NORDIC JOURNAL OF
BOTANY

Research

Nutlet micromorphology of Iranian *Nepeta* (Lamiaceae) species

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Nordic Journal of Botany
2019: e02441
doi: 10.1111/njb.02441

Suject Editor: Emma Ortunez
Editor-in-Chief: Torbjörn Tyler
Accepted 27 June 2019

To evaluate nutlets characteristics for systematic relationships, a comprehensive morphological and micro-morphological study of the nutlets of 16 Iranian taxa of *Nepeta* (Lamiaceae) was conducted using scanning electron microscopy (SEM). Differences in surface ornamentation, size, shape and color were observed between the species. The studied taxa were categorized in two basic types based on surface ornamentation: non-sculptured (smooth-type) and sculptured nutlets. The non-sculptured nutlets type could be further divided into four sub-types, including smooth, granulate, undulate-cellular and reticulate. In the sculptured nutlets, three subtypes of tuberculate, tuberculate-cellular and verrucate were recognized. The shape of nutlets were described as oblong, ovoid-oblong, oblong-linear, to elliptic-oblong and their size range are 1.2–2.0 mm in length and 0.5–2.0 mm in width. Based on micrographs, the areole form and location were defined as bi-lobed, straight, basal, sub-basal or lateral. Nutlet micromorphological characteristics such as surface ornamentation can be useful for classification and identification of e.g. medicinal species of *Nepeta* in Iran.

Keywords: Lamiaceae, *Nepeta*, sculpture, SEM, surface ornamentation, systematics

Introduction

Mentheae is an economically important tribe in the family Lamiaceae with several ornamental and medicinal plants (Moon et al. 2009). *Nepeta* L. is one of the largest genera of the tribe Mentheae and subfamily Nepetoideae (Harley et al. 2004). It comprises about 300 species which are often perennial herbs, rarely annual plants (Jamzad 2012).

Generally, *Nepeta* is distributed in the central and southern parts of Europe, Asia and the Middle East (Pojarkova 1954, Tzakou et al. 2000, Harley et al. 2004). Iran is one of the centers of diversity in *Nepeta* with 79 species naturally occurring in different regions of the country (Jamzad 2012, Chizzola et al. 2014). Economically, most *Nepeta* species are important due to their use as medicinal and aromatic plants (Proestos et al. 2013). *Nepeta* species are characterized by nepetalactones, iridoid monoterpen, flavonoid and phenolic compounds (Formisano et al. 2011, Misic et al. 2015), which show several activities such as an antimicrobial, insecticide, larvicide, cytotoxic,

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anticarcinogen, antioxidant, anticonvulsant, analgesic, anti-inflammatory agent and antidepressant, revealing its importance in medicine and agriculture (Formisano et al. 2011, Suntar et al. 2018).

Morphologically, identification of *Nepeta* species is difficult due to frequent hybridization (Kaya and Dirmenci 2008). Morphological characteristics such as leaf, calyx and corolla shape and size vary among *Nepeta* species.

Some *Nepeta* species are morphologically very similar, such as *N. teucriifolia* subsp. *teucriifolia* and *N. saccharata*. The genus *Nepeta* has been subdivided into six groups based on flower type, inflorescence characteristics and micromorphological features such as nutlet surface ornamentation, but the latter is often not mentioned or simply ignored (Jamzad 2012). Nutlet shape, size, surface ornamentation, color, direction and form of areoles provide important diagnostic characteristics of the species. Although areoles in *Nepeta* species are generally U- or V-shaped, there are useful variations among different species in the size and form of areoles (Budantsev and Lobova 1997, Jamzad et al. 2000, 2003).

Several studies have shown that nutlet micromorphology and seed surface anatomy may be useful for taxonomic classification of flowering plants (Satil et al. 2012, Yu et al. 2012, Jin et al. 2014). Nutlet micromorphology is also important to distinguish between *Nepata* species (Budantsev and Lobova 1997, Jamzad et al. 2000, Kaya and Dirmenci 2008, Hassan and Dar 2012). In addition, micromorphological analyses of the nutlets can provide useful information for identifying which species of *Nepeta* are being sold by the pharmaceutical industry (Kahkeshani et al. 2014) and authenticating purity of seed present in markets (Van et al. 2017).

The importance of nutlet surface micromorphology using scanning electron microscopy (SEM) has been reported in several studies of the family Lamiaceae (Husain et al. 1990, Demissew and Harley 1992, Marin et al. 1996, Guerin 2005, Akgul et al. 2008, Ozkan et al. 2009, Tabassi et al. 2016) and for the genus *Nepeta* specifically (Budantsev and Lobova 1997, Jamzad et al. 2000, Rapisarda et al. 2001, Mosquero et al. 2002, Padure et al. 2005, Kaya and Dirmenci 2008, Hassan and Dar 2012, Serpooshan et al. 2014). Also, Moon et al. (2009) have shown that micromorphology can be taxonomically useful at generic and specific level in tribe Mentheae.

This study aimed to demonstrate the micromorphological variation in nutlet surface ornamentation among selected

Table 1. *Nepeta* species investigated in this study and their collection data.

Taxa	Growth form	Collection locality	Collection date and elevation (m)	Herbarium voucher No.
N. bracteata Benth.	annual	Razavi Khorassan province, northwest of Torbate Heydarieh, mountain slopes	18.08.2015 (1800)	FUMH 46201
N. ispahanica Boiss.*	annual	Kerman province, 24 km from Jiroft towards Kerman	23.08.2015 (1650)	s.n.
N. eremophila Hausskn. & Bornm. ex Bornm.*	annual	Kerman province, 80 km from Kerman towards Sirjan	11.08.2015 (1886)	ANRRCI 3001
N. mahanensis Jamzad & M. Simmonds*	annual	Kerman province, Mahan, Sekonj, at roadside	17.08.2015 (1810)	ANRRCI 6853
N. saccharata Bunge	annual	Kerman province, Darb-e Behesht, mountain slopes of Bahrasman	25.08.2015 (3323)	ANRRCI 4875
N. assurgens Hausskn. & Bornm. ex Bornm.*	perennial	Kerman province, Darb-e Behesht, mountain slopes of Bahrasman	20.09.2017 (3323)	ANRRCI 8636
N. binaloudensis Jamzad*	perennial	Razavi Khorassan province, Golmakan, Cheshmeh Sabz	15.07.2015 (2600)	FUMH 45489
N. teucriifolia Willd. subsp. *teucriifolia*	perennial	Kerman province, Rayen, Hezar mountain, up to waterfall	29.09.2016 (3831)	s.n.
N. dschuparensis Bornm.*	perennial	Kerman province, Darb-e Behesht towards Babgorgi	20.09.2017 (2935)	ANRRCI 5373
N. glomerulosa Boiss. subsp. *glomerulosa*	perennial	Kerman province, Bardsir, Ghaleh-Askar	26.09.2015 (2749)	s.n.
N. glomerulosa Boiss. subsp. *carmanica* (Bornm.) Rech.f.*	perennial	Kerman province, Darb-e Behesht towards Babgorgi	27.09.2017 (2935)	ANRRCI 7690
N. kotschyi Boiss. var. *persica* (Boiss.) Jamzad*	perennial	Kerman province, 47 km from Darb-e Behesht towards Jiroft, mountain slopes	28.09.2017 (2350)	ANRRCI 7838
N. racemosa Lam. var. *crassifolia* (Boiss. & Buhse) A.L.Budantzev*	perennial	Tehran province	12.09.2016 (1250)	FUMH 46421
N. cataria L.	perennial	North Khorassan province, W Bojnord, Ghorkhod Mount	04.07.2011 (1400)	FUMH 44565
N. nuda L.	perennial	Gilan province, Astara, Heyran pass	16.06.1977 (1400)	FUMH 2314
N. bodeana Bunge	perennial	North Khorassan province, W Bojnord, Badranlu	12.06.1996 (1920)	FUMH 27266

* Endemic taxa to Iran.

taxa of *Nepeta* in Iran to improve the taxonomic information on the species and to evaluate the usefulness of SEM for systematic classification. We attempted to evaluate the possible taxonomic differentiation of the species on the basis of nutlet surface morphology.

Material and methods

Plant material

Nutlets of 16 *Nepeta* taxa including five annuals and 11 perennials species of medicinal plants growing in Iran were provided by the Agricultural and Natural Resources Research Center of Kerman Province, Iran (ANRRCI) and Ferdowsi University of Mashhad Herbarium, Iran (FUMH). Among these, nine taxa are endemic to the country. The plant specimens were collected from different regions of Iran, mainly Kerman and Khorassan provinces during 2015–2017. The collection data of the studied species are presented in Table 1. The infrageneric classification of *Nepeta* is based on Rechinger (1982), Budantsev (1993) and Jamzad et al. (2003).

Optical observation

Color and appearance features of the nutlets were studied with a BX51 stereomicroscope and a camera. To ensure the color, normal size and maturity of the nutlets, 10 samples of each species were observed under a stereomicroscope. The color chart of the Royal Horticultural Society was used for determination of the nutlet color (Appendix 1). We recognized nutlet shape following Clopton (2004).

Scanning electron microscopy (SEM)

For pre-treatment of the samples, the nutlets were rinsed with 100% ethanol and then placed in an ultrasonic bath for 5 min. Dry nutlets were mounted with aluminum stub double-sided adhesive tape and coated with a thin layer of a palladium using a sputter coater. Photographs were taken using a scanning electron micropscope (SEM) at the Central Laboratory of the Ferdowsi University of Mashhad, Iran. The measurements of nutlet size were also carried out under SEM. The terminology of nutlet sculpturing follows Barthlott (1981), Ryding (1992), Harris and Harris (1994), Budantsev and Lobova (1997), Padure et al. (2005), Kaya and Dirmenci (2008), Moon et al. (2009) and Hassan and Dar (2012).

Results

The examined nutlet morphological characteristics (shape, size, color, surface ornamentation and areole) of 16 *Nepeta* taxa, belonging to nine sections, are summarized in Table 2. The color optical observations of the nutlets are shown in Fig. 1A–P, and the scanning electron micrographs in Fig. 2–6.

Table 2. A comparison of morphological characteristics of the nutlets studied for 16 *Nepeta* taxa.

Species	Section	Seed shape	Size (length×width) in mm	Color	Areole form and position	Nutlet surface
N. bracteata	*Micronepeta*	ovoid	1.4–1.8×0.8–1	light brown-yellowish	bi-lobed sub-basal	smooth
*N. ispahanica**	*Micronepeta*	ovoid-oblong	1.4×0.7–0.8	light brown-yellowish	straight basal	smooth
*N. eremophila**	*Micranthae*	ovoid	1.3–2×0.6–1	Brown	bi-lobed sub-basal	verrucate
*N. mahanensis**	*Micranthae*	ovoid	1.2–1.8×0.6–1	Brown	bi-lobed sub-basal	undulate
N. saccharata	*Micranthae*	ovoid-oblong	1.6×0.8–0.9	Brown	bi-lobed sub-basal	reticulate
*N. assurgens**	*Spicatae*	ovoid-oblong	1.4–1.7×0.8–1.5	Brown	bi-lobed sub-basal	reticulate
*N. binaloudensis**	*Spicatae*	oblong-linear	1.2–1.4×0.7–1.5	Brown	straight basal	finely granulated
N. teucriifolia subsp. *teucriifolia*	*Schizocalyx*	elliptic-oblong	1.7×0.9	blackish-brown	bi-lobed sub-basal	reticulate
*N. dschuparensis**	*Psilonepeta*	oblong	1.8–2.1×1.1–2	dark brown	bi-lobed sub-basal	undulate
N. glomerulosa. subsp. *glomerulosa*	*Spartonepeta*	ovoid-oblong	1.8–2 ×0.9–1	brown	bi-lobed sub-basal	reticulate
N. glomerulosa subsp. *carmanica**	*Spartonepeta*	ovoid-oblong	1.5×0.7–0.8	brown	bi-lobed sub-basal	reticulate
N. kotschyi var. *persica**	*Stenostegiae*	oblong	1.7–2.3×1–1.4	dark brown, black	straight sub-basal	tuberculate
N. racemosa var. *crassifolia**	*Stenostegiae*	ovoid	1.9–2×0.8–1.1	black	bi-lobed sub-basal	reticulate
N. cataria	*Cataria*	ovoid-oblong	1.4–1.9×0.5–0.9	black	straight sub-basal	reticulate
N. nuda	*Orthonepeta*	ovoid-oblong	1.7×0.8	black	bi-lobed sub-basal	tuberculate-cellular
N. bodeana	*Orthonepeta*	ovoid-oblong	1.4–1.8×0.6–1.1	black	bi-lobed lateral	undulate

* Endemic taxa to Iran.

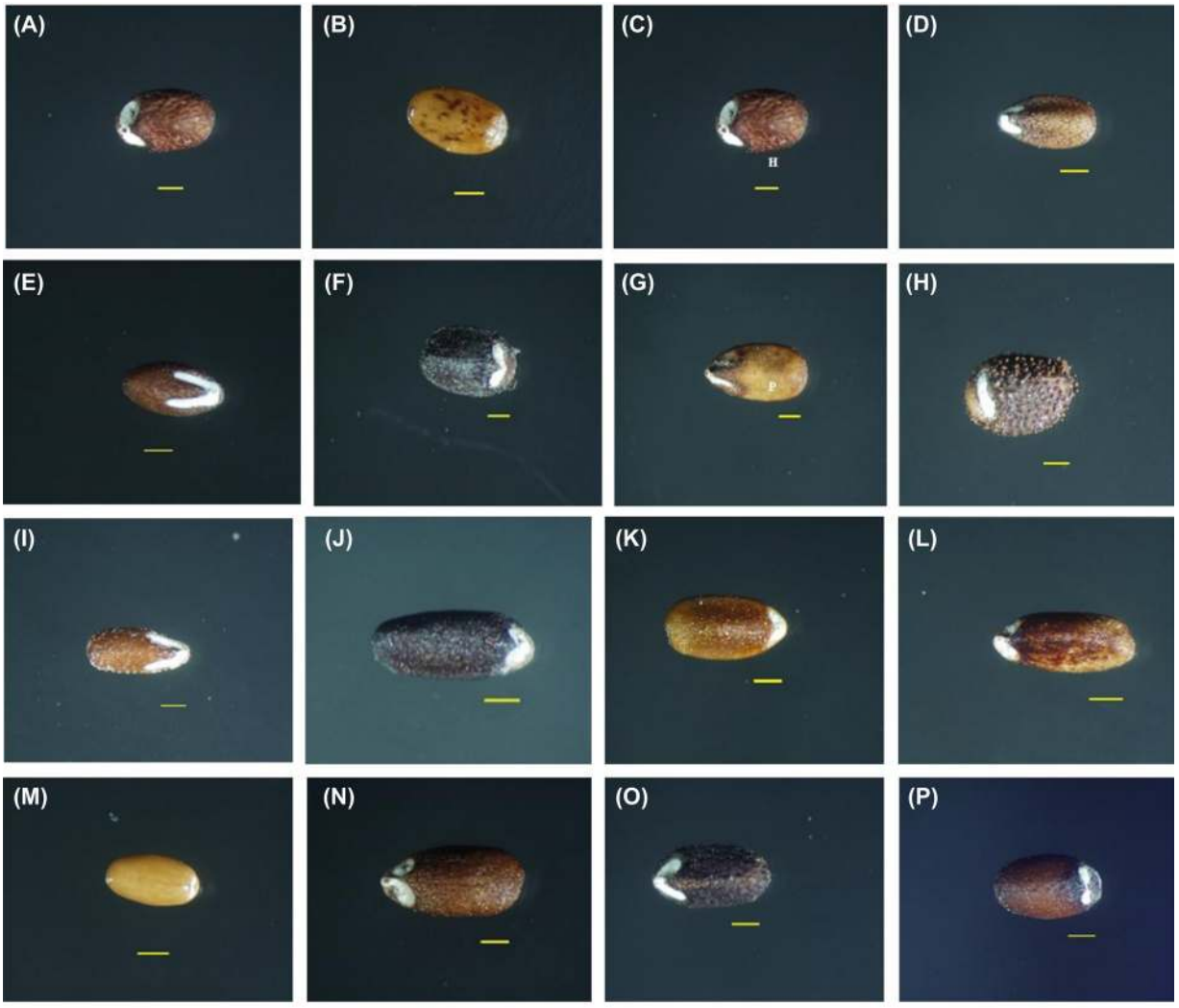

Figure 1. The color optical observations of *Nepeta* nutlets using a stereomicroscope. (A) *N. saccharata*, (B) *N. ispahanica*, (C) *N. racemosa* var. *crassifolia*, (D) *N. mahanensis*, (E) *N. eremophila*, (F) *N. bodeana*, (G) N. *glomerulosa* subsp. *glomerulosa*, (H) *N. kotschyi*, (I) *N.glomerulosa* subsp. *carmanica*, (J) *N. teucriifolia* subsp. *teucriifolia*, (K) *N. assurgens*, (L) *N. binaloudensis*, (M) *N. bracteata*, (N) *N. dsuparensiss*, (O) *N. nuda*, (P) *N. cataria*. Scale bars = 0.5 mm.

Variation in the shape and size of the nutlets

The nutlets are variable in their shape and size (Table 2, Fig. 1–6). Nutlet shapes are characterized as oblong, ovoid, ovoid-oblong, oblong-linear or elliptic-oblong; however, they were predominantly oblong to ovoid in the species of this study. The nutlets ranged in size from 1.2 mm (*N. binaloudensis* and *N. mahanensis*) to 2.3 mm (*N. kotschyi* var. *persica*) in length and from 0.5 mm (*N. cataria*) to 2 mm (*N. dschuparensis*) in width (Table 2). The largest nutlets on average were found in *N. racemosa* var. *crassifolia*, *N. dschuparensis*, *N. kotschyi* and *N. glomerulosa*. The smallest nutlets were those of *N. ispahanica*, *N. mahanensis* and *N. binaloudensis*. The nutlet size ranges for all species are presented in Table 2.

Variation in the color of nutlets

The nutlets in the studied taxa are variable in color (Fig. 1, Table 2), ranging from brown (*N. assurgens*, *N. glomerulosa* subsp. *carmanica*, *N. eremophila*, *N. saccharata*), light brown (*N. ispahanica*, *N. bracteata*), to dark brown/black (*N. kotschyi*), blackish-brown (*N. teucriifolia* subsp. *teucriifolia*) and black (*N. cataria*, *N. racemosa* var. *crassifolia*, *N. nuda*). Some taxa can be distinguished easily from the others by their nutlet color alone, such as *N. ispahanica* and *N. bracteata* with light brown/yellowish color and bright surface (Fig. 1B, M). Furthermore, *N. ispahanica* is distinguishable by dark brown spots on the surface (Fig. 1B).

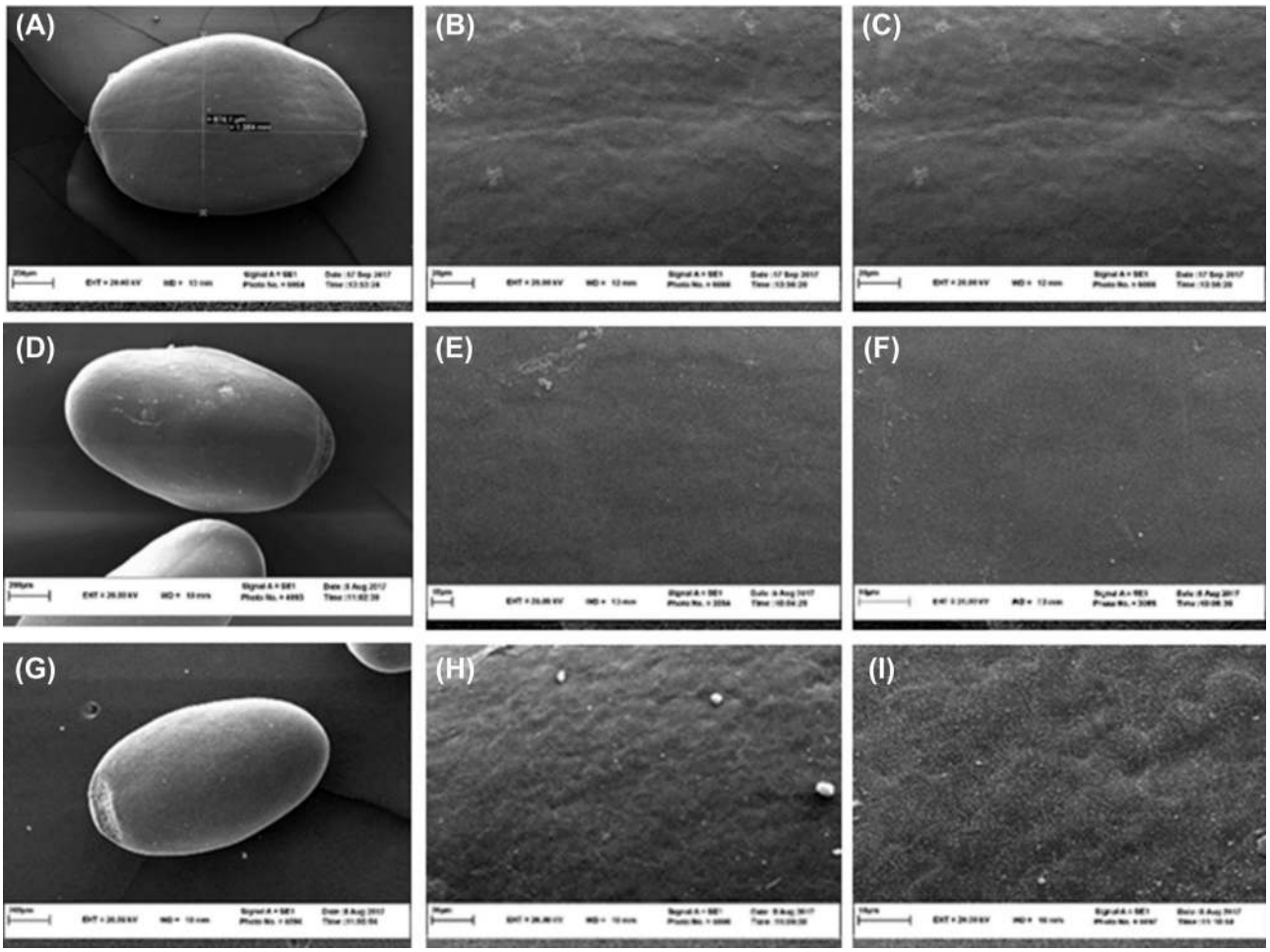

Figure 2. Nutlet micromorphology of *Nepeta* species. Smooth sub-type: (A–C) *N. bracteata*, (D–F) *N. ispahanica*. Granulate sub-type: (G–I) *N. binaloudensis*. Scale bars = 200 µm (A, D, G), 20 µm (B, E, H), 10 µm (C, F, I).

Variation in nutlets surface ornamentation

The results show that two main nutlet types can be distinguished in *Nepeta* species based on surface ornamentation: non-sculptured, and sculptured nutlets. Some sub-types are distinguishable within these main types.

Type 1 – Non-sculptured nutlets: in this type, there are no tubercles on nutlet surface, however, prominent undulate-cellular or reticulate patterns are formed by hexagonal and polygonal cells or with finely granulated surface. They can be divided into four sub-types: smooth, granulate, undulate-cellular and reticulate.

'Completely smooth': the surface in this sub-type of nutlets is smooth without any tubercles or ornamentation. This pattern is observed only in *N. bracteata* (Fig. 2A–C) and *N. ispahanica* (Fig. 2D–F).

'Granulate': this sub-type can be distinguished easily from the others by the finely granulated nutlet surface such as in *N. binaloudensis* (Fig. 2G–I). In this group, the surface of nutlets appears smooth, however, their granulate surfaces are visible using the higher magnifications.

'Undulate-cellular': in the undulate-cellular sub-type, the nutlet surface has a prominent undulate pattern shaped by hexagonal cells or with cellular pattern consisting of regular groups of polygonal cells. This pattern is only found in *N. mahanensis* (Fig. 3A–C), *N. bodeana* (Fig. 3D–F) and *N. dschuparensis* (Fig. 3G–I). There is a thick cuticle masking of the cell structure in the central area of the nutlets and under high magnification, cellular reticulations can be seen at the apex (*N. bodeana*).

'Reticulate': the reticulate sub-type consists of large oblong, rounded or polygonal cells. The cellular pattern consists of deep rounded or polygonal cells (*N. glomerulosa* subsp. *carmanica*, *N. glomerulosa* subsp. *glomerulosa*, *N. assurgens*, *N. racemosa* var. *crassifolia*) (Fig. 4) or of regular groups of polygonal cells (*N. saccharata* and *N. teucriifolia* subsp. *teucriifolia*) (Fig. 5). The nutlets of these species have a smooth pattern (regular or polygonal cells) and are easily distinguished from the other species. Furthermore, within this sub-type, the nutlets of *N. racemosa* var. *crassifolia* can be distinguished from any other by its deep polygonal pits on the surface which is recognized as pitted-granular (Fig. 4J–K).

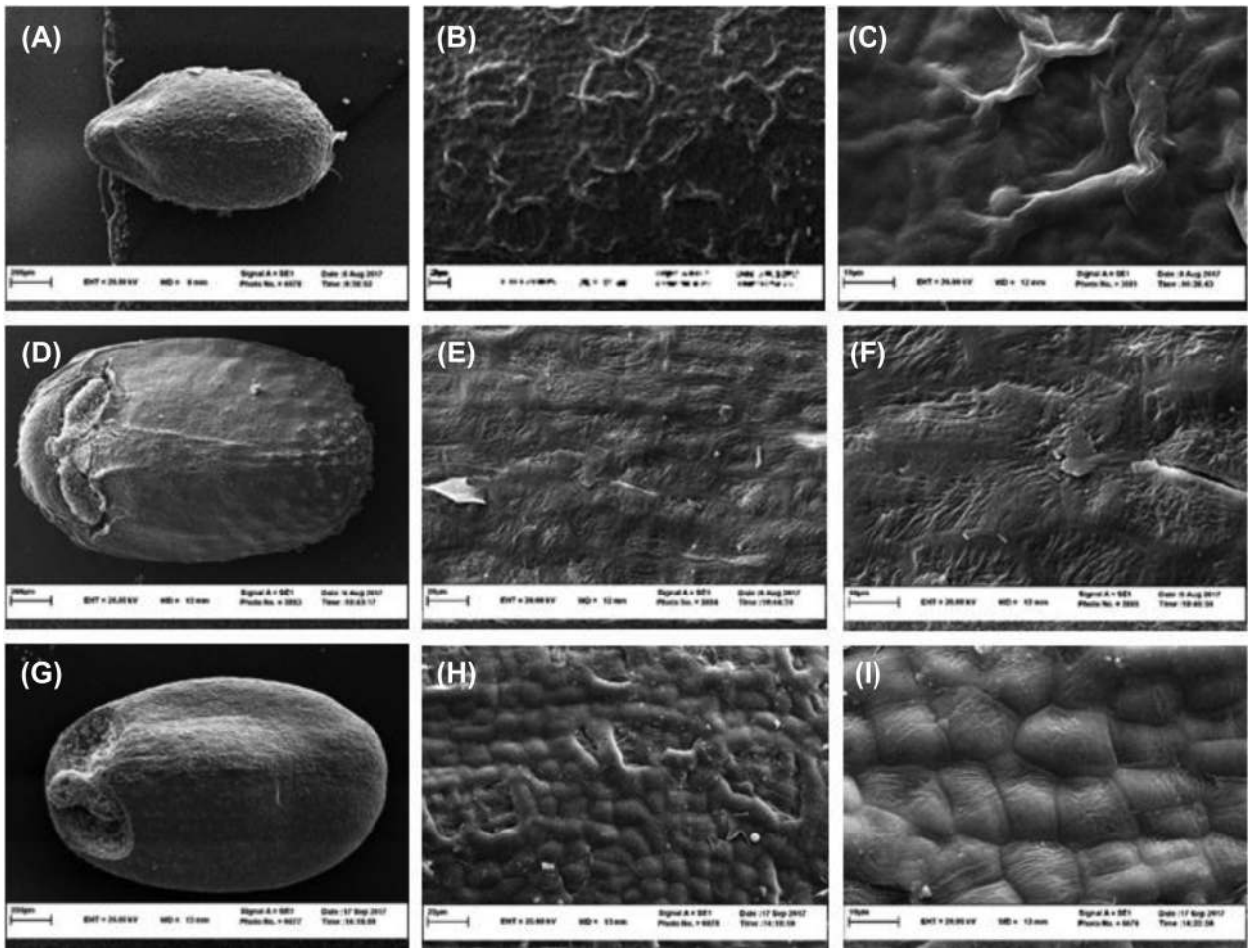

Figure 3. Nutlet micromorphology of *Nepeta* species. Undulate sub-type: (A–C) *N. mahanensis*, (D–F) *N. bodeana* and (G–I) *N. dschuparensis*. Scale bars = 200 µm (A, D, G), 20 µm (B, E, H), 10 µm (C, F, I).

Type 2 – Sculptured nutlets: this type is characterized by having rounded, oblong or papillate or cone-shaped tubercles located on all surfaces, especially towards the apex with a length of approximately 50–90 µm. Three sub-types are recognized based on the shape of the tubercles: tuberculate, tuberculate-cellular and verrucate.

'Tuberculate': this sub-type is characterized by having oblong- or rounded-shaped tubercles such as in *N. kotschyi* var. *persica* (Fig. 6A–C). *N. kotschyi* can be easily distinguished from all other taxa by its papillate protuberances pattern on the nutlet surface (Fig. 6A–C).

'Tuberculate-cellular': this sub-type is characterized by oblong or cone-shaped tubercles with a rounded or truncate apex. The nutlets of *N. nuda* are included in this sub-type (Fig. 6D–F).

In the 'verrucate' sub-type, the tubercles are formed by the ring of convex radial cells. The center of these tubercles is mostly depressed. *N. eremophila* nutlets show this pattern (Fig. 6G–I).

Differences in areole form and size

The areoles are in different positions (basal, sub-basal or lateral) and their forms are bi-lobed or straight in the studied taxa. A summary of the form of the areoles are given in Table 2. The nutlets in *Nepeta* species have generally U- or V-shaped, bilobed or straight areoles. The taxa with V-shaped areole are *N. mahanensis* and both subspecies of *N. glomerulosa*. U-shaped areoles can be observed in *N. eremophila* only, which is clearly differentiated from other species by its long areole (Fig. 1E, 6G). In non-sculptured nutlets, *N. mahanensis* has a narrow head and a V-shaped areole which can be easily distinguished from the others (Fig. 3A–C).

Discussion

In the present study, the micro-morphological characteristics of the nutlets 16 representative Iranian taxa of *Nepeta* were investigated, among which *N. racemosa* var. *crassifolia*, *N. kotschyi* var. *persica*, *N. assurgens*, *N. mahanensis* and *N. binaloudensis* are studied for the first time using scanning electron microscopy. The nutlets vary significantly in color, size, shape and surface ornamentation. The importance of nutlet morphology, especially its micro-morphological characters, has already been proved in the several genera of

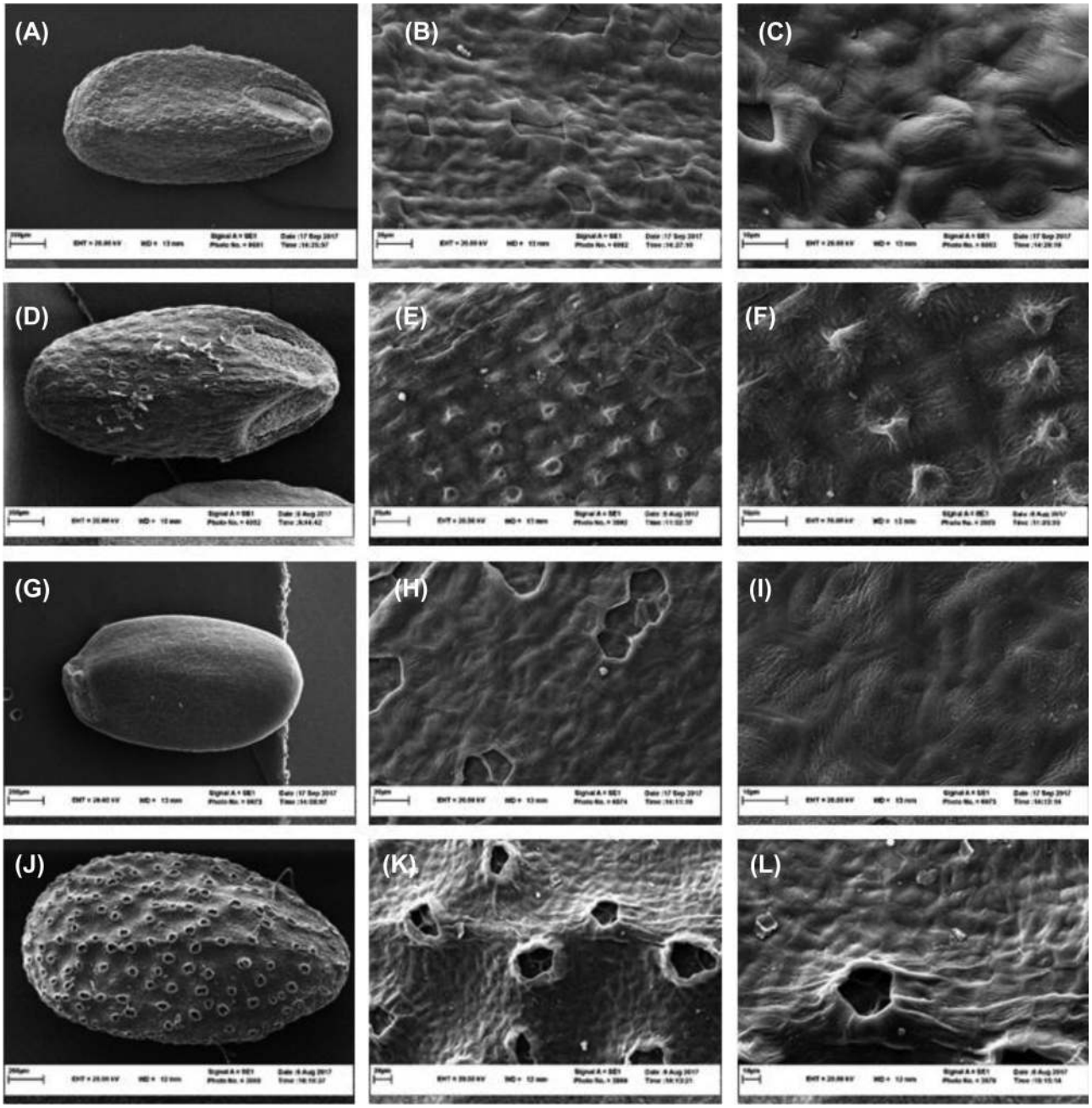

Figure 4. Nutlet micromorphology of *Nepeta* species. Reticulate sub-type: (A–C) *N. glomerulosa* subsp. *carmanica*, (D–F) *N. glomerulosa* subsp. *glomerulosa*, (G–I) *N. assurgens*, (J–L) *N. racemosa* var. *crassifolia*. Scale bars = 200 μm (A, D, G, J), 20 μm (B, E, H, K), 10 μm (C, F, I, L).

Lamiaceae including *Nepeta* (Budantsev and Lobova 1997, Jamzad et al. 2000, Padure et al. 2005, Kaya and Dirmenci 2008, Moon et al. 2009, Hassan and Dar 2012). Furthermore, nutlet surface morphology of some *Nepeta* species growing in Iran has been investigated previously (Jamzad et al. 2000, Serpooshan et al. 2014).

The mature nutlets of *Nepeta* species are usually brown, however, the color of the nutlets varies from light brown-yellowish and brown to blackish-brown and black in the studied species. A similar range in the color of nutlets in *Nepeta* species has been reported in the previous studies.

The nutlets of the some *Nepeta* species are easily distinguished from the others based on the color. *N. ispahanica* and *N. bracteata* can be distinguished from the other studied taxa by their bright nutlet surface (Fig. 1). According to Kaya and Dermenci (2008), *N. humulis* has nutlets with bright surface. Moreover, such variation in size and shape of the nutlets (Table 2, Fig. 1) indicates the taxonomic significance of nutlet

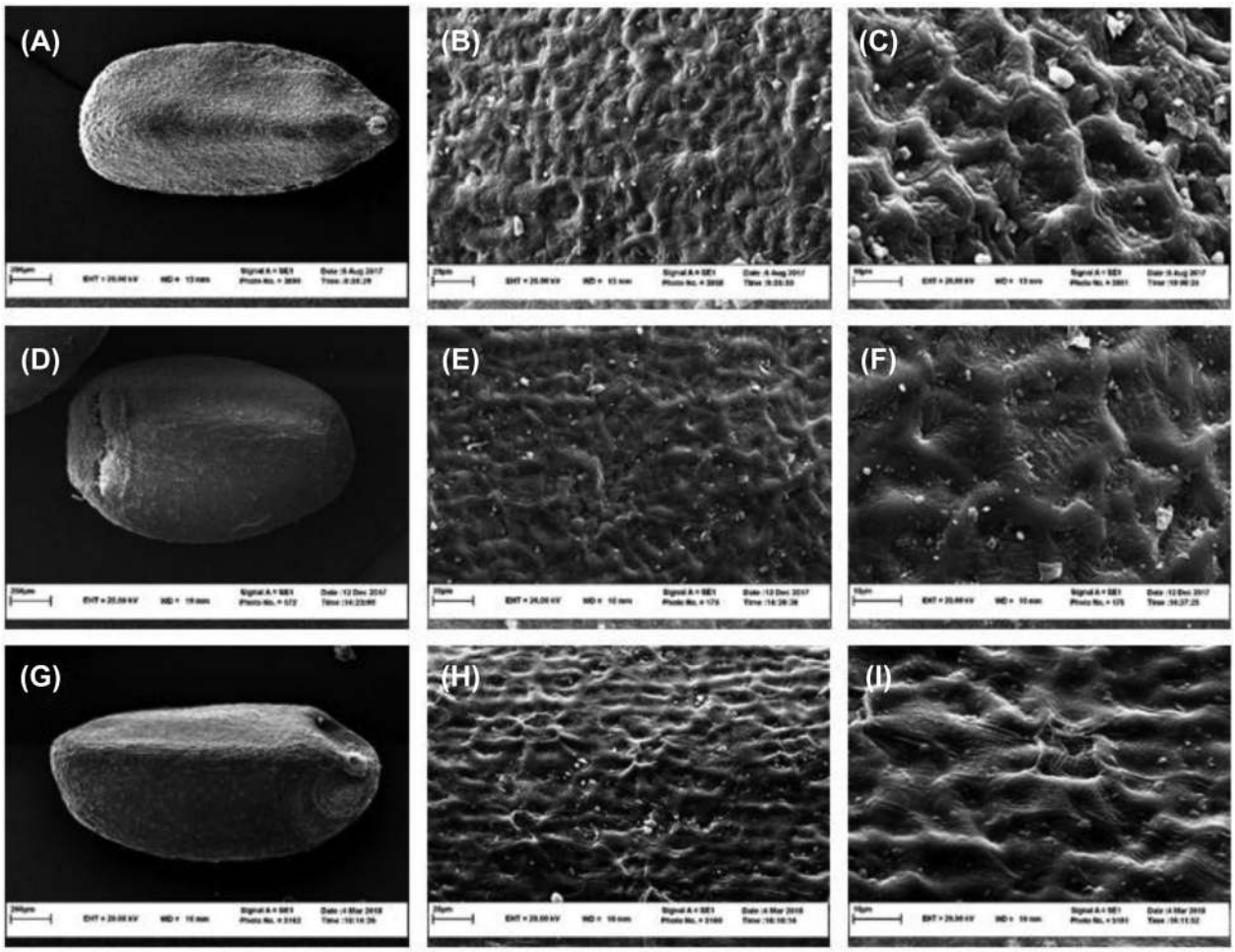

Figure 5. Nutlet micromorphology of *Nepeta* species. Reticulate sub-type: (A–C) *N. saccharata*, (D–F) *N. cataria*, (G–I) *N. teucriifolia* subsp. *teucriifolia*. Scale bars = 200 μm (A, D, G), 20 μm (B, E, H,), 10 μm (C, F, I).

morphology in *Nepeta* species. Among the studied taxa, *N. dschuparensis* and *N. kotschyi* var. *persica* have the largest nutlets and the smallest ones are those of *N. ispahanica*, *N. mahanensis* and *N. bracteata*. Kaya and Dirmenci (2008) studied nutlets of 39 taxa of Turkish *Nepeta*. Similar to our observations, they reported the nutlets as black, blackish-brown or brown in color, oblong (1.5–3.0 × 0.8–1.4 mm), broadly oblong (1.2–3.0 × 0.7–1.8 mm) and rounded (1.8–2.2 × 1.5–2.0 mm), trigonous in size. Husain et al. (1989), studied two *Nepeta* from Yugoslavia, the nutlets were broadly ovate, (1.8–2.0 mm) in lengh and (0.8–1.0 mm) in width and ligh brown in color. The taxonomic value of the areoles on nutlets is also remarkable; the size (short or long), form (straight or bi-lobed, and V- or U-shaped) and position (basal, sub-basal or lateral) of the areoles are notably varied among the studied species. Variation in areole morphology was also reported in previous studies on *Nepeta* (Jamzad et al. 2003, Kaya and Dirmenci 2008, Hassan and Dar 2012). In the present study, the positions of areoles on nutlets are basal, sub-basal, lateral, bilobed or straight. Although, the nutlets have generally U- or V-shaped areoles, there is useful variation among different species in their size and form. Jamzad et al. (2003) used the areole characters as diagnostic for some *Nepeta* species (*N. balouchestanica*, *N. hormozganica* and *N. ispahanica*); according to Jamzad et al. (2003), *N. hormozganica* differs from other species by its long areole lobes. Within the genus, only three species have nutlets with long areole lobes: *N. mirzayanii* (= *N. eriosphaera* Rech.f. & Koeie), *N. bellevii* Prain and *N. hormozganica* (Jamzad et al. 2003). In the present study, only *N. eremophila*, with U-shaped, whitish and long areoles, is clearly differentiated from other species. Therefore, the combination of different morphological characters of nutlets may be helpful for the classification and identification of the taxa.

Furthermore, our observations confirm the usefulness of the sculpturing patterns on nutlet surface for the taxonomy of *Nepeta* species. *N. bracteata* and *N. ispahanica* (sect. *Micronepeta*), among the annual species, have smooth surfaces without any tubercles or ornamentation. The features of nutlet surface in *N. bracteata* recorded by Jamzad et al. (2000) is the same as of our samples, however, Budantsev and Lobova (1997) recorded an undulate surface morphology

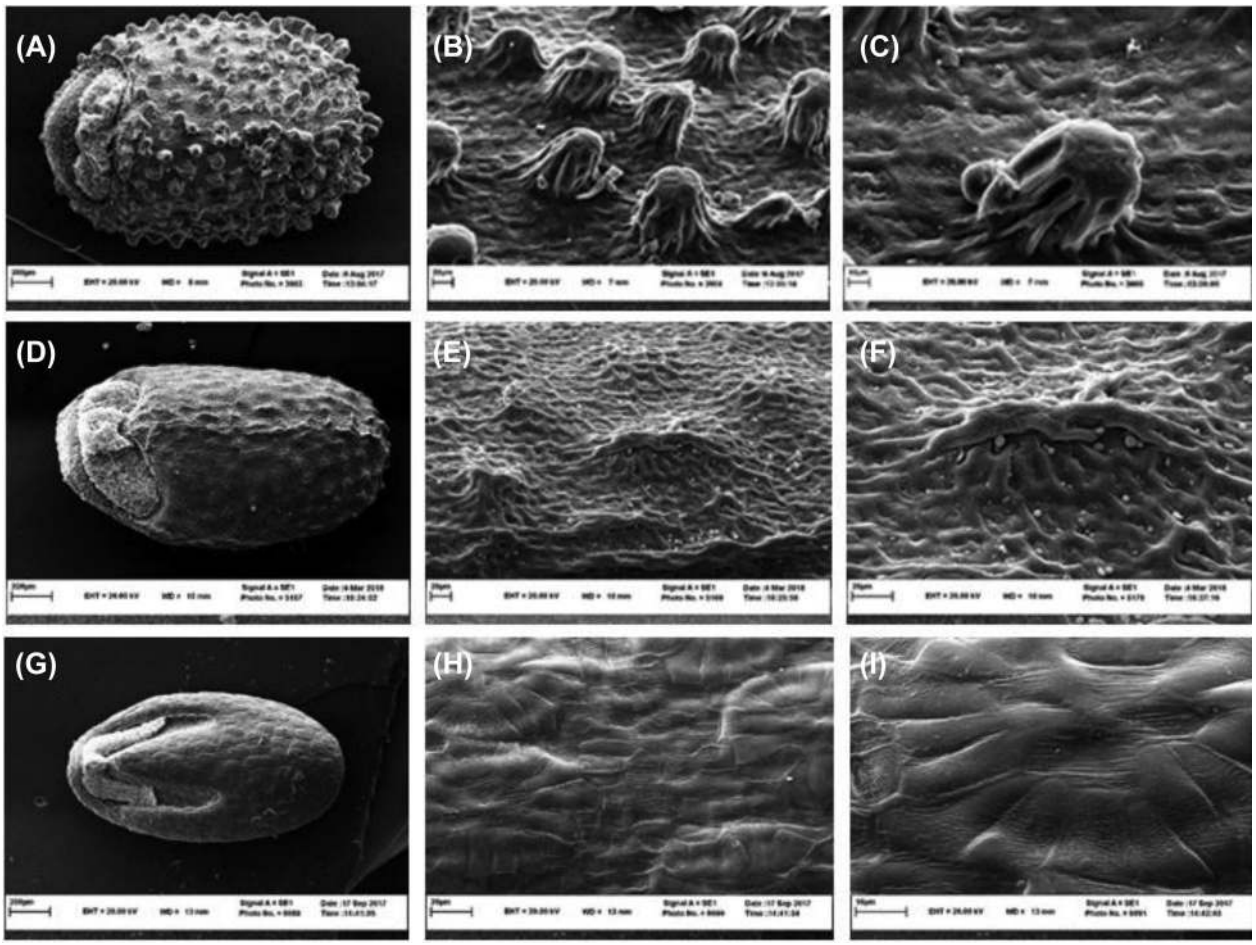

Figure 6. Nutlet micromorphology of *Nepeta* species. Sculptured nutlets: (A–C) tuberculate sub-type in *N. kotschyi* var. *persica*, (D–F) tuberculate-cellular sub-type in *N. nuda*, and (G–I) verrucate sub-type in *N. eremophila*. Scale bars = 200 μm (A, D, G), 20 μm (B, E, H), 10 μm (C, F, I).

for this species. The nutlets are not completely smooth in the other annual species, *N. saccharata*, *N. mahanensis* and *N. eremophila* (sect. *Micranthae*), but with different patterns including partly smooth (reticulate, undulate) and sculptured (verrucate), respectively. Kaya and Dirmenci (2008) recorded such a variation in the other annual species of the sect. *Micranthae*, i.e. *N. humilis* Benth and *N. meyeri* Benth. According to Jamzad et al. (2000), the species of *N.* sect. *Micronepeta* have nutlets with smooth surface. Jamzad (2003) described *N. mahanensis* as a new annual species endemic to Iran with an uncertain sectional placement. Moreover, phylogenetic analysis of *Nepeta* species based on ITS sequence data revealed grouping of *N. mahanensis* with annual and perennial sections (Jamzad et al. 2003). The first micromorphological study of *N. mahanensis* nutlets in the present work clearly shows that this species belongs to *N.* sect. *Micranthae* based on the annual growth form and its undulate nutlet surface. *Nepeta assurgens* and *N. binaloudensis*, both perennial and endemic species, are closely related based on molecular phylogenetic analysis and belong to *N.* sect. *Spicatae* (Jamzad et al. 2003). Budantsev and Lobova (1997) recorded that several Central and Southwest Asian species of this section have generally undulate or reticulate nutlets. However, *N. binaloudensis*, an endangered and endemic species to Khorassan-Kopet Dagh in NE Iran (Memariani et al. 2016), can be easily distinguished from the other species of the section by its smooth to partly granulated nutlets.

The studied taxa of *Nepeta* in the sections *Schizocalyx* (*N. teucriifolia* subsp. *teucriifolia* = *N. fissa* C.A.Mey.), *Psilonepeta* (*N. dschuparensis*) and *Spartonepeta* (*N. glomerulosa*) show a relative homogenous nutlet morphology i.e. in shape, size and color, form and position of areoles, and also in nutlet surface (Table 2). Our results for *N. teucriifolia* are the same as those recorded by Kaya and Dirmenci (2008) under the name *N. fissa*. However, Serpooshan et al. (2014) recorded a smooth surface by ridged cellular texture consisting of irregular cells, for the nutlets of the endemic species *N. dschuparensis*. Generally, the nutlet surface in this species is the same as non-sculptured or smooth type in our observations.

The taxa of *N. glomerulosa* (*N.* sect. *Spartonepeta*) have brown nutlets compared with two other sections which have darker ones. The endemic taxon *N. glomerulosa* subsp.

carmanica can be distinguished from the type subspecies by its smaller nutlets. *N. cataria* (*N.* sect. *Cataria*) also has similar morphological nutlets characters, however, it differs by the straight form of the areoles. Mosquero et al. (2002), Padure et al. (2005) and Kaya and Dirmenci (2008) recorded the same morphological features for the latter species. The taxa of the sections *Stenostegiae* and *Orthonepeta* show sculptured (tuberculate) to partly smooth (undulate, reticulate) nutlet surfaces. In *N.* sect. *Stenostegiae*, *N. kotschyi* var. *persica* (= *N. persica*) has tuberculate nutlets as already recorded by Budantsev and Lobova (1997). However, we observed reticulate nutlets in *N. racemosa* var. *crassifolia* (= *N. crassifolia* Boiss. & Buhse) which differs from the verrucate nutlets for *N. racemosa* recorded by Budantsev and Lobova (1997). Further studies are recommended to check if *N. racemosa* and *N. crassifollia* are really conspecific or not. In *N.* sect. *Orthonepeta*, based on our study, *N. nuda* has sculptured (tuberculate) nutlets similar to the three subspecies of *N. nuda* in the flora of Turkey recorded by Kaya and Dirmenci (2008). In this section, *N. bodeana* (= *N. sintenisii* Bornm.) differs by its lateral position of areoles and also by undulate surface.

Our results, in accordance with the previous works, demonstrate the taxonomic significance of nutlet micromorphology in the genus *Nepeta*. The morphological traits of nutlet surface may have a wide range of variation at sectional level; however, they are very helpful for classification and identification of *Nepeta* species and infraspecific taxa. Frequent hybridization and introgression as well as morphological variation between the ecotypes make *Nepeta* a complex genus (Kaya and Dirmenci 2008). So, nutlet morphology can play an important role as a method for fast and reliable detection of species authentication in pharmaceutical industries (Kahkeshani et al. 2014), especially for the local and regional endemic taxa.

Acknowledgements – This paper is part of results of MSc thesis of the first author supported by grant no. 3/45025 Vice President for Research and Technology of Ferdowsi University of Mashhad. We thank Mohammad Reza Joharchi for the species identification and his helpful comments, and Ms. Sadeghiyan and Mr. Khademi for their technical assistance with the scanning electron microscopy and photography.

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Appendix 1. Color chart of the Royal Horticultural Society.