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# MORPHOLOGY, PALYNOLOGY, NUTLET AND SEED MICROMORPHOLOGY OF REPORTED SALVIA VIRIDIS (LAMIACEAE) IN LIBYA

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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 hexacolpate, radially symmetrical, isopolar and suboblate. Their exine sculpturing is bireticulate. In addition, size, shape and ornamentation of nutlets and seeds are diagnostic.

#### **KEYWORDS**

Salvia virids, Lamiaceae, Morphology, Palynology, Nutlet, Seed and Libya.

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### INTRODUCTON

The genus Salvia L. from Lamiaceae is one of the largest genera in this family (Wang 2013<sup>1</sup>, Cvetkovikj et al, 2015)<sup>2</sup>. The plant name Salvia (sage) comes from the Latin word salvare, which means healer (Topcu et al, 2013)<sup>3</sup>. The genus Salvia L. belongs to the Mentheae tribe within the Nepetoideae subfamily (Kharazian 2014)<sup>4</sup>. Includes around 1000 species that have almost cosmopolitan distribution (Walker and Sytsma, 2007<sup>5</sup>, Salehi et al, 2014<sup>6</sup>, Saravia et al, 2018)<sup>7</sup>; In Libya, it is represented by 10 species; out of which 3 are cultivated (Jafri 1985)<sup>8</sup>. Some of these species are annual, perennial, herbaceous, suffruticose, fruticose and subshrubby (Kharazian 2014)<sup>4</sup>. The main speciation centers of these taxa are considered to be the eastern Mediterranean region; the southwestern,

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western, eastern and central regions of Asia; Southern Africa and Central and South America (Esra *et al*, 2011<sup>9</sup>, Kahraman *et al*, 2010<sup>10</sup>, Kharazian 2014<sup>4</sup>). Saravia *et al*, 2018)<sup>7</sup>. 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)<sup>1</sup> and some species of *Salvia* have been cultivated worldwide for use in folk medicines (Tohamy *et al*, 2012)<sup>11</sup>.

Salvia species are used in traditional medicines all around the world, possessing antioxidant, antiantibacterial, antitumor diabetic. and antiinflammatory features. Annual, with white hairs and sessile glands, sometimes scabrous or glabrescent. Stem erect, simple or branched from base or above. Leaves 1.2-4.2cm in length while, it 0.5-2cm in width, ovate to oblong, obtuse, crenate, crenate or rounded to cordate at base; petioles length of both basal and upper leaves 0.3-4cm 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 6-8mm, corolla 10-14 mm, purplishpink, rarely white  $(Odeh 2014)^{12}$ .

Annual or biennal herb, 20-50cm tall, erect simple or branched, with short to long eglandular hairs intermixed with capitate glandular hairs. Leaves  $5\times2.5$ 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 1-7cm apart; bracts linear, up to  $15\times0.5$ mm. calyx tubular 7mm, accrescent in fruit, up to 10mm with 13 nerves; upper lip with two, 1.5mm lateral and cusp like median teeth; lower lip with 2, acuminate, 3mm teeth. Corolla pink or violet, 14-18mm. Nutlets oblong-trigonous,  $3\times1.5$ mm, pale orange brown (Jafri 1985)<sup>8</sup>.

The genus Salvia was studied by several investigators (Yildiz *et al*, 2009, Doaigey *et al*, 2018<sup>13</sup>) who reported the pollen characters of species were useful for their identification. (Celenk *et al*, 2008)<sup>14</sup>. Kahraman and Doghan (2010)<sup>10</sup> and Al-Watban *et al*, 2015<sup>15</sup> reported that the pollen size, shape and exine ornamentation in the genus Salvia

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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 and 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<sup>16</sup>, Khosroshahi and Salmaki, 2018<sup>17</sup>). Seed surface micromorphology was found to have a systematic value at the generic and specific levels (Marin *et al*, 1996<sup>18</sup>, Hedge 1970<sup>19</sup>).

The studied plant reported from El Merj as *S.horminum* L. var. *viridis* (L.) Briquet by Keith (1.c.) (Jafri *et al*, 1985)<sup>8</sup>. The macromicromorphological 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 were prepared manually. and stems Size measurements for the pollen grains were taken according to Erdtman (1971).

### **RESULTS AND DISCUSSION**

Annual herbs, 8.5-32.5cm long. Stem 1-15.5cm, erect, simple, much branched below or upper and unbranched, quadrangular, solid, glandular and eglandular hairy, retrorse (Figure No.1.A and 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- $5.5 \times 1.7$ -3cm, petiole 3-4.6cm; rosette leaves 3- $4 \times 1.4$ -3cm, petiole 2.7-5cm; sessile leaves 3- $5 \times 1.5$ -

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2.6cm. Verticils up to 11; 2-6 flowered; Flowers are at the base of bracts. Peduncle 8-30cm. Bracts 12-21  $\times 8$  -15mm, 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 (Figure No.2. D and E) Calyx tubular, 12×3-4mm, accrescent in fruit, 8-10×2.5-3mm in flowering, with 13 nerves; upper lip with two, c.1mm lateral and cusp like median teeth; lower lip with 2, acuminate, 4-4.5mm teeth. Pedicels erect or suberect, 3-5mm in fruit; 2.5-3 in flowering. Corolla 11-12mm, pink or violet, with hairs and oil globules (Figure No.2 D and E). Another 2×0.25mm; pollen yellow, longitudinal, versatile, diandrous, filament 4-4.5mm with hairs. Style 10mm; stigma 1.5mm, bifurcate, pink or violet (Figure No.1).

### **Pollen Morphology**

The pollens of *S. viridis* are monad, suboblate shape and 6-zonocolpate. Polar axis (P) is  $29\mu$ m, equatorial axis (E) 37.6 $\mu$ 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 3-3.5mm, width 2mm, oblong shape and brown color. Nutlets surface ornamentation are regular prominences in chain form with tangled strands on the surface. The seed  $3 \times 1.5$ mm, obovate shape with acute apex and dark brown color, reticulate, Anticlinal wall narrow and depress.

# Discussion

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)<sup>8</sup> several differences were found here. It was reported that the leaf was to 5 x 2.5cm, the number of flowers in verticilis was 4-8, the bract was c. 15 x 0.5mm, the corolla was 14-18mm and the nutlet was 3 x 1.5mm in size. According to our study, the plant length was 8.5-32.5cm, the bract was 12-21 x 8-15mm, the corolla was 11-12mm, the petiole was 3-4cm, the pedicel 3-5mm was in length and number of

verticillasters was 2-6 flowered, the nutlet was 3.5-4.5 x 3-3.5mm 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,  $(1992)^{20}$  revised the classification of all genera in Labiatae and placed Salvia within the subfamily Nepetoideae as the genus Salvia has hexa colpate pollen grains. The pollens of S. viridis are hexacolpate, 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)<sup>10</sup>. Nutlets length 3-3.5mm, width 2mm, oblong shape and brown color. ornamentation Nutlets surface are regular prominences in chain form with tangled strands on the surface. Kahraman *et al*,  $(2009)^{21}$  pointed the size, shape and ornamentation of S. ballsiana, S. macrochlamys and S. hedgeanaare diagnostic.

The seed  $3 \times 1.5$ mm, obovate shape with acute apex and dark brown color, reticulate, Anticlinal wall narrow and depress. Seed surface micromorphology was found to have asystematic value at the generic and specific levels (Marin *et al*, 1996<sup>18</sup>, Hedge 1970<sup>19</sup>).



Figure No.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 No.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



Figure No.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

#### CONCLUSION

This study will be convenient for other investigations about this species, because it is the first morphological, palynological and micromorphological study of nutlets and seeds of this taxon in Libya. We concluded that it is important to recommend anatomical and chemical studies about the studied taxon and other related species to show the differences and its importance.

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### **CONFLICT OF INTEREST**

We declare that we have no conflict of interest.

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