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Comprehensive Evaluation of Morphological, Anatomical, and Palynological Characteristics of some Selected Taxa of Zygophyllaceae in Egypt.

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Abstract

This study presents a comprehensive comparative analysis of six Egyptian taxa from the family Zygophyllaceae, representing the genera Fagonia, Zygophyllum, and Tribulus. An integrative approach was employed, combining morphological, anatomical, and palynological investigations using both light microscopy (LM) and scanning electron microscopy (SEM). The main objective was to evaluate the taxonomic significance of these characters and enhance species identification and classification within the family. Morphological assessments included leaf structure, floral and fruit morphology, and seed characteristics. Anatomical studies focused on the tissue organization of stems, petioles, and leaf blades, with emphasis on epidermal features, vascular patterns, and crystal distribution. Palynological analysis revealed significant variation in pollen size, polarity, aperture types, and exine ornamentation. A total of 219 characters were scored and analyzed statistically using Minitab software. Cluster analysis and dendrogram construction revealed a clear separation of Tribulus species from Fagonia and Zygophyllum simplex, which conversely showed closer affinities. These findings highlight the diagnostic value of integrating morphological, anatomical, and palynological data, and support the recognition of Tribulus at a distinct taxonomic rank within Zygophyllaceae. Collectively, these characters provide valuable tools for the accurate delimitation and identification of taxa at both generic and specific levels.

Keywords: Anatomical characters, Cluster analysis, Macromorphological characters, Palynological characters, Zygophyllaceae.

Introduction

Zygophyllaceae R.Br. (s.l.) is a large and heterogenous family comprising approximately 285 species across 22 genera, mostly distributed in arid and semi-arid regions of the Old World, particularly in Africa, and extending into warm temperate zones. Most species inhabit xerophytic and halophytic environments (Christenhusz and Byng, 2016). The delimitation of taxa within the family has changed multiple times due to the structural diversity and variable plasticity of its members. As a result, the family has long been a subject of taxonomic controversy. Additionally, some of its genera were even separated and treated as distinct families (Engler, 1931; Takhtajan 1969, 1986 & 2009; Cronquist 1988; Heywood et al., 2007; Shipaunov, 2012). In light of recent molecular phylogenetic analyses, integrated with morphological and anatomical data, the taxonomic position of Zygophyllaceae has been reassessed and redefined. Based on the recent molecular findings of Sheahan and Chase (1996, 2000), Beier et al. (2003), and APG III (2009), the family has been reclassified under the order Zygophyllales, alongside Krameriaceae. Currently, Zygophyllaceae is divided into five subfamilies viz.: Zygophylloideae, Morkillioideae, Tribuloideae, Seetzenoideae and Larreoideae**;** in which the Zygophylloideae recognized as the largest and strongly supported as a monophyletic group. This subfamily includes four genera: *Zygophyllum* L., *Fagonia* L., *Roepera* A. Juss., and *Tetraena* Maxim (Stevens 2001, onwards).

From a palynological perspective, Zygophyllaceae is recognized as a eurypalynous family (Erdtman, 1952; Punt et al., 2007). Accordingly, the study of pollen morphology within this family plays a significant role in elucidating taxonomic relationships and affinities among its taxa. The pollen morphology of the Zygophyllaceae has been extensively studied by several authors. Early investigations were conducted by Erdtman (1952), Kuprianova and Alyoshina (1978), and Yunus and Nair (1988), with further contributions from more recent investigations by Perveen and Qaiser (2006), Bukhari et al. (2014), El-Atroush et al. (2015) and Taia et al. (2021). Perveen and Qaiser (2006) studied the pollen of 14 species representing five genera from Pakistan, recognizing four distinct pollen types and highlighting the diagnostic value of palynological characters at both generic and specific levels. Similarly, Bukhari et al. (2014) investigated selected species from Saudi Arabia, adding valuable insights to the regional palynological record. In a more focused study, Taia et al. (2021) examined 12 taxa of *Fagonia* from Libya and concluded that pollen characteristics provide limited taxonomic delimitation within the genus.

Several studies have highlighted the significance of anatomical characters in the taxonomic classification of the family Zygophyllaceae. Metcalfe and Chalk (1957) established detailed anatomical characters of stems, petiole and leaves, while subsequent studies have highlighted the significance of internal vegetative features in distinguishing genera and species. Sheahan and Cutler (1993) examined 37 species across 19 genera, identifying diagnostic traits such as cuticle thickness, hypodermis, crystal types, pith structure, and venation patterns that distinguish subfamilies like Tribuloideae and Zygophylloideae. In *Tribulus* and *Fagonia*, various studies (e.g., Khalifa, 1968; Ahmed and Khafagi, 1997; Ahmed and Mohamed, 2005b) have investigated stem, leaf, petiole, and stipule anatomy, emphasizing their role in supporting generic boundaries and aiding reclassification. Additional investigations focusing on seed morphology, trichome types, and petiole vasculature (Ahmed, 1991; Khafagi, 2004; Abdel Khalik and Hassan, 2012) have further supported species-level distinctions. Regional anatomical studies from Saudi Arabia, Libya and Sudan (Waly et al., 2011; Elkamali et al., 2016; Taia et al., 2017; Gabr and Ragab, 2023) emphasized the taxonomic value of mesophyll structure, stele type, and crystal distribution in distinguishing *Tribulus*, *Fagonia*, *Zygophyllum*, and *Tetraena* species, further confirming the systematic significance of vegetative anatomy in Zygophyllaceae. Based on numerical analyses, Abdel Khalik (2012) investigated Egyptian species of Zygophyllaceae using morphological, palynological, and seed characteristics. The study revealed that, although the family is generally heterogeneous, the subfamilies Tribuloideae, Zygophylloideae, Seetzenioideae, and Tetradiclidoideae are relatively homogeneous. It also suggested that traditional groupings within *Tribulus* and *Fagonia* seem to be artificial. Similarly, Shamso et al. (2013) analysed morphological data from eight genera and identified three distinct groups: one corresponding to Tribuloideae (*Tribulus*), a second to Zygophylloideae (*Zygophyllum* and *Fagonia*), and a third comprising *Seetzenia*, *Peganum*, and *Nitraria*, further supporting broader taxonomic distinctions within the family.

Furthermore, chemosystematic studies and DNA fingerprinting conducted by Draz et al. (2024; 2025) supported the taxonomic delimitation of species within the genera *Fagonia*, *Tribulus*, and *Zygophyllum*. Their study also investigated the genetic variability of *T. terrestris* across different populations, providing valuable insights into its genetic plasticity and adaptability.

In Egypt, the family Zygophyllaceae *s.s.* is represented by 33 species across five genera (Boulos, 2000, 2009). In the present study, six taxa belonging to three genera (*Fagonia*, *Tribulus*, and *Zygophyllum*) were investigated in detail. Morphological, palynological, and anatomical characteristics were examined using both light microscopy (LM) and scanning electron microscopy (SEM). The main objective of this study is to assess the taxonomic significance of these traits in selected species of Zygophyllaceae and to provide additional data to support species identification and classification within the family. Furthermore, the study aims to evaluate the degree of affinity and interrelationships among the investigated taxa through the application of numerical analysis.

Materials and methods

Plant materials

The present study was based on the examination of specimens kept in major Egyptian herbaria: Cairo University (CAI), the Agriculture Museum, Flora and Phytotaxonomy Researchs (CAIM), and National Research Centre (CAIRC), (Herbaria acronyms according to Thiers (2024); continuously updated). Additionally, fresh specimens were collected from various locations between March and April 2022 (Table 1). The identification of these specimens was confirmed by comparing them to collections located at the Herbarium of Cairo University and using several regional floras (Täckholm, 1974; Boulos, 2000; Zohary, 1972; Chaudary, 2001). The nomenclature of all taxa was updated according to several online databases, including *The Plant List* (www.theplantlist.org) and the *International Plant Names Index* (www.ipni.org). For taxa belonging to the genus *Fagonia*, the nomenclature followed additional sources such as *iNaturalist* (www.inaturalist.org), *Tropicos* (www.tropicos.org), and the *Euro+Med PlantBase*.

Table 1: List of studied taxa, localities, and data of collection

Taxa	Locality, Date & collector	Voucher No.
Tribulus pentandrus	- Nasr City, Azhar Univ. for girls, 12/4/2022, *Draz et al.* s.n. (CAIRC) - Helwan, 31/1/1975, *H. Hosni et al.* s.n. (CAI) - Wadi Angabiya, Suez Road 16/3/1956, *El Hadidi & Batanouny* s.n. (CAI)	- A313 - cai. 49.249.783.139 - cai. 49.249.783.140
Tribulus terrestris var. *terrestris*	-Nasr City, Azhar Unv. for girls, 12/4/2022, Draz *et al.* s.n. (CAIRC) - N Qostul, Nubia, 29/12/1963, *L. Boulos* s.n. (CAI) -Gebel Catherine, Sinai, 21/8/1982, *El Hadidi et al.* s.n. (CAI)	- A307 - cai. 49.249.784. 213 - cai. 49.249.784. 214
Tribulus terrestris var. *orientalis*	-Oases Road, 6th October City, Dream Park zone; 23/4/2022, *Draz et al.* s.n. (CAIRC) -80 Km Cairo-Fayoum desert road; 27/4/2022, *Draz et al.* s.n. (CAIRC) -Cairo University, 2/7/1976; *Sisi* s.n. (CAI) -Abu Zaabal, 31/8/1967, *El Hadidi & Khattab* s.n. (CAI)	- A308 - A309 - cai.49.249.784.02 - cai.49.249.784.02
Fagonia glutinosa	-Near Alameen desert, Matrouh governorate,18/3/2022, *Draz et al.* s.n. (CAIRC) -Along Cairo-Alex desert road, South Tahrir province; 26/2/1968, *Romee* s.n. (CAI) -Wadi Natroun, 1/5/ 1976; *Chertek* s.n. (CAI) -Burg El Arab, Maruit, 23 Apr 1929, *Drar* s.n. (CAIM)	- A311 - cai.48.246.757.136 - cai. 48.246.757.137 - s.n.
Fagonia scabra	-Near Alameen desert, Matrouh governorate, 18/3/2022, *Draz et al.* s.n. (CAIRC) -Sinai, Mittla Pass; 7/4/1990; I. *El Garf* s.n. (CAI) -Wadi Hof; 24/2/1956; *M. Imam* s.n. (CAI) - Wadi Seyal, Red Sea, 4 Mar 1960, *Hilaly & Khattab* s.n. (CAIM)	- A312 - cai. 48.246.764.87 - cai. 48.246.764.88 - s.n.
Zygophyllum simplex	-Dabaa road, 72 km from Cairo, 18/3/2022, *Draz et al.* s.n. (CAIRC) -Alex-Cairo desert road, 85 Km from Cairo, 23/7/1970, *Ibrahim & Mahdi* s.n. (CAI) - Siwa-Matruh road, 25/1/1987, *A Fahmy*,39 (CAI)	- A310 - cai. 48.248.269.232 - cai. 48.248.269.233

Morphological examination

Morphological data were obtained from both fresh and herbarium specimens. For each taxon, at least 3–4 specimens (if available) were selected to reflect the morphological diversity observed within the species. The examined characters included habit, vegetative structures, as well as floral and fruit features (Table 2).

Pollen grains and seed examinations

Pollen samples and seeds were obtained from the anthers of mature unopened flowering buds, while the seeds were collected from mature fruits. Pollen grains for light microscopy (LM) were produced following Erdtman's (1960) method and subsequently mounted in drops of glycerin jelly. Light microscopic examinations were performed using a Serico research microscope at (E 40, 0.65) with a 16x eyepiece, and the seeds were examined with a dissecting microscope. For SEM examination, Pollen and seeds were fixed onto stubs using double-sided tape. The stubs were subsequently coated with gold utilizing an Ion-sputter JFC-1100 apparatus. The samples were examined using a FE-SEM QUANTA FEG250Y at the Electron Microscopy Unit, National Research Centre, Dokki, with an accelerating voltage of 20 KV. A minimum of 15-20 pollen grains per specimen was measured. Pollen characteristics, such as shape, size, aperture type, and exine ornamentation, were evaluated (Table 3). Pollen terminology is followed Punt et al. (2007), Hesse et al. (2009), and Halbritter et al. (2018), whereas seed coat terminology is based on Barthlott (1981).

Anatomical examination

For anatomical analysis, 3–5 specimens of the fourth internodes from the apex, mature blade, and petiole (if present) were selected and preserved in Formalin Acetic Alcohol (FAA) to preserve internal tissue structures. The samples were processed using the paraffin wax method following Johansen (1940) to prepare them for microtome sectioning at a thickness of 10–15 µm. Sections were mounted on glass slides using Haupt's adhesive, prepared by dissolving 1 g of gelatin in 50 ml of warm distilled water, followed by the addition of 7.5 ml of glycerol and a small phenol crystal, and refrigerating for 24 hours until solidified. After drying for 24 hours, the sections were stained using the standard Safranin-Fast Green double staining technique and mounted in Canada balsam (Sass, 1958). Sometimes, hand sectioning was also performed.

For epidermal studies, three pieces of lamina were treated with 5% KOH for 24 – 48 hr. to clear the tissues. Both the upper and lower epidermal layers were examined under a light microscope for stomatal and trichome characteristics. Terminology followed Metcalfe and Chalk (1957) and Evert 2006, and anatomical descriptions are summarized in Tables (4-6).

Statistical analysis

A multivariate clustering analysis was performed on a data matrix comprising 219 morphological, palynological, and anatomical character states of six studied taxa using Minitab statistical software (version 2022). After the analysis, a dendrogram would be plotted, displaying the similarity and distance levels among the investigated taxa.

Results

Using both light microscopy (LM) and scanning electron microscopy (SEM), six taxa representing three genera within the family Zygophyllaceae were investigated for their morphological, anatomical and palynological characteristics in order to identify the most important characters for taxonomic identification and differentiation. The diagnostic features observed are summarized in cumulative tables (2-6) and in Plates (1-6), with each character encoded in binary form (0/1) to facilitate comparative analysis. The studied taxa exhibited considerable variation in both macro- and micro-morphological traits, supporting their clear delimitation at both interspecific and intergeneric levels.

Macro-morphological variations

The most informative and diagnostic morphological characters among the studied taxa were observed in both vegetative and reproductive structures, including growth habit, stem characteristics, leaf composition, and surface texture, as well as floral and fruit morphology (Table 2). *Tribulus* taxa characterized by an herbaceous habit, paripinnate leaves with scarious stipules, and eglandular trichomes. In contrast, *Fagonia* species exhibit a suffrutescent growth form, unequal trifoliate leaves with spiny stipules, and glandular trichomes. *Zygophyllum simplex* is morphologically distinct from both genera, possessing sessile, unifoliate succulent leaves with scarious stipules, glabrous and a decumbent habit.

Floral morphological characters also showed significant variation among the studied taxa, particularly in flower size, sepal persistence, petal color, and structural traits at the stamen base. *Tribulus* taxa were distinguished by relatively large flowers (>10 mm in diameter), persistent sepals, yellow petals, and the presence of intrastaminal glands, while *Fagonia* species featured medium-sized flowers with mucronate, persistent sepals and violet petals. In contrast, *Zygophyllum simplex* exhibits the most distinct floral morphology, characterized by the smallest flowers (<5 mm in diameter), caducous sepals, clawed yellow petals, and the presence of filament scales. At the generic level, *Tribulus* species distinguished by their schizocarpic fruits, with tuberculate, hairy surfaces and prominent features such as dentate wings or spines. In contrast, *Fagonia* and *Zygophyllum* produce capsular fruits with variable shapes and surface textures, ranging from smooth to moderately hairy. Seed morphology provided additional micro-level diagnostic features, with distinct differences in seed shape, hilum position, and surface sculpturing. *Tribulus* species exhibited a reticulate seed surface, with prolonged ellipsoid to obovoid shapes and a terminal hilum; while *Fagonia* showed puncticulate seed surfaces with globular projections, suborbicular seed shapes, and a subterminal hilum. In contrast, *Zygophyllum simplex* characterized by smooth, ellipsoid seeds and a terminal hilum, further emphasizing its unique taxonomic identity within the group (Plate 1).

Table 2: Data matrix of Morphological characters for six studied taxa of Zygophyllaceae. (0 = absent, 1 = present):

Organ	Character	Character state	*Tribulus pentandrus*	*T. terrestris* var. *terrestris*	*T. terrestris* var. *orientalis*	*Fagonia glutinosa*	*F. scabra*	*Zygophyllum Simplex*
Plant	Habit	Herb	1	1	1	0	0	1
		Shrub or Low shrub	0	0	0	1	1	0
	Duration	Annual	1	1	1	0	0	0
		Biennial	1	0	0	0	0	0
		Perennial	0	0	0	1	1	1
Stem	direction	Prostrate	1	1	0	1	0	0
		Ascending	0	0	1	0	0	0
		Procumbent	0	0	0	0	1	0
		Decumbent	0	0	0	0	0	1
	Surface	Pubescent	1	0	0	0	0	0
		Densely hairy	0	1	1	0	0	0
		Glandular hairs	0	0	0	1	1	0
		Glabrous	0	0	0	0	0	1
Leaf or leaflet	Division of leaves	Simple	0	0	0	0	0	1
		compound	1	1	1	1	1	0
	Size	Equal	1	1	1	0	0	1
		Unequal	0	0	0	1	1	0
	Number of leaflets	Many-foliolate	1	1	1	0	0	0
		Trifoliate	0	0	0	1	1	0
		Unifoliate	0	0	0	0	0	1
	Shape	Ovate-rhombical	0	0	0	1	0	0
		Elliptic-lanceolate	1	1	1	0	0	0
		Ovate-oblong	0	0	0	0	1	0
		Ellipsoidal	0	0	0	0	0	1
	Surface	Hairy on both sides	1	0	0	1	0	0
		Lower side hairy	0	1	1	0	1	0
		Glabrous	0	0	0	0	0	1
	Texture	Papery	1	1	1	1	1	0
		Succulent	0	0	0	0	0	1
	Type of hairs	Eglandular	1	1	1	0	0	0
		Glandular	0	0	0	1	1	0
	Apex	Obtuse	1	0	1	0	0	1

		Acute	0	1	1	0	0	0
		Mucronate	0	0	0	1	1	0
	Base	Symmetric	0	0	0	1	1	1
		Asymmetric	1	1	1	0	0	0
Petiole	Presence	Present	1	1	1	1	1	0
		Absent	0	0	0	0	0	1
Stipule	Shape	Scarious	1	1	0	0	0	0
		Membranous	0	0	1	0	0	1
		Spiny	0	0	0	1	1	0
Flower	Diameter	Less than 5 mm	0	0	0	0	0	1
		6-8 mm	0	0	0	1	0	0
		More than 10 mm	1	1	1	0	1	0
Sepal	Persistence	Persistent	1	1	1	1	1	0
		Caducous	0	0	0	0	0	1
	Shape	Lanceolate	1	1	1	0	0	0
		Ovate	0	0	0	1	1	1
	Surface	Eglandular	1	1	1	0	0	0
		Glandular	0	0	0	1	1	0
		Glabrous	0	0	0	0	0	1
	Apex	Acute	1	1	1	0	0	1
		Mucronate	0	0	0	1	1	0
Petal	Colour	Violet	0	0	0	1	1	0
		Yellow	1	1	1	0	0	1
	Shape	Spathulate	1	0	0	1	0	1
		Clavate	0	1	1	0	0	0
		Clawed	0	0	0	1	1	1
Stamens	Filament base	Without basal scales	1	1	1	1	1	0
		With basal scales	0	0	0	0	0	1
	Intrastaminal glands	Present	1	1	1	0	0	0
		Absent	0	0	0	1	1	1
Ovary	Shape	Globoid	1	0	0	0	0	0
		Oblong	0	1	1	0	0	1
		angular ovoid	0	0	0	1	0	0
		Winged conical	0	0	0	0	1	0
	Style	Present	0	0	0	1	1	1
		Absent	1	1	1	0	0	0
	Stigma	5-lobed	1	1	1	0	0	0
		Simple	0	0	0	1	1	1
	Surface	Hairy	1	1	1	1	1	0
		Glabrous	0	0	0	0	0	1
Fruit	Fruit stalk	Shorter or long as the fruit	0	0	0	1	0	1
		Much longer than fruit	1	1	1	0	1	0
	Size (diameter)	2-3 mm	0	0	0	1	0	1
		4-6 mm	0	0	0	0	1	0
		Over 6 mm	1	1	1	0	0	0
	Type	Schizocarp	1	1	1	0	0	0
		Capsule	0	0	0	1	1	1
	Surface	Pubescent	0	1	0	0	1	0
		Densely hairy	1	0	1	1	0	0
		Tuberculate	1	1	1	0	0	0
	Shape	Obovoid	0	0	0	0	0	1
		Obconical	0	0	0	0	1	0
		Spheroidal	0	0	0	1	0	0
		Pyramidal	1	1	1	0	0	0
	Appendage	5- lobed	0	0	0	1	1	0
		Narrowly winged	0	0	0	0	0	1
		2-4 spines	0	1	1	0	0	0
		Dentate triangular wings	1	0	0	0	0	0
Seeds	Colour	Brown	0	1	1	1	1	1
		Creamy	1	0	0	0	0	0
	Shape	prolonged ellipsoid	1	0	0	0	0	0
		Obovoid	0	1	1	0	0	0
		Suborbicular	0	0	0	1	1	0
		Ellipsoid	0	0	0	0	0	1
	Hilum position	Terminal	1	1	1	0	0	1
		Subterminal	0	0	0	1	1	0
	Sculpture	Reticulate	1	1	1	0	0	0
		puncticulate with globular particles	0	0	0	1	1	0
		Smooth	0	0	0	0	0	1

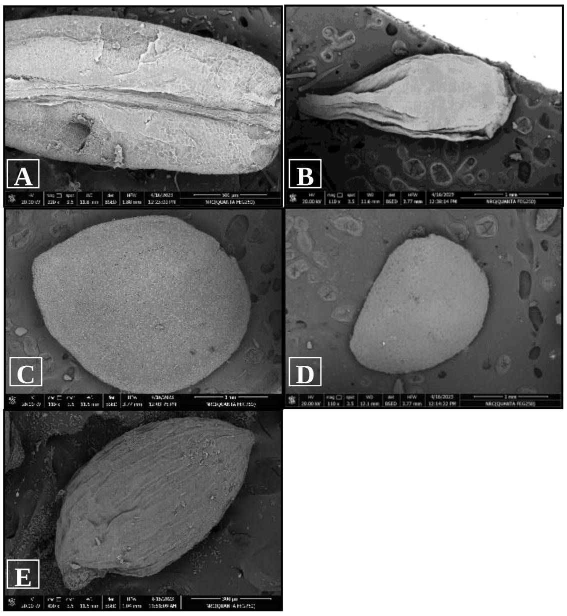

Plate 1: SEM photographs of the studied taxa seeds: (A) *Tribulus pentandrus*; (B) *Tribulus terrestris*; (C) *Fagonia glutinosa*; (D) *Fagonia scabra*; (E) *Zygophyllum simplex*

Micro-morphological variations

Palyno-morphological characters

Pollen characteristics of the studied taxa exhibited significant morphological variation, particularly in aperture type, pollen size, and exine sculpture, reflecting clear intergeneric differentiation. The findings are summarized in Table (3) and representative photographs of pollen grains are shown in Plates (2)

Among the studied genera, *Tribulus* exhibited the largest pollen grains (35–45 µm in diam.). the grains were apolar, poly-pantoporate, and spheroidal although some appeared cup-shaped due to the presence of a monocryptic pore, with a semitectate exine and coarsely reticulate to coarsely reticulate -cristate ornamentation pattern (Plate 2, A-D) In *T. pentandrus*, the tectum showed straight muri and regular hexagonal or pentagonal lumina, while *T. terrestris* featured winding muri with prominent pyramidal thickenings at the intersections and irregularly polygonal lumina. In contrast, *Fagonia* species displayed smaller to medium-sized pollen grains, with mean polar axes ranging from 22.36–26.65 µm and equatorial diameters from 18.46–21.13 µm. The grains were isopolar, tricolporate, and subprolate in shape, with slit-like to fusiform colpi and a tectate exine with microreticulate to foveolate sculpturing pattern (Plate 2, E & H). *Zygophyllum simplex* exhibited the smallest grains among all examined taxa, with a mean polar axis of 9.96 µm and a mean equatorial diameter of 8.88 µm. The grains were prolate-spheroidal, tricolporate, with slit-like colpi and a microreticulate–foveolate tectate exine (Plate 2, I & J).

Table 3: Data matrix of Palynological characters for studied taxa (0 = absent, 1 = present)

Character	Character state	*T. pentandrus*	*T. terrestris* var. *terrestris*	*T. terrestris* var. *orientalis*	*F. glutinosa*	*F. scabra*	*Z. simplex*
P/E ratio	1.00	1	1	1	0	0	0
	1.21-1.26	0	0	0	1	1	0
	1.12	0	0	0	0	0	1
Polarity	Isopolar	0	0	0	1	1	1
	Apolar	1	1	1	0	0	0
Pollen shape	Prolate -spheroidal	0	0	0	0	0	1
	Subprolate	0	0	0	1	1	0
	Spheroidal	1	1	1	0	0	0
Pollen size	Very small	0	0	0	0	0	1
	Small	0	0	0	0	1	0
	Medium	1	1	1	1	0	0
Aperture type	Poly-pantoporate	1	1	1	0	0	0
	Tricolporate	0	0	0	1	1	1
Exine sculpture	Coarsely Reticulate-cristatate	0	1	1	0	0	0
	Coarsely reticulate	1	0	0	0	0	0
	microreticulate to faveolate	0	0	0	1	1	1

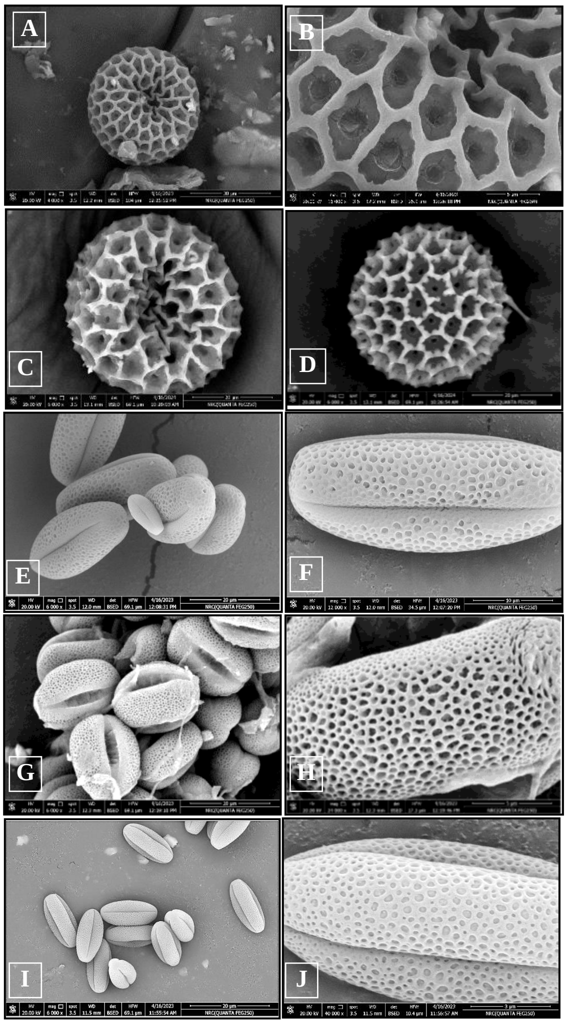

Plate (2): SEM photographs of the studied taxa pollen grains of: (A & B) *Tribulus pentandrus*; (C & D) *Tribulus terrestris*; (E & F) *Fagonia glutinosa*; (G & H) *Fagonia scabra*; (I & J) *Zygophyllum simplex*.

Anatomical characters

Comparative anatomical features, such as the presence or absence of petioles, stomatal positioning, trichome types, leaf outlines, mesophyll differentiation and vascular bundle arrangements, exhibit distinct patterns that are both taxonomically and adaptively significant, allowing clear differentiation among genera and species. Significant variation in anatomical characters was observed among the studied taxa, as presented in Table (4-6) and illustrated in Plates (3-6).

Stem Anatomy

The anatomical features of the stem of the present study showed significant variations in stem outline, cuticle thickness, cortex structure, pith characteristics, and stele type (Table 4 & Plate 3). The outline of the stem cross section was useful for distinguishing between the studied taxa. It varies from terete in *Tribulus* and *Z. simplex* to terete with ridges and furrows in *Fagonia* species. A thick cuticle was observed in all studied taxa except *Z. simplex*, which uniquely possesses stem stomata. The cortex comprised parenchyma in *Tribulus* and *Zygophyllum*, while *Fagonia* exhibited both collenchyma and parenchyma with scattered patches of cortical fibers (Plate 3, D & E).

The vascular system differed significantly, was a eustele with 15-25 discrete vascular bundles in *Tribulus*, and an ectophloic siphonostele with continuous vascular rings in *Fagonia* and *Z. simplex* (Plate 3, D-F). The pith in all taxa composed of thin-walled parenchymatic cells, wide and centric in *Tribulus*, and narrow, excentric or crescent-shaped in the *Fagonia* and *Z. simplex*. Notably, lignified pith parenchyma was observed only in *Z. simplex* (Plate 3, F). Druses crystals were present in *Tribulus* and *Z. simplex*, but absent in *Fagonia* species. Within *Tribulus*, crystals were restricted to the pith of *T. terrestris* var. *terrestris* and were absent in var. *orientalis*. Additionally, crystals were observed in the vascular tissue only in *Z. simplex*.

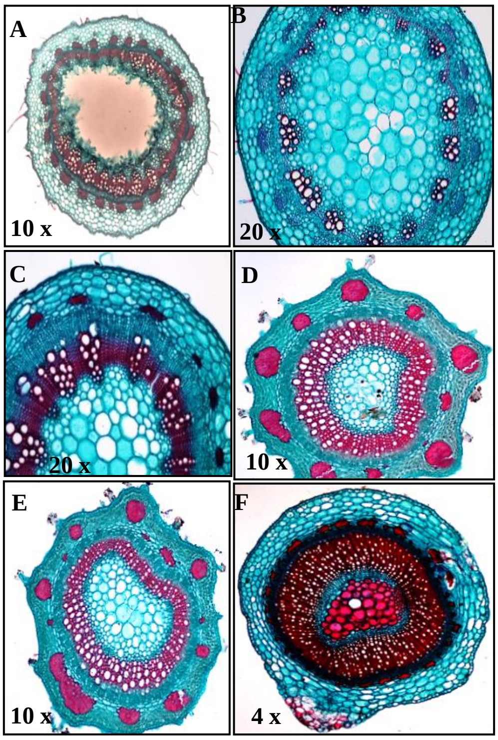

Plate 3: Photographs of transverse stem sections of: (A*) T. pentandrus*; (B)*T. terrestris var. terrestris*; (C) *T. terrestris var. orientalis*; (D) *F. glutinosa*; (E) *F. scabra*; (F) *Z. simplex*.

Table 4: Data matrix of stem anatomical characters for studied taxa (0 = absent, 1 = present)

Character			Character state	T. pentandrus	T. terrestris var. terrestris	T. terrestris var. orientalis	F. glutinosa	F. scabra	Z. simplex
Outline in T.S.			Terete	1	1	1	0	0	1
			±Terete with ridges & furrows	0	0	0	1	1	0
Dermal system	Cuticle		Thin	0	0	0	0	0	1
			Thick	1	1	1	1	1	0
	Trichomes	Presence	Present	1	1	1	1	1	0
			Absent	0	0	0	0	0	1
		Type	Eglandular unicellular	1	1	1	0	0	0
			Glandular	0	0	0	1	1	0
	Epidermal cells		Radial	1	0	0	0	1	1
			Tangential	0	1	1	1	0	0
	Stomata		Present	0	0	0	0	0	1
			Absent	1	1	1	1	1	0
Ground system	Cortex	No. of tissues	One	1	1	1	0	0	1
			Two	0	0	0	1	1	0
		Type of tissues	Polyhedral parenchyma	1	1	1	1	1	1
			Collenchyma	0	0	0	1	1	0
		Crystal presence	Present	1	1	1	0	0	0
			Absent	0	0	0	1	1	1
	Pith	Position	Centric	1	1	1	0	0	0
			Excentric	0	0	0	1	1	1
		Thickness	Hollow	1	0	0	0	0	0
			Solid	0	1	1	1	1	1
		Width	Narrow	0	0	0	1	1	1
			Wide	1	1	1	0	0	0
		Shape	Circular	1	1	1	0	0	0
			Crescent	0	0	0	1	1	1
		Types of parenchyma	Non-lignified	1	1	1	1	1	0
			Lignified (with pitted walls)	0	0	0	0	0	1
		Crystals presence	Present	1	1	0	0	0	0
			Absent	0	0	1	1	1	1
Vascular system		Type of stele	Siphonostele	0	0	0	1	1	1
			Eustele	1	1	1	0	0	0
		No. of V.B.	Cycle	0	0	0	1	1	1
			15-25	1	1	1	0	0	0
		2ry xylem components in interfascicular region	Fibers only	1	1	1	0	0	0
			Vessels & fibers	0	0	0	1	1	1
		2ry xylem components in fascicular region	Vessels	1	1	1	0	0	0
			Vessels & fibers	0	0	0	1	1	1
		Crystals	Present	0	0	0	0	0	1
			Absent	1	1	1	1	1	0

Petiole anatomy

All examined taxa possessed well-developed petioles except *Zygophyllum simplex* which lacked a distinct petiole (Table 5, Plate 4). The diagnostic significance of petiole morphology is evident in their distinct outlines, which varied among the studied species from arc-shaped in *Fagonia scabra,* circular in *F. glutinosa* and circular with ridges in *Tribulus* species (Plate 4, A-E). Notably, these ridges were divergent in *Tribulus pentandrus* and adjacent in *T. terrestris.* Cuticle thickness also varied, being relatively thick in *Fagonia* species and thinner in *Tribulus* species. Cortical tissue structure also varied among species; *F. glutinosa* having multiple layers of polyhedral parenchyma, while the remaining taxa featured an outer layer of elongated chlorenchyma followed by parenchyma. In *Tribulus*, chlorenchyma confined to the ridges, whereas in *F. scabra* it formed a continuous ring. Vascular bundle arrangement further served as a reliable distinguishing feature; *Tribulus* species exhibited four main oval-shaped vascular bundles with two smaller subsidiary bundles located at the ridges (Plate 4, A-C). Meanwhile, *Fagonia* species had a single arch-shaped vascular bundle, with *F. scabra* also showing distinctive scleridic patches beneath the vascular tissue (Plate 4, E). Additionally, druses crystals were observed in *Tribulus* taxa but were absent in *Fagonia* species.

Table 5: Data matrix of leaf petiole characters for studied taxa (0 = absent, 1 = present)

System	Character	Character state	*T. pentandrus*	*T. terrestris* var. *terrestris*	*T. terrestris* var. *orientalis*	*F. glutinosa*	*F. scabra*	*Z. simplex*
	Occurrence	Present	1	1	1	1	1	0
		Absent	0	0	0	0	0	1
	Outline in T.S.	Circular with 2- slightly ridges	0	1	0	0	0	0
		Circular with 2-adjacent ridges	0	0	1	0	0	0
		Circular with 2-divergent ridges	1	0	0	0	0	0
		± Circular	0	0	0	1	0	0
		Arc shaped	0	0	0	0	1	0
Dermal system	Cuticle thickness	Thin	1	1	1	0	0	0
		Thick	0	0	0	1	1	0
	Trichomes	Glandular	0	0	0	1	1	0
		Eglandular	1	1	1	0	0	0
	Epidermis arrangement	Radially isodiametric	1	0	0	0	0	0
		Tangential	0	1	0	0	0	0
		Mixed	0	0	1	1	1	0
Ground system	No. of tissue	One	0	0	0	1	0	0
		Two	1	1	1	0	1	0
	Types of tissue	Elongated chlorenchyma (palisade)	1	1	1	0	1	0
		Polyhedral parenchyma	1	1	1	1	1	0
	Position of elongate chlorenchyma	In complete ring	0	0	0	0	1	0
		Restricted at the ridges	1	1	1	0	0	0
Vascular system	No. of V.B.	One	0	0	0	1	1	0
		Six (4-main V.B. & 2-subsidary)	1	1	1	0	0	0
	Shape of main V.B.	Arch shaped	0	0	0	1	1	0
		Oval	1	1	1	0	0	0
	Druses	Present	1	1	1	0	0	0
		Absent	0	0	0	1	1	0
	Sclerieds	Present	0	0	0	0	1	0
		Absent	1	1	1	1	0	0

Leaf Blade Anatomical Characters

The anatomical characters of the leaf blades of the studied taxa are summerized in Table (6) and illustrated in Plate (5). These features revealed distinct differences among the studied taxa.

The outline of the leaf blade in cross-section was found to be valuable for species differentiation, ranging from plano -convex to oblong in *Zygophyllum simplex*. Based on mesophyll structure, three main types were identified: dorsiventral (bifacial) leaves with a subdermal hypodermis and Kranz anatomy, as observed in *Tribulus* species; isobilateral leaves, in *Fagonia* species; and radial or centric leaves, in *Z. simplex*. In *Tribulus* species, the palisade tissue was uniseriate and confined on the adaxial surface (Plate 5, A-F), whereas in *Fagonia* species it was biseriate on both adaxial and abaxial surfaces. Spongy mesophyll consisted of 2–3 layers in *Tribulus* species, and was reduced to 1–2 layers in *Fagonia* species (Plate 5, G-J). The blade anatomy of *Z. simplex* was unique, consisting of a uniseriate epidermis of isodiametric cells enclosing a centric mesophyll. This mesophyll was further differentiated into an outer palisade layer and an inner water-storage parenchyma composed of 3–4 layers of large, thin-walled cells (Plate 5, K-L). The midrib anatomy also showed taxonomic significance. In *Tribulus* species and *Fagonia glutinosa*, the abaxial outline of the midrib was U-shaped; flattened-ribbon in *Fagonia scabra*; while in *Z. simplex*, the midrib was indistinct. A single central vascular bundle was present in all species except *F. scabra*, which exhibited two main vascular bundles (Plate 5, I-J). The number of lateral vascular bundles ranged from 10–18 in most taxa, and reached up to 20–22 in *T. terrestris I*. In *Z. simplex*, several small peripheral bundles were embedded within the inner palisade layer, with the xylem oriented outward and the phloem inward. Druses crystals were observed in all *Tribulus* species and absent in *Fagonia* species and *Z. simplex*, further distinguishing these genera.

Table 6: Data matrix of lamina anatomical characters for studied taxa (0 = absent, 1 = present)

Character			Character state	*T. pentandrus*	*T. terrestris* var. *terrestris*	*T. terrestris* var. *orientalis*	*F. glutinosa*	*F. scabra*	*Z. simplex*
Dermal system		Stomata level	Raised	1	1	1	0	1	0
			Sunken	0	0	0	1	0	1
	Trichomes	Occurrence	Present	1	1	1	1	1	0
			Absent	0	0	0	0	0	1
		Types	Eglandular hairs	1	1	1	0	0	0
			Glandular hairs	0	0	0	1	1	0
	Epidermal cells	Cuticle thickness	Thick	0	0	0	1	1	1
			Thin	1	1	1	0	0	0
		Arrangement	Radial – tangential	1	1	1	1	1	0
			Radial	0	0	0	0	0	1
		Anticlinal walls	Recessed, ± straight	1	1	1	0	0	1
			Recessed, undulate	0	0	0	0	1	0
			Raised, straight	0	0	0	1	0	0
		Periclinal walls	Striated tabular to concave	0	1	1	0	0	0
			Smooth tabular to convex	1	0	0	1	1	0
			Papillate ± tabular	0	0	0	0	0	1
Mesophyll		Type	Dorsiventral	1	1	1	0	0	0
			Isobilateral	0	0	0	1	1	0
			Radial or centric	0	0	0	0	0	1
		palisade tissue	One row in one side	1	1	1	0	0	1
			2-rows in each side	0	0	0	1	1	0
		spongy tissue	1-2 rows	0	0	0	1	1	0
			2-3 rows	1	1	1	0	0	1
		Wate storage layer	Present	1	1	1	0	0	0
			Absent	0	0	0	1	1	1
Midrib		Abaxial outline	Flattened- ribbon	0	0	0	0	1	0
			U- shaped	1	1	1	1	0	0
			Not obvious	0	0	0	0	0	1
Vascular system		main vascular bundle	Single	1	1	1	1	0	1
			Two	0	0	0	0	1	0
		No. of lateral vascular bundles	10 – 18	1	0	1	1	1	0
			20 – 25	0	1	0	0	0	1
Crystals			Present	1	1	1	0	0	0
			Absent	0	0	0	1	1	1

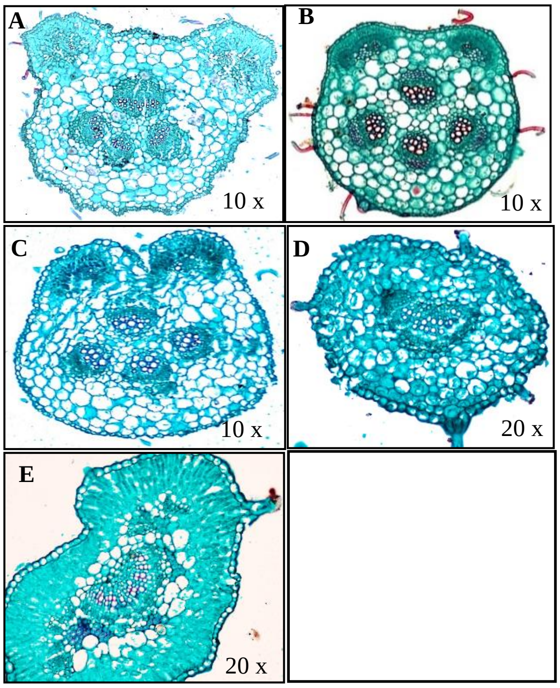

Plate 4: Photographs of transverse petiole sections of: (A) *Tribulus pentandrus*; (B) *Tribulus terrestris var. terrestris*; (C) *Tribulus terrestris var. orientalis*; (D) *Fagonia glutinosa*; (E) *Fagonia scabra*.

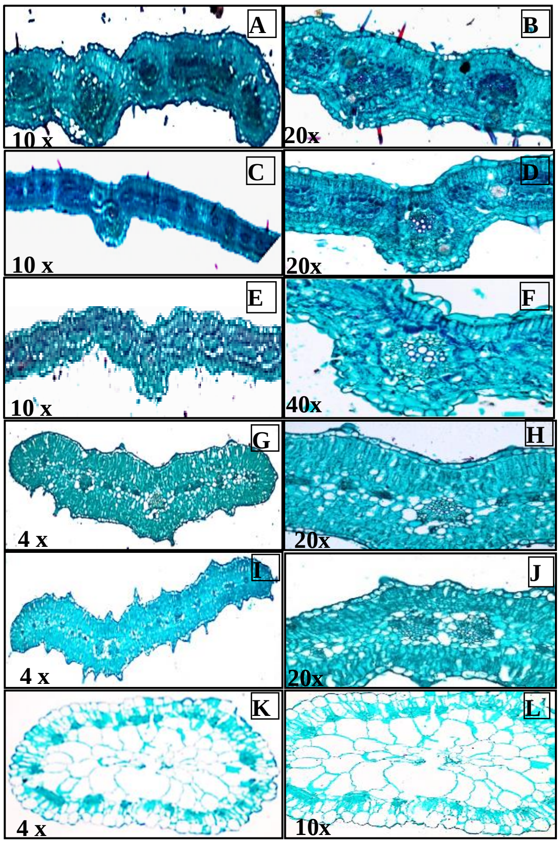

Plate 5: Photographs of transverse lamina sections of: (A-B) *Tribulus pentandrus*; (C-D) *Tribulus terrestris var. terrestris*; (E-F) *Tribulus terrestris var. orientalis;* (G-H) *Fagonia glutinosa*; (I-J) *Fagonia scabra*; (K-L) *Zygophyllum simplex.*

Epidermal features and Indumentum

The current study focuses on micro-morphological features of trichomes, epidermal cells, and stomata. The data on the foliar epidermal characters of the taxa examined are presented in Table (6) and Plate (6).

Among the studied taxa, *Zygophyllum simplex* was the only completely glabrous species, while *Tribulus* and *Fagonia* species exhibited varying degrees of indumentum, from pubescent to densely hairy. Two distinct trichome types were observed: eglandular unicellular hairs in *Tribulus* taxa and glandular hairs in *Fagonia* species. The epidermal cells were polygonal in *Tribulus* taxa and *Z. simplex* (Plate 6, A-C & F), but irregular and indistinct in *Fagonia* species (Plate 6, D, E). Anticlinal wall patterns also varied, with *Tribulus* taxa and *Z. simplex* showing recessed, ± straight walls, while *F. scabra* had recessed, undulate walls (Plate 6, E), and *F. glutinosa* displayed raised, straight walls. Regarding periclinal walls, *T. terrestris* had striated tabular to concave forms, *Fagonia* species had smooth tabular to convex surfaces, and *Z. simplex* exhibited papillate, ± tabular walls. Stomatal position was another distinguishing feature; raised in *Tribulus* taxa and *F. scabra*, whereas *F. glutinosa* and *Z. simplex* exhibited sunken stomata (Plate 6, D & F).

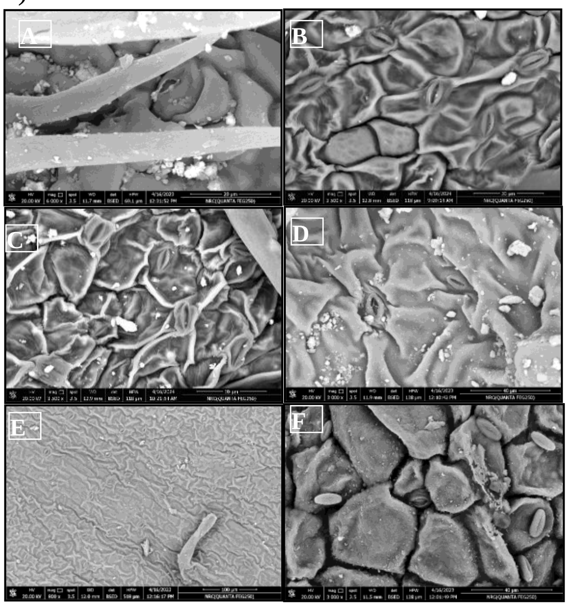

Plate 6: SEM Photograph of the lamina adaxial surface showing epidermal characteristics of: (A) *Tribulus pentandrus*; (B) *Tribulus terrestris* var. *terrestris*; (C) *Tribulus terrestris* var. *orientalis*; (D) *Fagonia glutinosa*; (E) *Fagonia scabra*; (F) *Zygophyllum simplex*.

Cluster analysis

A multivariate statistical analysis was performed using 219 morphological, palynological, and anatomical character states, each represented by binary codes (0, 1), across the six studied taxa belonging to the family Zygophyllaceae. The analysis aimed to elucidate patterns of similarity and divergence among the studied taxa and to evaluate their taxonomic affinities. The cumulative data presented in Tables (2-6) and the resulting phenetic relationships are illustrated through a dendrogram (Fig. 1). The dendrogram shows that the studied taxa were separated into two main series (I, II), which included four clusters (I, II, III, and IV) at a similarity level of 32.08%. Series I comprised all representatives of the genus *Tribulus*, which characterized by many foliate leaves, schizocarpic fruits, pantoporate apolar pollen with coarse reticulate sculpturing, dorsiventral mesophyll with kranz anatomy and eglandular hairs. This series was further subdivided into two clusters at a higher similarity level of 84.32%. Cluster I included *T. pentandrus*, whereas Cluster II grouped the two varieties of *T. terrestris* (*T. terrestris* var. *terrestris* and *T. terrestris* var. *orientalis*) showing the highest degree of similarity (93.07%). Series II included taxa from the genera *Fagonia* and *Zygophyllum*, which were divided into two distinct clusters at a similarity level of 45.71%. This series is characterized by uni-or trifoliate leaves, capsule fruits, tri-colpate or colporate pollen, isobilateral or centric mesophyll without kranz anatomy and glandular hairs or glabrous. Cluster III comprised *Fagonia glutinosa* and *F. scabra*, exhibiting a high internal similarity of 81.93%, indicating close morphological and anatomical affinities. In contrast, Cluster IV was composed solely of *Zygophyllum simplex*, indicating its relative distinctiveness within this group.

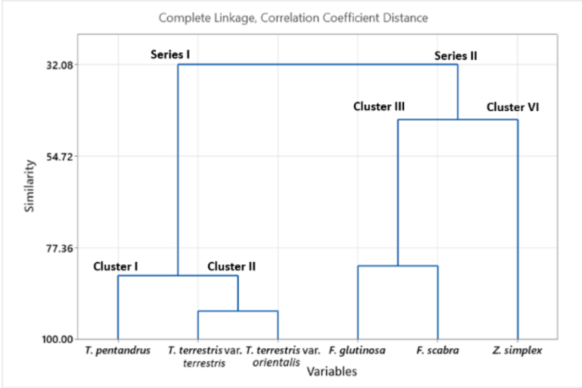

Fig. 1 Dendrogram indicating the relationships among the six studied taxa of Zygophyllaceae based on combined morphological, palynological, and anatomical data analysis using Minitab statistical software

Discussion

The present study provides a comprehensive comparative analysis of six taxa from three genera within the family *Zygophyllaceae*, focusing on a range of morphological, palynological, and anatomical traits. The diagnostic features examined include leaf architecture, floral morphology, fruit and seed characteristics, as well as palynological and anatomical variations. When considered collectively, these characters hold significant taxonomic value and contribute to the accurate identification and delimitation of taxa at both the genus and species levels.

A Comparative analysis of *Tribulus* species, *Fagonia* species, and *Zygophyllum simplex* revealed distinct morphological differences that support their generic separation within Zygophyllaceae, Vegetative traits, particularly leaf structure and trichome type, as well as floral morphology, fruit and seed characteristics, proved to be taxonomically informative. These findings are consistent with previous studies by El-Hadidi (1974, 1978), Hosni (1988), Beier (2005), and Abdel Khalik & Hassan (2012). This distinction is supported by the findings of Shamso et al. (2013), who conducted a numerical analysis of morphological characters across various species within Zygophyllaceae. Their study revealed the clear separation of *Tribulus* as a distinct phenon, apart from the cluster that encompassed both *Fagonia* and *Zygophyllum* species. Draz et al. (2024) documented two distinct forms of *Tribulus terrestris*, identified as *T. terrestris* var. *terrestris* (Form I) and *T. terrestris* var. *orientalis* (Form II), highlighting the notable morphological plasticity of this species. Based on our findings, the morphological traits largely overlap between the two varieties, with the exception of differences in fruit surface characteristics, appendages, and the number of vascular bundles in the stem. Overall, the morphological diversity observed supports the current taxonomic delineation of these taxa and reflects ecological adaptations. These characters may serve as reliable diagnostic features for identification and classification.

Pollen grain characteristics have proven valuable for evaluating the taxonomy of Zygophyllaceae (Perveen & Qaiser, 2006; Bukhari et al., 2014). Significant variation was observed across the three studied genera *Tribulus*, *Fagonia*, and *Zygophyllum*, particularly in aperture type, pollen size, and exine sculpturing. In Tribulus, pollen grains exhibited a high degree of ultrastructural uniformity, especially regarding aperture type, exine ornamentation, and overall shape and size. These observations are in agreement with previous findings reported by Praglowski (1987), Perveen & Qaiser (2006), Ben Nasri-Ayachi & Nabli (2009), Semerdjieva (2011), and El-Atrush et al. (2015). Tai et al. (2021) reported dimorphic pollen in Libyan accessions of *Fagonia*, characterized by the presence of two distinct aperture types within a single taxon. In contrast, the current study observed monomorphic pollen in Egyptian populations of *F. glandulosa*, exhibiting only a single aperture type. This variation may be attributed to geographic or environmental influences on pollen development and highlights the need for further molecular and biogeographic investigation. The two Egyptian *Fagonia* species examined in this study exhibited prolate pollen shapes, tricolporate apertures, and microreticulate-foveolate exine sculpturing, whereas *Zygophyllum simplex* displayed the smallest pollen grains among all taxa studied. These results are consistent with previous findings by El-Ghamery et al. (2002), Perveen & Qaiser, (2006); Abd El Khalik, (2012); Bukhari, (2014). Overall, the distinct palynological differences observed among *Tribulus*, *Fagonia*, and *Zygophyllum* validate their separation at the generic level within Zygophyllaceae. These results highlight diagnostic the value of pollen morphological traits in resolving taxonomic relationships and underscore their potential as supplementary tools in future phylogenetic and ecological studies.

Anatomical characteristics are valuable taxonomic traits that play a significant role in resolving some taxonomic challenges at both the genera and species levels (Metcalfe & Chalk 1957; Ganeva et al. 2009; Song & Hong 2018; Sathee & Jain, 2022; Gabr & Ragab 2023). They also reflect a plant adaptation to the extreme environmental conditions (Hameed et al. 2009; Ashraf & Harris, 2013; Elkharbotly, 2016; Taia et al., 2017).

The outline of the stem cross section varied from terete in *Tribulus* taxa and *Zygophyllum simplex* to terete with ridges and furrows in *Fagonia* species, the same shape of the stem outline has been described by many authors: El Hadidi (1966); Taia et al. (2017). The vascular system showed notable variation among the studied taxa. *Tribulus* exhibited an eustele, while both *Fagonia* and *Zygophyllum simplex* possessed an ectophloic siphonostele characterized by continuous vascular rings. These findings are consistent with those reported by Ahmed & Mohamed (2005b) and Gabr & Ragab (2023). In the present study, the pith consisted of thin-walled parenchymatous cells in all taxa, being wide and centric in *Tribulus*, but narrow, excentric, or crescent-shaped in *Fagonia* species and *Z. simplex* (Elkamali et al., 2016). Notably, lignified pith parenchyma with pitted walls was observed only in *Z. simplex* (Sheahan & Cutler, 1993). This finding supports the explanation proposed by El Hadidi (1966), who stated that excentric pith formation may result from abnormal secondary xylem growth and lignification of pith cells, with pitted walls developing after the initiation of secondary growth.

The anatomical characteristics of the petiole also showed considerable variation among the studied taxa, providing valuable diagnostic features for species delimitation. Distinct petiole outlines, variations in cortical tissue structure, and the arrangement of vascular bundles served as reliable distinguishing features. These anatomical characteristics features are consistent with previous findings reported by Metcalf & Chalk (1957) and Gabr & Ragab (2023), highlighting the taxonomic significance of petiole morphology.

Leaf anatomical features provided a significant taxonomic distinction among the studied taxa.

A distinct abaxial layer of water storage cells (hypodermal layer) was observed only in Tribulus taxa, aligning with the findings of Metcalfe & Chalk (1957), Sheahan & Cutler (1993), and Nikolova & Vassilev (2011). The variability in mesophyll types ranging from dorsiventral and isobilateral to centric, along with differences in vascular tissue organization, has proven useful for both species and genus identification, and supporting its relevance in taxonomic studies. In Tribulus, the dorsiventral mesophyll comprises a single palisade layer and 2–3 compact spongy layers with reduced intercellular spaces, a structure associated with efficient CO_2 transfer in the C4 pathway (Welkie & Caldwell, 1970). In contrast, Zygophyllum simplex exhibits a centric mesophyll, differentiated into an outer photosynthetic palisade layer and an inner water storage parenchyma composed of 3–4 rows of cells, consistent with the observations of Waly et al. (2011). These anatomical adaptations are characteristic of xerophytic environments, as supported by Zaman & Padmesh (2009), Abd Elhalim et al. (2016), and El Kharbotly (2016). The consistent presence of Kranz anatomy strongly indicates that C_4 photosynthesis is a defining feature of both *Tribulus* taxa and *Z. simplex*. In *Tribulus*, a complete Kranz sheath was observed encircling the vascular bundles, whereas in *Z. simplex*, the sheath appeared arc-shaped (Crookston & Moss, 1972; Sheahan & Cutler, 1993; Sheahan & Chase, 1996; Sage et al., 2011; Edwards & Voznesenskaya, 2011; Lauterbach et al., 2016). These findings are in accordance with Taia et al., (2017), who reported that the leaves of *Fagonia* species lack Kranz anatomy. In addition to the previous findings, druses crystals were absent in all *Fagonia* species but present in *Tribulus* and *Zygophyllum simplex*. In *Tribulus,* crystals were observed in the petiole, leaflets, and the stem pith of var. *terrestris*. In *Z. simplex,* druses were restricted to the vascular tissue of the stem. The complete absence of both Kranz anatomy and crystals in *Fagonia* further supports its anatomical distinction from *Tribulus* and *Zygophyllum*.

The present findings support the generic specificity of trichomes, as noted by Sheahan & Cutler (1993) and Gabr (2023). *Zygophyllum simplex* was completely glabrous, while *Tribulus* and *Fagonia* species showed varying degrees of indumentum. Eglandular unicellular hairs were observed in *Tribulus*, and glandular hairs in *Fagonia*, both of which provide valuable taxonomic characters. These epidermal features, consistent with the observations of Abdel Khalik & Hassan (2012) and Gabr et al. (2013), support species and genus-level distinction within the family.

Cluster analysis of the combined characters revealed high intra-generic similarity, particularly between the two *Tribulus* varieties and between *Fagonia glutinosa* and *F. scabra*, and lower inter-generic similarity. *Tribulus* formed a distinct cluster, clearly separated from *Fagonia* and *Zygophyllum*, which grouped together. The separation of *Tribulus* from the other taxa supports its distinctiveness, characterized by several unique morphological (many foliate leaves and schizocarpic fruits), anatomical (dorsiventral mesophyll with Kranz anatomy and the presence of eglandular hairs), and palynological (pantoporate, apolar pollen with coarse reticulate sculpturing) characters. These findings align with traditional taxonomic classifications and previous numerical studies (Abdel Khalik, 2012; Shamso et al., 2013; Gabr, 2023), supporting the placement of *Tribulus* in its own subfamily (*Tribuloideae*) or even a separate family (*Tribulaceae*), distinct from *Zygophylloideae*, which includes *Fagonia* and *Zygophyllum* (Sheahan & Cutler, 1993; El Hadidi, 1978; Saleh et al., 1982). Additionally, this statistical analysis is consistent with previous chemosystematic and fingerprinting studies by Draz et al. (2024, 2025), further supporting the distinct separation of *Tribulus* from other taxa. *Fagonia* species showed closer affinity to *Zygophyllum simplex*, suggesting a possible evolutionary relationship. Overall, these results confirm clear taxonomic distinctions among the studied genera.

Conclusion

The combined morphological, palynological, and anatomical data of studied taxa of *Tribulus*, *Fagonia*, and *Zygophyllum* revealed clear intergeneric and interspecific distinctions within Zygophyllaceae *s. l*. Morphological traits such as leaf form, trichome type, floral and fruit structures, along with palynological features, particularly pollen aperture and exine patterns, provide valuable diagnostic characters for taxonomic delimitation. *Tribulus* is notably distinct in its pollen and fruit morphology, supporting its recognition at a higher taxonomic level. Anatomical characters, including stomatal position, leaf outline, mesophyll differentiation, vascular arrangement, and crystal distribution, further reinforce these distinctions. By integrating morphological, palynological, and anatomical investigations with previously performed chemosystematic and fingerprinting analyses, this study provides strong evidence supporting the placement of *Tribulus* in a distinct subfamily (Tribuloideae) or even a separate family (Tribulaceae), apart from Zygophylloideae, which comprises *Fagonia* and *Zygophyllum*. These findings highlight the importance of integrative anatomical, palynological, and morphological studies in resolving taxonomic relationships and understanding ecological adaptations within the family Zygophyllaceae.

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الملخص العربي

عنوان البحث: التقييم الشامل للصفات المورفولوجية و التشريحية و حبوب اللقاح لبعض الأنواع المختارة من الفصيلة الرطريطية فى مصر

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تقدّم هذه الدراسة تحليلاً مقارنًا شاملاً لستة من الأنواع المصرية التابعة للعائلة الرطريطية ، و الممثلة للأجناس *Fagonia*، و*Zygophyllum*، و*Tribulus*. و قد تم عمل دراسة متكاملة تضم دراسات مورفولوجية، وتشريحية، وصفات حبوب اللقاح بإستخدام كلا من المجهر الضوئي(LM) والمجهر الإلكتروني الماسح(SEM) . و يعد الهدف الرئيسي للدراسة هو تقييم الأهمية التصنيفية لهذه الصفات المدروسة وتعزيز دقة تعريف وتصنيف هذه الأنواع داخل العائلة.
وقد اشتملت الدراسات المورفولوجية صفات تركيب الأوراق ، والصفات المظهرية للأزهار والثمار، بالإضافة إلى خصائص البذور، و على الجانب الاخر ركزت الدراسات التشريحية على تنظيم و توزيع الأنسجة في السيقان والعناقيد الورقية وأنصال الأوراق ، مع التركيز على صفات البشرة الخارجية للأوراق، وأنماط الأنسجة الوعائية، وتوزيع البلورات. أما تحليل حبوب اللقاح فقد أظهر تباين واضح في حجم حبوب اللقاح، وقطبيتها، وأنواع الفتحات، وأنماط زخرفة السطح الخارجى لها.
كما تم تسجيل وتحليل ٢١٩ صفة إحصائيا بإستخدام برنامج Minitab ، و نتج عن هذا التحليل شجرة Dendrogram مبينة انفصال أنواع *Tribulus* عن باقي الأنواع المدروسة، في حين أظهرت تقاربا بين كلا من أنواع *Fagonia* و *Zygophyllum simplex* . كما أوضحت هذه الدراسة القيمة التصنيفية للتكامل بين النتائج المورفولوجية والتشريحية وحبوب اللقاح فى اعتبار جنس *Tribulus* كوحدة تصنيفية متميزة داخل عائلة Zygophyllaceae.
وعليه فإن هذه الصفات المجتمعة تعد قيم تصنيفية في تحديد الأنواع وتصنيفها بدقة على المستويين الجنسي والنوعي.