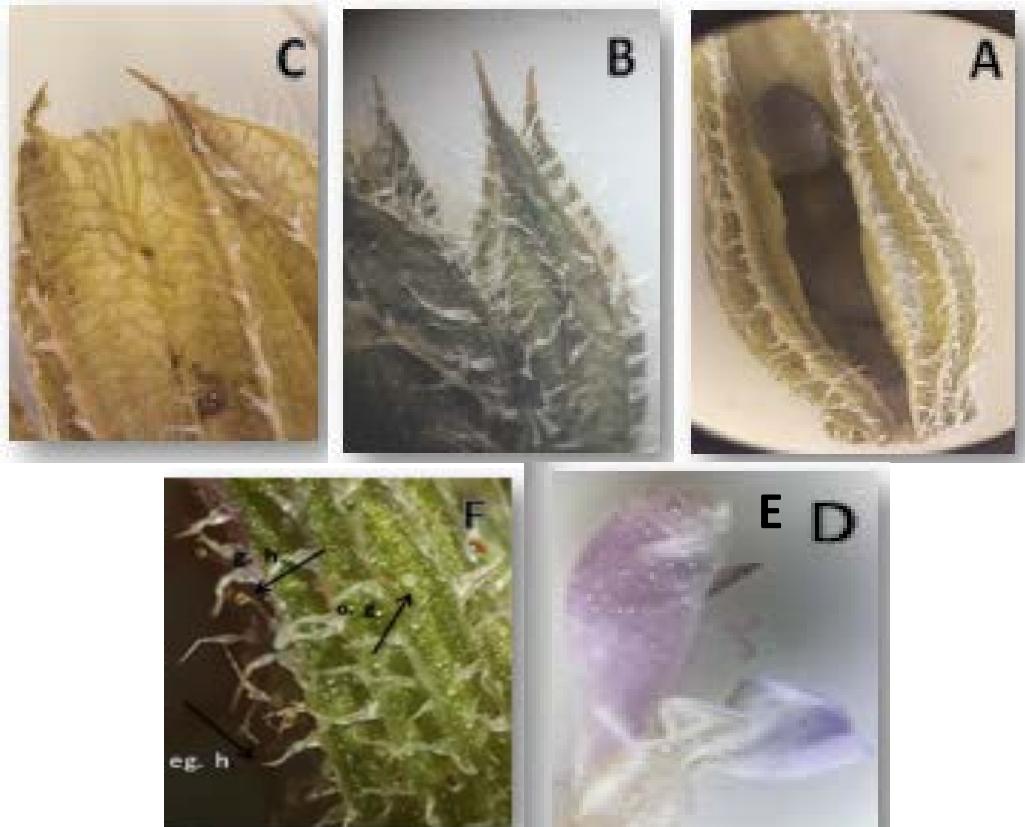


Figure 2: A) Mature Nutlet; B: lower lip with two teeth; C: upper lip with two lateral and cusp like median teeth; D: Oil globules on the corolla; E: showing glandular, eglandular hairs and oil globules on the calyx

Pollen Morphology

The pollens of *S. viridis* are monad, suboblate shape and 6-zonocolpate. Polar axis (P) is 29 μ m, equatorial axis (E) 37.6 μ m, and P/E rate 0.77. The ornamentation is bireticulate type of exine sculpturing, with 1-2 large central secondary lumina per primary lumen.

Nutlets and Seed Morphology

Nutlets length 3-3.5 mm, width 2 mm, oblong shape and brown color. Nutlets surface ornamentation are regular prominences in chain form with tangled strands on the surface. The seed 3x1.5 mm, obovate shape with acute apex and dark brown color, reticulate, Anticlinal wall narrow and depress.

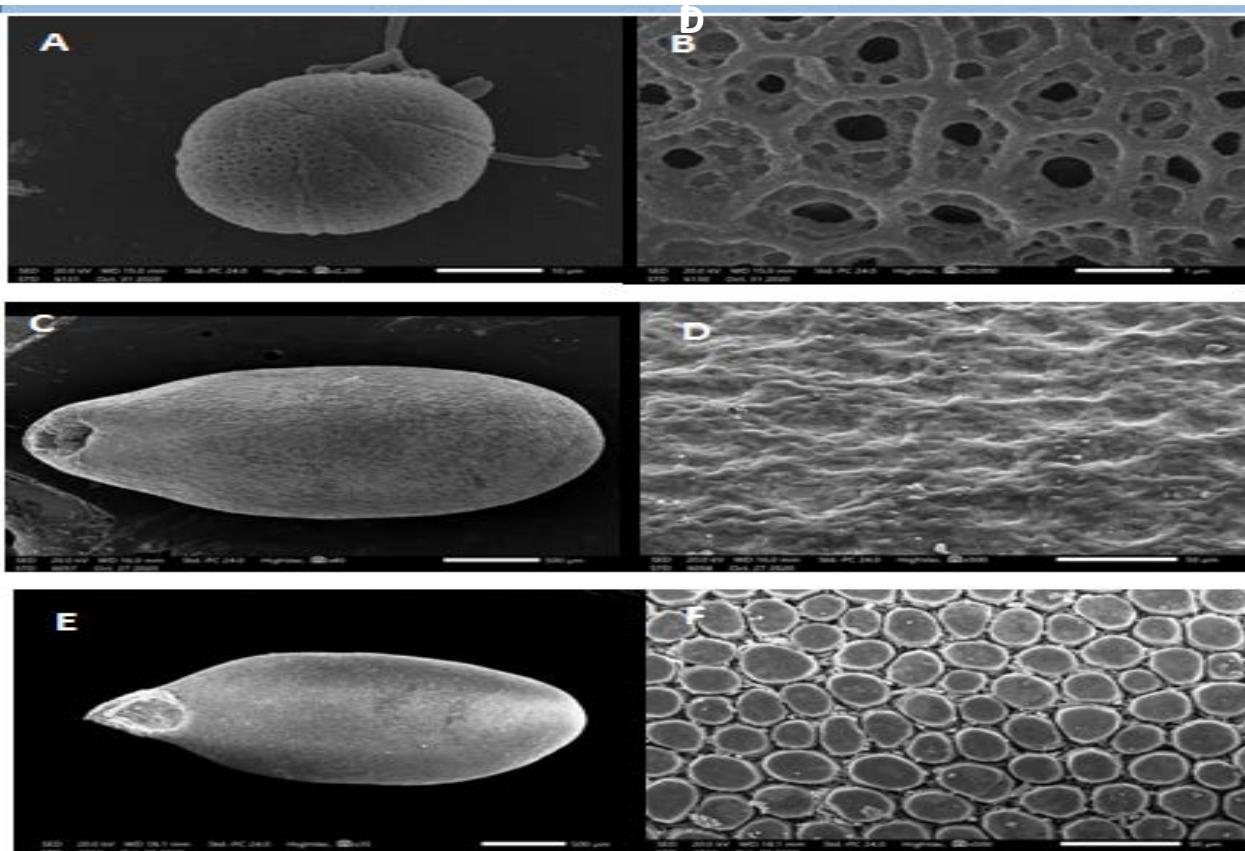


Figure 3: Scanning electron micrographs of *Salvia viridis* : A-Pollen grain shape; B- Pollen grain ornamentation; C- Nutlet shape; D- Nutlet surface ornamentation ; E-Seed shape; F- Seed ornamentation

Morphological characteristics such as leaf size and corolla characteristics are taxonomically significant to identify the species. *S. viridis* morphologically differs from the other members of *Salvia* in terms of plant length, leaves, bracts and corolla. Although the present results usually correspond with the description recorded in the Flora of Libya (Jafri, 1985) several differences were found here. It was reported that the leaf was to 5 x 2.5 cm, the number of flowers in verticillaris was 4-8, the bract was c. 15 x 0.5 mm, the corolla was 14-18 mm, the nutlet was 3 x 1.5 mm in size. According to our study, the plant length was 8.5-

3.5 cm, the bract was 12-21 x 8-15 mm, the corolla was 11-12 mm, the petiole was 3-4 cm, the pedicel 3-5 mm was in length and number of verticillasters was 2-6 flowered, the nutlet was 3.5-4.5 x 3-3.5 mm in size. We also measured other morphological characters of the species. Research findings reveal that the morphological characteristics of *S. viridis* provide some additional information to those data reported in Flora of Libya. Cantino et al. (1992) revised the classification of all genera in Labiateae and placed *Salvia* within the subfamily Nepetoideae as the genus *Salvia* has hexa colpate pollen grains. The

pollens of *S. viridis* are hexocolpate, radially symmetrical and isopolar. Its shape is suboblate. The ornamentation is bireticulate type of exine sculpturing, with 1–2 large central secondary lumina per primary lumen. The shape of the pollen and the sculpturing of the exine in the genus *Salvia* may be significant in separating the species (Kahraman, et al. 2010). Nutlets length 3–3.5 mm, width 2 mm, oblong shape and brown color. Nutlets surface ornamentation are regular prominences in chain form with tangled strands on the surface. Kahraman et al. (2009) pointed the size, shape and ornamentation of *S. balliana*, *S. macrochlamys* and *S. hedgeana* are diagnostic. The seed 3×1.5 mm, obovate shape with acute apex and dark brown color, reticulate, Anticinal wall narrow and depress. Seed surface micromorphology was found to have asystematic value at the generic and specific levels (Marinet al. 1996, Hedge 1970).

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Cite this article as:

El Rabiai G.T. and Elfaidy K. K. (2021). Morphology, Palynology, Nutlet and Seed micromorphology of reported *Salvia viridis* (Lamiaceae) in Libya, *Int. J. of Pharm. & Life Sci.*, 12(2): 11-16.

Source of Support: Nil

Conflict of Interest: Not declared

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