Palynotaxonomic studies on Boraginaceae in Saudi Arabia and its taxonomic significance

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Abstract: The present study assesses the taxonomic significance of pollen morphology and micro - and macro-morphological characters of the 21 Taxa representing 12 genera collected mainly from Saudi Arabia, belonging to 2 subfamilies (Heliotropoideae and Boragonoideae) with 5 tribes of (Echieae, Lithospermeae, Cynoglosseae, Boraginea and Eritricheae). Pollen morphology was examined by scanning electron microscopy (SEM) and the data were analyzed using SPSS. The pollen and morphological data of the studied taxa were investigated using cluster analysis to find whether or not pollen characteristics can support the classifications based only morphological characters and explore variations in both pollen and morphological characters. The cluster analysis of 24 characters and 60 character states of pollen leads to the recognition of 8 pollen types. The pollen of the studied taxa were divided into four major groups based mainly on exine ornamentation, size, symmetry, polarity of pollens and number ,type, shape, position of apertures .The results show that Groupings based only on pollen characteristics alone did not align completely with those based on morphological data. The palynological results ensure the eurypalynous type of the family and support the proposal that *Echium* belongs to tribe Lithospermeae rather than tribe Echieae. Morphological results also suggest that *Paracaryum*, *Microparacaryum*, *Lappula*and *Trichodesma* are included in the Subfamily: Cynoglossoideae rather than being different tribes of Subfamily Boragonoideae.
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1. Introduction

Boraginaceae is one of the most eurypalynou sfamilies (Clarke ,1977), in which

a large proportion of the species can be individually recognized by their palynological characters (Díez and Valdés,1991and Khatamsaz,2001).Pollen morphology, therefore, has great potential as a means of classification, and is frequently utilized to clarify taxonomic questions(Clarke,1997;Nowicke and Miller, 1989andDíez and Valdés, 1991).

Boraginaceae includes ca.2450 species distributed among ca. 100-142 genera, (Nowicke and Miller,1990;Mabberley,1997;Långström and Chase, 2002;APG II, 2003;Mabberley,2008andCohen,2014).It occurs worldwide, especially in the tropics and subtropics.

Johnston (1957) and Cronquist (1981)treated the Boraginaceaebroadly as composedof five subfamilies: Cordioideae; Ehretioideae; Heliotropioideae; Boraginoideae and Wellstedtioideae, The subfamilies are distinguished from one another on the basis of differences in plant habit, gynoecial morphology, and fruit characters. Takhtajan (1987) elevated three of the five subfamilies to family status; namely, Ehretiaceae; Cordiaceaeand Wellstediaceae. Alternatively (Böhle and Hilger,1997; Gottschlingand Hilger, 2001and Hilger *et al*., 2004)on the basis of DNA chloroplast supported the treatment of the subfamilies of the Boraginaceae, s.l., as the separate families Boraginaceae, s.s.; Cordiaceae; Ehretiaceae; and Heliotropaceae.By this latter classification, the Boraginaceae is essentiallyequivalent to the subfamily Boraginoideae. Stevens (2001)recognizedfive subfamilies within Boraginaceae s.l., Boraginoideae, Cordioideae, Ehretioideae, Heliotropioideae, and Hydrophylloideae.

Weigend and Hilger (2014) advocated the recognition of the morphologically well-differentiated clades of Boraginales at family rather than subfamily level and therefore, Boraginales consists of a total of seven families: Boraginaceae s.str., Codonaceae, Cordiaceae, Heliotropiaceae, Hydrophyllaceae, Lennoaceae and Wellstediaceae.

In Boraginaceae, tribes frequently have been recognized based on a combination of style division, stigma number, position of nutlet attachment, and nutlet sculpture. However, this tribal classification has been considered weak(Al Shehbaz, 1991) mainly because fruits from this group are suggested to be under evolutionary selective pressures, thus, being changeable characters (Johnston ,1924and Selvi *et al.*,2011) unsuitable for taxonomic purposes. This has led to the acceptance between four (Långström and Chase, 2002) and 13 tribes and eight subtribes (Popov,1953) depending on the author, and has resulted in increased taxonomic complexity within the family(Cohen ,2014).Therefore phylogenetic analyses(Långström and Chase, 2002; Mansion, 2009; Selvi *et al*., 2011 and Nazaire and

Hufford,2012) have led to the identification of four to five tribes-Boragineae, Cynoglosseae, Echiochileae Lithospermeae, and Trichodesmeaethat are congruent with the traditional taxonomic system of Boraginaceae. The phylogeneticrelationships among tribes are becoming betterresolved and more supported, but relationships within each tribe remain largelyunresolved (Hilger, 2004 and Selvi *et al*., 2011).

The pollen morphology of the family Boraginaceae have been studied by several authors (Clarke ,1977and Díez and Valdés, 1991). From previous studies, it was noted that two tribes, Cynoglosseae and Eritricheae, have a specialized pollen morphology (Sahay, 1973;Ahn and Lee, 1986 and Díez, 1994). Pollen grains of these tribes were described as being heterocolpate, consisting of three normal, "true" apertures and three pseudoapertures. Bigazzi and Selvi (1998) in their comparative survey on pollen morphology in the Boragineae, recognized 15 pollen morphotypes and showed that tectum sculpture and aperture morphology is a discriminatory character, while the number of aperture appear variable within genera and even single species.

Långström and Chase (2002) revised the tribes within subfamily Boraginoideae onthe basis of *atp*B plastid gene and parsimony analysis and recognized fourtribes, Boragineae (well supported with a bootstrap of 100), Lithospermeae (with bootstrap support of 70), andboth Eritrichieae and Cynoglosseae with moderately supported clade, with genera from both tribes paraphyletic with respect to each other. They alsoused the occurrence of heterocolpate pollen and single style with an undivided stigma to distinguish the tribe from others within subfamily Boraginoideae. In addition, a novel clade, Echiochileae, was recognized at the tribal level which was distinguished by square or spherical pollen, a bifid style with two stigmas, nutlets with a basal attachment scar (areole), and a flat to pyramidal gynobase.(Långström and Chase 2002 and Luebert, 2010)

In addition, Boraginoideae has some confusing genera such as Echiolion Desf and which has uncertain position within Boraginoideae .Johnston (1924) placed Echiochilon in Lithospermeae, Johnston (1957) stated its relationships to be unclear. Sauvage (1954) and Verdcourt (1991)temporarily placed Echiochilon in Eritrichieae. UntillJohnston (1957), none of the species of Echiochilon with actinomorphic flowers were placed in Echiochilon but rather in Sericostoma s.lato; they were treated together because morphological characters indicate that they might be closely related Johnston (1957). However, Riedl (1967) kept Echiochilon in Eritrichieae. Verdcourt (1991) have clearly expressed their doubts regarding the tribal position of Echiochilon..

Moreover; Genus *Echium* L. had been included in the tribe Echieae for a long time

(DE Candolle *et al*., 1844 and Gurke, 1893); however many authors(Al Shehbaz ,1991;Riedl ,1997;Hilger*et al.*, 2005 and Gottschling, 2001) placed *Echium* in the tribe Lithospermeae. This was confirmed by molecular data, from plastid at pB DNA sequences (Romeiras *et al.*,2008).

The purpose of this study is to provide a survey of pollen types of the studied taxa, discuss some aspects that may contribute to a better insight in the difficult taxonomy of the studied taxa and to shed further light on the systematic relationships occurring among genera in order to clarify the systematic relationships within the tribe. The study also investigates the detailed characters of both pollens and micro and macro morphological characters to find whether or not pollen characteristics can support the classifications based only morphological characters.

2. Materials and Methods

Plant samples were collected from natural populations in Saudi Arabia. A list of specimens investigated is given in Table1.Taxonomic nomenclature follows of Saudi Arabia(Migahid, 1978),Flora of Eastern Saudi Arabia (Mandaville, 1990) and Flora of Egypt (Boulos, 2000).Pollen samples were collected from the field in Saudi Arabia, the samples included 21 Taxa representing 12 genera collected mainly from many geographic regions. For SEM, the acetolysed pollen grains were air dried mounted on aluminium stubs and coated with a 30 nm layer of gold/palladium for eight minutes at high vacuum in a sputtering chamber (Jeol JFC-1100 Ion Sputter). After coating, the specimens were viewed with a Jeol JSM 5300 electron microscope at 25 KV. T. Olympus sz61 Stereomicroscope equipped with Camera was used for morphological data.

Palynological description of pollen types were made based on the quantitative and qualitative morphologic results. The terminology used is in accordance with (Erdtman, 1952;Reitsma, 1970; Frenguelli, 2003;Punt*et al*., 2007andHesse *et al.*, 2009). Morphological were studied from fresh materials and dried specimens.

Data analysis

Analysis of pollen characters as a tool to recognize morphotypes has been applied to palynology by many authors (Huysmans*etal*.,1994) due to the low dependency of the pollen stereo structural features on environmental factors. Cluster analysis relies on the discriminate analysis to check if the groups are statistically significant and if the variables significantly discriminate between the groups.

Forhierarchical cluster analysis, the data were analyzed using the SPSS 14.0 for windows 10 program. The characters were scored as character

and character state for multi-state qualitative characters and continuous quantitative measures were treated as such; were averaged on 5-10 measures for each taxon.

The output was plotted in the form of dendrogram .The dendrogram was based on Word method (square Euclidean distance).Each taxon was scored for a set of 24 characters for palynological data and 19 characters for morphological data.

Table.1 List of taxa used in this study and their distribution in Saudi Arabia along with their sub familial positions in different systems of classification

No	Studied Taxa	Distribution	Subfamily
1	*Alkanna orientalis (L.) Boiss.*	KSA, Alasyaha Al-qassimregion , 14.3.2013, I. Tolba *et al.*	Boraginoideae
2	*Anchusa aegyptiaca* (L.)A.DC	KSA , Wadi El Rumah NW of Uniaza, 3.2012, Gazer *et al.*	Boraginoideae
3	*Arnebia decumbens* (Vent.)Coss.& Kralik *Arnebia hispidissima* (Lehm.)	KSA, Alshamassia (Al-qassim region)- Wadi ElRumah Zalat *et al.*, 2.2015.	Boraginoideae
4	A.DC.	Alshmassia- Wadi El Rumah , Zalat *el at*.,2.2015	
5	*Arnebia linearifolia* A.DC.	KSA ,Wadi El Rumah NW of Uniaza , 2.2010 , El Melegi *et al.*	Boraginoideae
6	*Arnebia tinctoria* Forssk.	KSA,Gizan, 17.4.2013, L. Shalabey.	Boraginoideae
7	*Echiochilon jugatum* I.M.Johnst.	Alasyaha Al-qassim region ,14.3.2011 , Gazer *et al.*	Boraginoideae
8	*Echium angustifolium* Mill.	KSA, Alasyaha :63 KmNE of Burayda ,3.2011 , Gazer *et al.*	Boraginoideae
9	*Echium horridum* Batt.	KSA, Al Qassim Al Madina Road,2.2011, El Melegi *et al.*	Boraginoideae
10	*Echium rauwolfii* Delile	KSA, Riyadh Al-Khabra:West of Qassim Region,2.2006, El Melegi *et al.*	Boraginoideae
11	*Heliotropium bacciferum* Forssk.	KSA ,hell slopes at al-Darb providence, north of Jazan region,2014, L. Shalabi,	Heliotropoideae
12	*Heliotropium crispum* Desf.	KSA ,Alshamasya :20 Km E. of Burayda, 4.2010, El Melegi *et al.*.	Heliotropoideae
13	*Heliotropium digynum* (Forsk.) Aschers. ex C. Christ	KSA , Alshamasya :20 Km E. of Burayda,4.2010, Gazer *et al.*	Heliotropoideae
14	*Heliotropium lasiocarpum* Fisch. & C.A.Mey.	KSA ,Riyadh Al-Khabra:West of Qassim Region ,2.2013, I.Talba *et al.*	Heliotropoideae
15	*Heliotropium supinum* L.	KSA ,Wadi El Rumah NW of Uniaza,3.2012 , El Melegi *et al.*	Heliotropoideae
16	*Hormuzakia aggregata* (Lehm.) Guşul	KSA, Unaiza- Burayd Road, 2.2010, El Melegi *et al.*	Boraginoideae
17	*Lappula spinocarpos* (Forssk.) Asch. ex Kuntz	KSA, El Bakria :15Km W. of Burayda,2.2009, Gazer *et al.*	Boraginoideae
18	*Moltkiopsis ciliata*(Forssk.) I.M. Johnst	KSA , Wadi El Rumah NW of Uniaza, 4.2013 El Gazali *et al.*	Boraginoideae
19	*Microparacaryum intermedium (Fresen.)* Hilger& Podl.	KSA, Al Qassim Al Madina Road,2.2011, Gazer *et al*.	Boraginoidea
20	*Paracaryum rugulosum* (DC.) Boiss.	KSA ,Wadi El Rumah NW of Uniaza,3.2012 Gazer *el al.*	Boraginoideae
21	*Trichodesma ehrenbergii* Schweinf. ex Boiss.	KSA,Gizan,2.4.2013, L. Shalabey.	Boraginoideae

Table 2. Data matrix based on 24 quantitative and qualitative characters for 21 species belonging to family Boraginaceae .

Taxa	P(µm)	E(µm)	P/E	pollen shape	AMB	Ape	Na	polarity	symmetry	Aty	Ends	AW
Alkanna orientalis	16.4-13.7	12.1-11.6	1.2-1.1	subprolate	triangular convex	stephanoaperture	3	heteropolar	radial	colporate	lalongate	broad
Anchusa aegyptiaca	26.9-32.3	19.1-25.2	1.2-1.4	subprolate	quadriangular convex	stephanoaperture	4	isopolar	radial	colporate	lalongate	broad
Arnebia decumbens	32-36.8	20.8-17.6	1.53-2.09	prolate	circular to polygonal	stephanoaperture	5	isopolar	radial	colpate	absent	broad
Arnebia hispidissima	34.29-35.48	16.90-17.29	2.03-2.1	perprolate	polygonal concave	stephanoaperture	5	isopolar	radial	colpate	absent	broad
Arnebia linearifolia	28.05-31.42	11.46-13.97	2.45-2.73	perprolate	polygonal concave	stephanoaperture	5	isopolar	radial	colpate	absent	broad
Arnebia tinctoria	17.1-23.7	8.5-10.2	2.1-2.3	perprolate	circular to polygonal	stephanoaperture	5	isopolar	radial	colpate	absent	broad
Echiochion jugatum	17.15-18.83	10.0-11.66	1.88-1.7	prolate	circular	stephanoaperture	2	isopolar	bilateral	porate	absent	broad
Echium angustifolium	16.13-17.11	10.11-10. 34	1.59-1.65	prolate	triangular concave	stephanoaperture	3	heteropolar	radial	colpate	absent	thin
Echium horridum	13.55-14.68	10.81-10.48	1.25-1.4	subprolate	triangular concave	stephanoaperture	3	heteropolar	radial	colporate	lalongate	broad
Echium rauwolfia	12.2-13.3	9.2-9.7	1.33-1.37	prolate	triangular concave	stephanoaperture	3	heteropolar	radial	colporate	lalongate	broad
Hormuzakia aggregata	24.32-26.19	21.14-22.38	1.15-1.17	perprolate	circular	stephanoaperture	8	isopolar	radial	colpate	absent	broad
Heliotropium bacciferum	28.86-36.59	15.68-17.95	1.84-2.04	prolate	polygonal convex	stephanoaperture	6	isopolar	radial	heterocolporate	lalongate	thin
Heliotropium crispum	21.72-32.86	18.86-17.73	1.16-1.34	subprolate	polygonal convex	stephanoaperture	6	isopolar	radial	heterocolporate	lalongate	thin
Heliotropium digynum	26.59-32.00	15.32-15.94	1.75-2	prolate	polygonal convex	stephanoaperture	6	isopolar	radial	heterocolporate	lalongate	thin
Heliotropium lasiocarpum	22.27-23.64	21.59-22.27	1.03-1.04	**Prolate**	polygonal convex	stephanoaperture	6	isopolar	radial	heterocolporate	lalongate	thin
Heliotropium supinum	18.39-19.84	12.26-13.68	1.35-1.62	prolate	polygonal convex	stephanoaperture	6	isopolar	radial	heterocolporate	lalongate	thin
Lappula spinocarpos	20.48-21.66	9.29-9.77	2.2-2.22	perprolate	polygonal convex	stephanoaperture	6	isopolar	radial	heterocolporate	lalongate	broad
Moltikiopsis ciliata	12.78-14.19	9.00-9.84	1.42-1.44	prolate	circular	polar	2	isopolar	**radial**	heterocolpate	lalongate	broad
Microparacaryum intermedium	7.91-8.36	4.55-4.64	1.7-1.84	prolate	circular	stephanoaperture	6	isopolar	radial	heterocolpate	lalongate	broad
Paracaryum rugulosum	10-10.44	7.11-7.77	1.41-1.34	prolate	circular	stephanoaperture	6	isopolar	radial	heterocolpate	lalongate	broad
Trichodesma ehrenbergii	15.00-16.67	14.19-14.83	1.05-1,12	prolate	triangular concave	anguaperture3	3	heteropolar	radial	heterocolporate	lalongate	broad

Table 2 Continue

Taxa	Ect l.	Ectl\P	colpus shape	pollen outline	pollen ornamentation	Mesd
Alkanna orientalis	15.6-11.98	long	fusiform	triangular	psilate	not differentiated
Anchusa aegyptiaca	9.77-14.11	short	linear	oblong	minutely perforate	differentated
Arnebia decumbens	19.09-21.45	long	long elliptic	oblong	psilate	differentated
Arnebia hispidissima	32.33-32.38	long	long elliptic	oblong	psilate	differentated
Arnebia linearifolia	22.72-25.28	long	linear long	constricted at equator	psilate	differentated
Arnebia tinctoria	16.43-22.8-	long	constricted at equator	constricted at equator	psilate	differentated
Echiochion jugatum	10.2-11.3	long	broad elliptic	circular	Fossulate	not differentiated
Echium angustifolium	14.33-14.40	long	linear long	triangular	microscabrate-perforate	not differentiated
Echium horridum	9.834-12.26	long	elliptic	triangular	microscabrate-perforate	not differentiated
Echium rauwolfia	10.1-10.9	long	elliptic	triangular	microscabrate-perforate	not differentiated
Hormuzakia aggregata	10.9-12.38	short	linear	oblong	perforate	differentated
Heliotropium bacciferum	23.86-25.91	long	linear	oblong lobed	ruglate to psilate	not differentiated
Heliotropium crispum	16.36-20.68	long	linear	oblong lobed	ruglate to psilate	not differentiated
Heliotropium digynum	18.18-24.8	long	linear	oblong lobed	psilate	not differentiated
Heliotropium lasiocarpum	17.27-20.23	long	linear	oblong lobed	ruglate to psilate	not differentiated
Heliotropium supinum	12.90-14.06	long	linear	oblong lobed	ruglate to psilate	not differentiated
Lappula spinocarpos	16.36-17.38	long	constricted at equator	constricted at equator	psilate	differentated
Moltikiopsis ciliate	6.5-7.3	long	broad elliptic	circular	Fossulate	not differentiated
Microparacaryum intermedium	4.45-5	short	fusiform	oblong	psilate	differentated
Paracaryum rugulosum	4.44-4.56	short	fusiform	oblong	psilate	differentated
Trichodesma ehrenbergii	8.83-10.32	long	fusiform	triangular	granulate	not differentiated

Table 2 Continue

Taxa	Meso	Mo	Clm	Apel	psuedocolpi	Aco
Alkanna orientalis	not differentiated	tuberculated	granulated	sunken	absent	psilate
Anchusa aegyptiaca	areolate	no membrane	smooth	sunken	absent	minutely perforate
Arnebia decumbens	granulated at equator	tuberculated	smooth	sunken	absent	tuberculated
Arnebia hispidissima	granulated at equator	tuberculated	smooth	sunken	absent	tuberculated
Arnebia linearifolia	granulated at equator	tuberculated	smooth	sunken	absent	tuberculated
Arnebia tinctoria	granulated at equator	tuberculated	smooth	sunken	absent	tuberculated
Echiochion jugatum	not differentiated	tuberculated membrane	smooth	elevated	absent	fossulate
Echium angustifolium	not differentiated	tuberculated	smooth	sunken	absent	microescabrate perforate
Echium horridum	not differentiated	tuberculated	smooth	sunken	absent	microescabrate perforate
Echium rauwolfia	not differentiated	tuberculated	smooth	sunken	absent	microescabrate perforate
Hormuzakia aggregata	areolate	no membrane	smooth	sunken	absent	perforate
Heliotropium bacciferum	not differentiated	no membrane	smooth	sunken	present	psilate
Heliotropium crispum	not differentiated	no membrane	smooth	sunken	present	psilate
Heliotropium digynum	not differentiated	no membrane	smooth	sunken	present	psilate
Heliotropium lasiocarpum	not differentiated	no membrane	smooth	sunken	present	psilate
Heliotropium supinum	not differentiated	no membrane	smooth	sunken	present	psilate
Lappula spinocarpos	granulated at equator	tuberculated	granulated	elevated	present	psilate
Moltikiopsis ciliate	not differentiated	tuberculated	smooth	sunken	absent	fossulate
Microparacaryum *intermedium*	transverse ectocingulus	tuberculated	thick granulated	sunken	present	psilate
Paracaryum rugulosum	transverse ectocingulus	tuberculated	thick granulated	sunken	present	psilate
Trichodesma ehrenbergii	not differentiated	tuberculated	granulated	elevated	absent	granulate

P=polar view; E= Equatorial view; AMB=outline in polar view;Ape= Aperture position; Na=number of apertures ;Aty= Aperture type; Ends=Endoapertureshape, Alt= width of apreture, Ectl = Ectoaperture length = L, Ectl\P= Ectoaperture to polar axis, Mesd= Mesocolpium differentiation., Meso = Mesocolpium ornamentation; Mo = membrane ornamentation., Clm= colpi margin, Apel= aperture elevation, Aco = Apocolium fieldornamentation.

3. Results:

Morphological Results

The studied taxa collected from Saudi Arabia belong mainly to two subfamilies, Heliotropoidea and Boragonoideae. Boraginaceae is readily recognized by the vegetative, floral and fruit characters. Morphological characters (19 characters and 55 character states) have been represented in table: **5**.which was chosen for cluster analysis.From the results it is evident that the studied taxa either perennials as in *Alkanaorientalis* and *Moltkiopsis ciliata*or annuals as in *Arnebia*spp., but *Heliotropium spp* show the two types of duration, leaf margin either entire in most species or undulate in *Heliotropium* and ciliate caulous in *Moltkiopsisciliata*. Most studied species have bulbs simple hair (Fig. 10 e), but sometimes in addition to simple hair, glandular hair is present as in *Alkanaorientalis* and *Anchusaa egyptiaca*. Bracts present in most studied species but absent in *Microparacaryum intermedium* and *Paracaryum rugulosum*. Most studied taxa have actinomorphic but flower Zygomorphic in *Alkana orientalis* , *Echium* spp and *Hormuzakia aggregta*. Petalcolor vary from yellow or blue or violet. Corolla shape either hypocratiform as in *Anchusa aegyptiaca* or infundibular as in *Echiumspp*. or tubular as *Microparacaryum intermedium* and*Paracaryum rugulosum* .Petals may be glabrous as in *Trichodesma ehrenbergi* or hairy as in *Heliotropium* spp and *Lappula spinocarpos*. Some taxa such*Alkana orientalis*, *Anchusa aegyptiaca* and *Lappula spinocarpos* have faucal appendages but the remaining taxa have no faucal appendages. Petal length vary from small (1-5 mm) as in *Heliotropium* spp. to large (more than 10mm) as in *Arnebia* spp. Stamens either cryptantherous in most studied taxa or phanerantherous as in *Trichodesma ehrenbergi* , but in *Moltkiopsis ciliata* has stamens 4 exerted stamens and the posterior one inserted. Staminal filament reduced in most studied taxa but well developed in*Arnebia* spp. Style simple in most studied taxa, but forked twice in some species like*Arnebia* spp and *Echium* spp. Style insertion is terminal in *Heliotropium* spp. and gynobasic in the remaining studied taxa. Gynobase either absent as in *Echium* spp. or pyramidal as in *Microparacaryum intermedium* and *Paracaryum rugulosum* (Fig.10.**d**) or flat as in *Heliotropium bacciferum.* Nut shape mostly ovoid or obliqueovoid (Fig. 9 and 10). Or cup shaped with scarious margin as in *Microparacaryum intermedium*and *Paracaryum rugulosum* (Fig. 10 **b**) or ovoid with appendiculate disc (Fig. 10 c) The areole of nutlets mostly basal in studied species as in *Alkana orientalis* or ventral as in *Microparacaryum intermedium* and *Paracaryum rugulosum* (Fig. 10).

Pollen results

Detailed investigation of pollen Characters Table: 2 revealed presence of 24 pollen characters and 60 character state as shown in Table: 4.The mean length of the polar axis (P) vary greatly between studied taxa, where ranged from 8.14 µmin *Microparacaryum intermedium*to 34.86 µm in *Arnebia hispidissima*. The mean of equatorial diameter ranged from 7.4 µm in *Microparacaryum intermedium* to 22.15 µm in *Anchusa egyptiaca*.Thepollen shape according to P/E value either subprolate as in *Alkana orientalis* and *Anchusa aegyptiaca* or perprolate as in *Arnebia* spp. and *Lappula spinocarpos* andprolate in the remaining studied species. The outline in polar (AMP) view vary from triangular as in *Alkana orientalis* and *Trichodesma ehrenbergi* and *Echium* spp. to polygonal as in *Heliotropium* and *Arnebia* spp and circular in *Microparacaryum intermedium, Paracaryum rugulosum* and *Moltkiopsis ciliate.* Aperture position stephanoaperture in most studied taxa but it is polar *Moltkiopsis ciliata* or anguaperture in *Trichodesma ehrenbergi* (Fig.1-5). Aperture number vary greatly between species , where 2 apertures in *Echiochilon jugatum* and *Moltkiopsis ciliata*and 3aperturesin *Echium* spp.and *Alkana orientalis* , 4 *a*pertures in *Anchusa aegyptiaca*,5aperture in *Arnebia* spp.,6aperture in *Heliotropium* spp., *Microparacaryum intermedium* and *Paracaryum rugulosum*, and 8 apertures in *Hormuzakia aggregate.* Pollen polarity isopolar in all studied taxa but heteropolar in *Trichodesma ehrenbergi* and *Echium* spp., All studied pollens have radial symmetry while, *Echiochilon jugatum* and *Moltkiopsis ciliate* possess bilateral symmetry. Aperture type either colporate as in *Alkana orientalis*,or colpate as in *Arnebia* spp., porate in *Echiochilon jugatum* or Heterocolporate in the remaining studied taxa. Endoaperture absent in *Arnebia* spp. and lalongate in the remaining studied taxa. Aperture width mostly broad in all studied taxa except in *Heliotropium* spp. have thin aperture. The mean of Ectoaperture length vary from 4.5 µm in *Microparacaryum intermedium* to 32.35 µm in *Arnebia hispidissima* . Colpus shape either fusiform as in as in *Alkana orientalis* and Paracaryum spp., and constricted as present in Lappula *spinocarpos* and linear in *Anchusa egyptiaca*. The pollen shape varies greatly between species; it is triangular as in *Alkana orientalis* or *Trichodesma ehrenbergi*or oblong as in *Anchusa egyptiaca* and *Hormuzakia aggregate*; and constricted at equator as in *Lappula spinocarpos* .Ornamentation either psilate as in *Alkana orientalis* and *Paracaryum rugulosum* and *Microparacaryum intermedium* or microcabrate in *Echium* spp.or fossulate as in *Moltkiopsis ciliata*and granulated in *Trichodesma ehrenbergi*. Mesocolpium either not differentiated or differentiated into transverse ectocingulus as in *Paracaryum rugulosum* and *Microparacaryum intermedium*. The aperture membrane is tuberculated as in most studied species or has no membrane as in *Heliotropium* spp. They aperture may be elevated as in *Trichodesma ehrenbergi* or sunken as in the remaining species. Pseudoaperture is found in *Paracaryum rugulosum* and *Microparacaryum intermedium* and *Heliotropium* spp. Apocolium field either psilate in *Paracaryum rugulosum* , *Microparacaryum intermedium* and *Heliotropium spp* or microcabrate in *Echiumspp*or tuberculated as in *Arnebia* spp.

Table 3. Infrafamilial and Tribal treatment in Boraginoideae and Heliotropodeae by selected authors to show how the studied genera in this paper have been placed by different authors.

	De Candolle 1846	Bentham & Hooker 1873	Baillon 1888	GÜrke 1897	Al-Shehbaz 1991	Riedl 1997	Takhtajan 1997	La¨ngstro¨m and M. W. Chase 2002	cón &al. 2016
Alkana	Lithospermeae	Boragineae	Boragineae	Boragineae	Lithospermeae	Lithospermeae	Lithospermeae		Lithospermeae
Anchusa	Boragineae	Boragineae	Boragineae	Boragineae	Boragineae	Boragineae	Boragineae	Boragineae	Boragineae Subtribe: boragininae
Aenebia	Lithospermeae	Lithospermeae	Lithospermeae	Lithospermeae	Lithospermeae	Lithospermeae	Lithospermeae		Lithospermeae
Echium	Echieae	Lithospermeae	Echieae	Echieae	Lithospermeae	Lithospermeae	Lithospermeae		Lithospermeae
Echiochilon	Echieae	Eritrichieae	Echieae	Echieae	Eritrichieae	Lithospermeae		Echiochileae	Subfamily: Echiochiloideae
Heliotropium	Heliotropoideae	Heliotropoideae	Heliotropoideae	Heliotropoideae	Heliotropoideae	Heliotropoideae	Heliotropoideae		Heliotropoideae
Lappula		Eritrichieae	Eritrichieae	Eritrichieae	Eritrichieae	Eritrichieae	Eritrichieae		Subf.:Cynoglosoideae Tribe:Rochelieae Subtribe:Eritrichiinaeeae
Moltkiopsis					Trigonotideae	Trigonotideae			Lithospermeae
Hormuzakia					Boragineae	Boragineae		Boragineae	Boragineae Subtribe: boragininae
Paracaryum		Cynoglosseae	Cynoglosseae	Cynoglosseae	Cynoglosseae	Cynoglosseae	Cynoglosseae		Subf.:Cynoglossoideae Tribe: Cynoglosseae Subtribe:Cynoglossinae
Microparacaryum							*Cynoglosseae*		
Trichodesma	Cynoglosseae	Cynoglosseae	Cynoglosseae	Cynoglosseae	Trichodesmeae	Trichodesmeae	Cynoglosseae		Subf.:Cynoglosoideae Tribe: Trichodesmeae

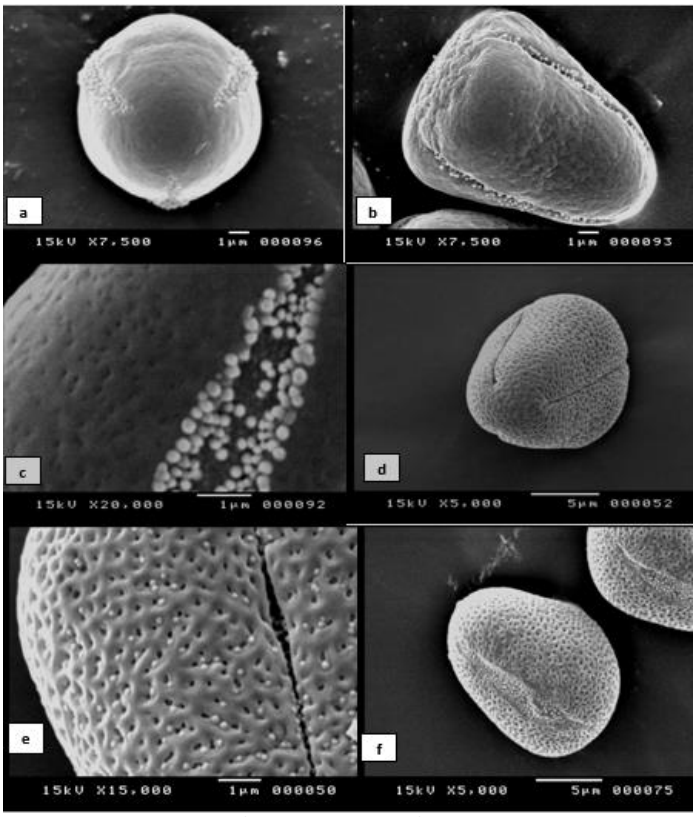

Fig.1:a-c.Scanning electron microscopy (SEM) images of the pollen grains of *Alkana oriantalis***a**-polar view; **b**-equatorial view, triangular, psilate ornamentation; **c**- enlarged colpi with granulated margo.**d-e** .Pollen of *Echium angustifollium*; **d**- oblique polar view showing tricolpate aperture, triangular pollen. **e**- enlarged equatorial view showing microdcabrate-perforate surface ornamentation. **f**-Pollen of *Echiumrauwolfia* , equatorial view showing colporate aperture,and surface ornamentation

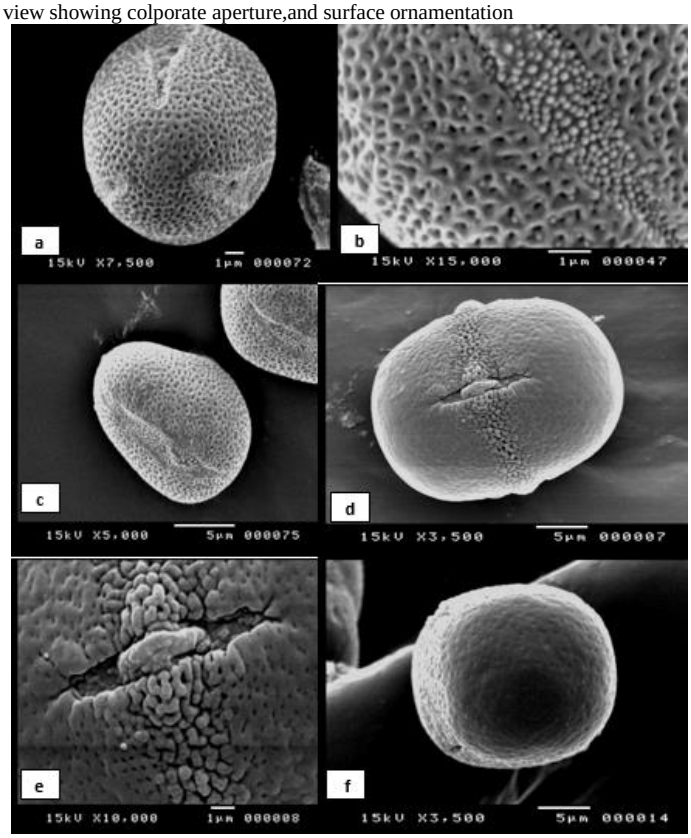

Fig.2:- a-b .Pollen of *Echium horridum;* **a**-polar view, **b**-enlarged colpi showing tuberculated aperture membrane and surface ornamentation. **c**- Pollen of *Echiumrauwolfia*: equatorial view showing colporate aperture,and surface ornamentation. d-f .Pollen of *Anchusa aegyptica*; **d**- equatorial view showing short colpi ; **e**- magnified aperture ; **f**- polar view.

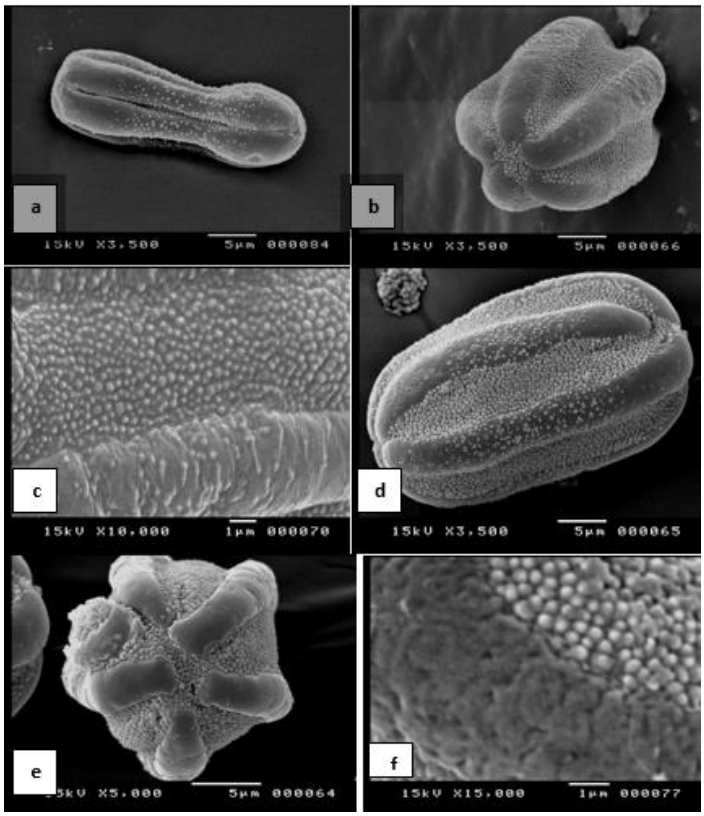

Fig.3. a-Pollen of *Arenbia linearifoli:* equatorial view showing mesocolpium ornamentation.**b-c** Pollen of *Arnebia decumbens*; **b-** overall shape with apertures ; **c-** enlarged aperture with associated ornamentation. **d-e** Pollen of *Arnebia hipidissim*;**d-** oblique equatorial view, rectangular-fusiform apertures with granulated membrane; **e-** polar view.**f-**Enlarged Pollen of *Echiochilon jugatum*showing sculptureof colpus membrane.

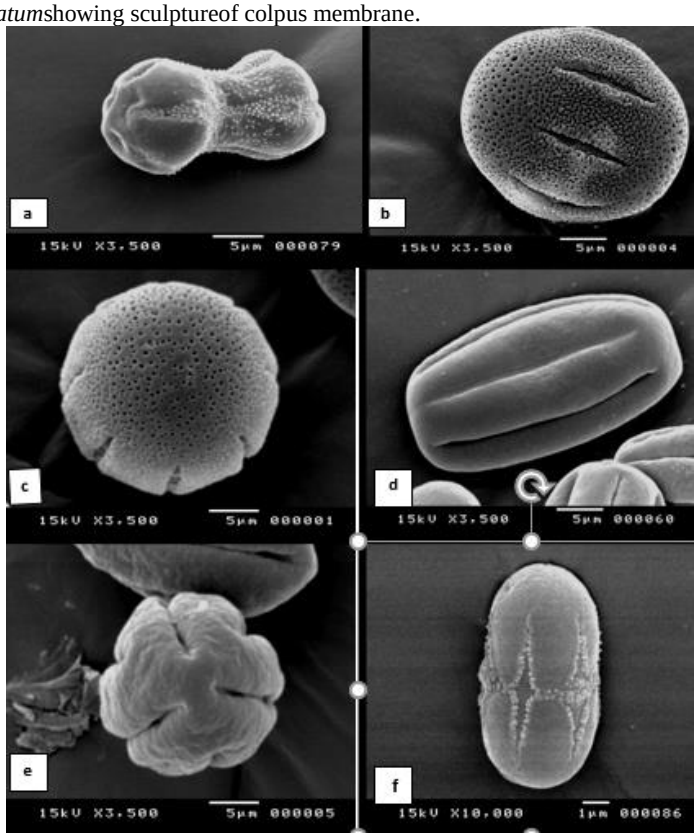

Fig.4.a-b Pollen of*Arnebia tinctoria;* **a-** equatorial view showing constriction at equator (Dumbell- shape). **b-**enlarged apertures showing ornamentation. **c-d**.Pollen of *Hormuzakia aggregate*; **c-** oblique equatorial view showing reticulate ornamentation in mesocolpium and short colpi; **d-** polar view showing details of ornamentation.**e-** Pollen of *Heliotropium digynum* ; equatorial view showing colpi and psilate sculpture.**f-** Pollen of *Heliotropium bacciferum* ,polar view

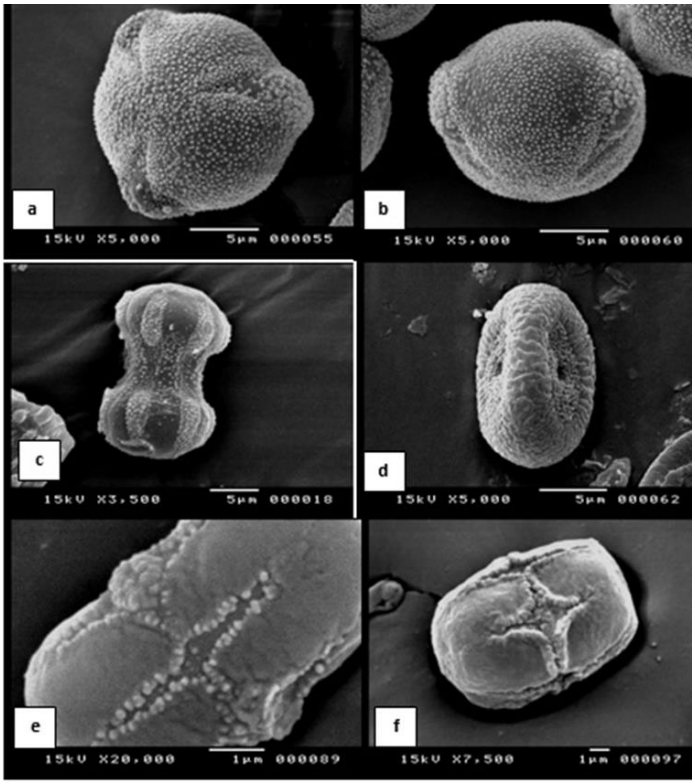

Fig.5.**a-b**- Pollen of *Trichodesma ehrenbergi***a-** polar view show in gangulaperture with protruding ora; **b**-equatorial view showing densely granulated ora.**c**- Pollen of *Lappula spinocarpos*equatorial view showing constriction at the equator. **d**-pollen of *Moltikiopsis ciliata* , equatorial view showing ornamentation and granulated membrane. **e-f**Pollen of *Microparacaryum intermedium*, an enlarged part of equatorial view showing ectocingulus and apertures with thickened margins

Table 4. Characters and character states of pollen for analysis based on the hierarchial

Pollen characters	
1-Mean the length of the polar(P) axis (µm)	13-Ectoaperture mean length
2-Meanof E equatorial diameter (µm)	14-Ectoaperture length to polar axis: long (1);short (2)
3- P/E	15-Colpus shape:fusiform(1);constricted at equator(2);elliptic(3);linear(4)
4-pollen shape : Subprolate(1);perprolate,(2);prolate(3)	16-Pollen shape in equatorial view: triangular (1);oblong(2);constricted ate equator (3);circular(4)
5-AMB : Triangular(1)quadriangular(2);Polygonal(3);Circular (4)	17-pollen ornamentation: psilate(1);perforate(2);microcabrate perforate(3);regulate to psilate(4);granulate(5);fossulate(6)
6-Aperture position:stephanoaerture(1);polar(2);anguaperture	18- Mesocolpium differentiation:not differentiated(1);differentiated (2)
7-Aperture number	19- d mesocolpium ornamentation: not differentiated(1);granulated(2);areolate(3);transverse ectocingulus(4)
8-Polarity:heteropolar(1);isopolar(2)	20- aperture membrane ornamentation:no membrane(1);tuberculated membrane(2)
9-Symmetry:radial(1);Bilateral(2)	21- Colpi margin: smooth(1);thin granulated(2);thick granulated(3)
10-Aperturetype:colporate(1);Colpate(2);heterocolpate(3);porate(4);hetrocolpate(5)	22- Aperture elevation:sunken(1);elevated(2)
11-Endoaperture:lalongate(1);absent(2)	23- Psuedocolpus: absent(1);present(2)
12-Aperture width: broad(1); thin(2)	24-Apocolium field ornamentation::psilate(1);minute perforate(2);tuberculate(3);fossulate(4);microcabrate perforate(5);granulate(6)

The previous results were scored as character and character states Table (3), and analyzed using SPSS 14.0 programme. The resulting 8 clusters were assumed to represent distinct morphotypes differing for combination of characters.

These clusters constitute 8 pollen types as shown in Fig.6. The resulting dendogram as in Fig.6 reveals 3 main clusters **a,b** and **c**. The cluster **c**separate *Heliotropium*spp.(Sub family Heliotropoideae Pollen type 8 characterized by Heterocolporate pollen , lobate and Psilate ornamentation. While clusters a and**b** comprise the tribes of subfamily Boragonoideae. The cluster a subdivided into 5 sub-clusters represent 5 pollen types.Pollen type(1)characterized by 6 heterocolporate,prolate, psilate with transverse ectocingulus includes*Paracaryum rugulosum* and *Microparacaryum intermedium*. Pollen type 2 includes*Lappula spinocarpos* characterized by 6 hetrocolporate aperture, constricted at equator. Pollen type 3 include *Moltkiopsis ciliata* and *Echiochilon jugatum* which characterized by dicolporate, circular pollen, bilateral symmetry and fossulate ornamentation. Pollen type 4 spate the studied species of genus *Arnebia* characterized by 6 – heterocolporate, granulated sculpture, densely granulated membrane aperture, pollen type 5 comprise *Anchusa aegyptiaca* and *Hormuzakia aggregata*characterized by 4-8 zonocolporate, brevi colpi, perforated ornamentation.The main cluster **b** subdivided into 2 subclusters contain two pollen types 6 and 7. The pollen type 6 comprise *Echium* spp.and *Alkana orientalis* which characterized by tricolporate sunkenaperture and granulated membrane. The pollen type 7 comprise*Trichodesma ehrenbergi*characterized by tri-anguaperture, and elevated granulated membrane

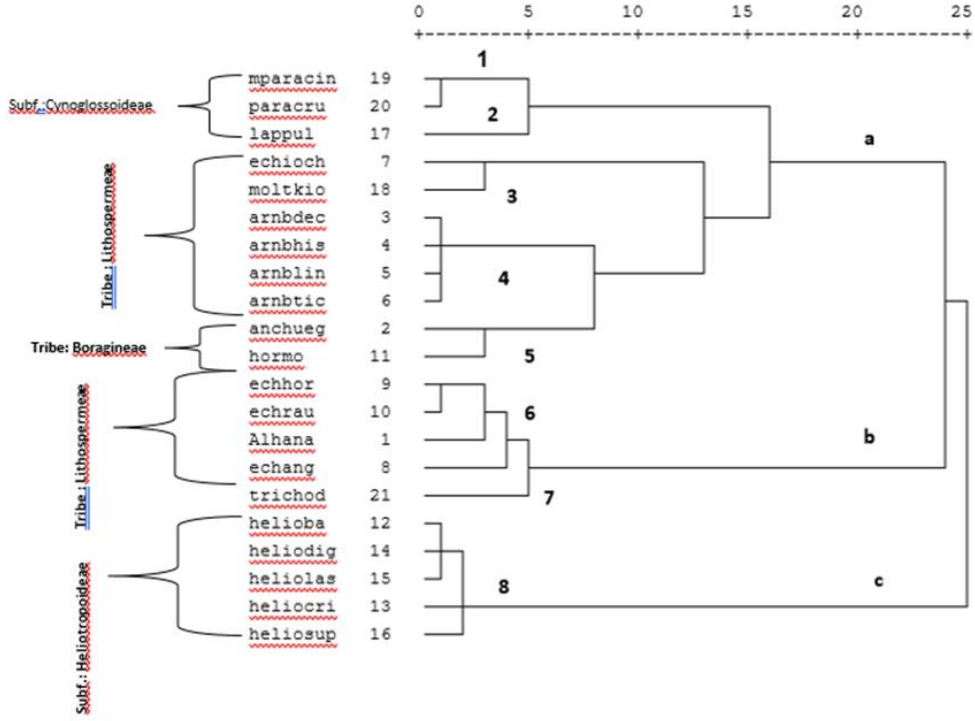

Fig.6 Dendogram derived from the hierarchical cluster analysis of pollen characters.

For investigation of morphological characters of the studied taxa, 19 characters and 55 character states were selected for vegetative and floral parts and nutlets Tab.4 (Fig. 8, 9 and 10)

The dendogram of Morphological data resulted in 3 main clusters. As in the dendogram of pollen Heliotropium spp. were separated in main cluster **A** based on the undulated leaf margin, reduced filament, terminal style , and basal areole .The main cluster **B** subdivided into three sub-clusters 2,3 and 4. The cluster 2 include*Anchusa aegyptiaca* and *Hormuzakia aggregata*. Based on presence of glandular hair, scales in corolla and oblique ovoid nutlets. The cluster 3 separated *Alkana orientalis*due tooblique ovoid nutlets ,tuberculated nutlet surface and areole flat. The subcluster 4 comprise *Paracaryum rugulosum* and *Microparacaryum intermedium*, *Lappula spinocarpos* and *Trichodesma ehrenbergi* based on pyramidal gynophore and nutletattachment to the gynobase by their whole length . Cluster 5 includesonly Arnebiaspp. due to well-developed filamentand twice forked style. The cluster 6 include*Echium* spp., while cluster 7 includes *Echiochilon jugatum* and *Moltkiopsis ciliata*based on basal areole and nut shape.

Table: 5. Selected morphological characters and their character states

Morphological characters	
1-Plant duration annual(1) ; perennial(2)	11-flower length (1-5 mm) small (1); (5-10 mm) medium (2); more than 10mm large (3)
2- Leaf margin : entire (1); undulate,(2);prolate(3)	12-stamens exsertion: cryptantherous(1); phaneraantherous (2);4 exserted and the posterior inserted(3).
3- shape of trichomes: simple (1); glandular and simple (2);bulbs simple hair(3)	13-filament length:reduced (1);well developed(2)
4-bracts : bracteate (1); ebracteate (2)	14-style shape: simple (1); twice forked.(2)
5-flower symmetry :actinomorphic(1); zygomorphic (2).	15-style insertion: gynobasic (1);terminal (2).
6-flower pedicel: pedicelled (1);sessile(2).	16- gynobase shape: olique ovoid (1); cupshaped with scarious margin (2);ovoid with appendiculate disc (3); ovoid or triquetrous (5).
7-corolla color: yellow (1); blue,(2);violet(3)	17- nutlet shape: ornamentation: not differentiated(1);granulated(2);areolate(3);transverse ectocingulus(4)
8-corolla shape:hypocratiriform (1);infundibular(2);bilabiate (3);tubular(4)	18- nutlet surface ornamentation:tuberculate (1);reticulate(2); smooth (3); rugulose with margin incurved glochidate-dentate (4);smooth in outer margin and inner margin dentate (5);glochidate (6)
9-corolla texture : hairy(1);glabrous(2); scaly (3)	19- areole position: basal (1);ventral (2)
10-Faucal appendages: present (1);absent(2).	

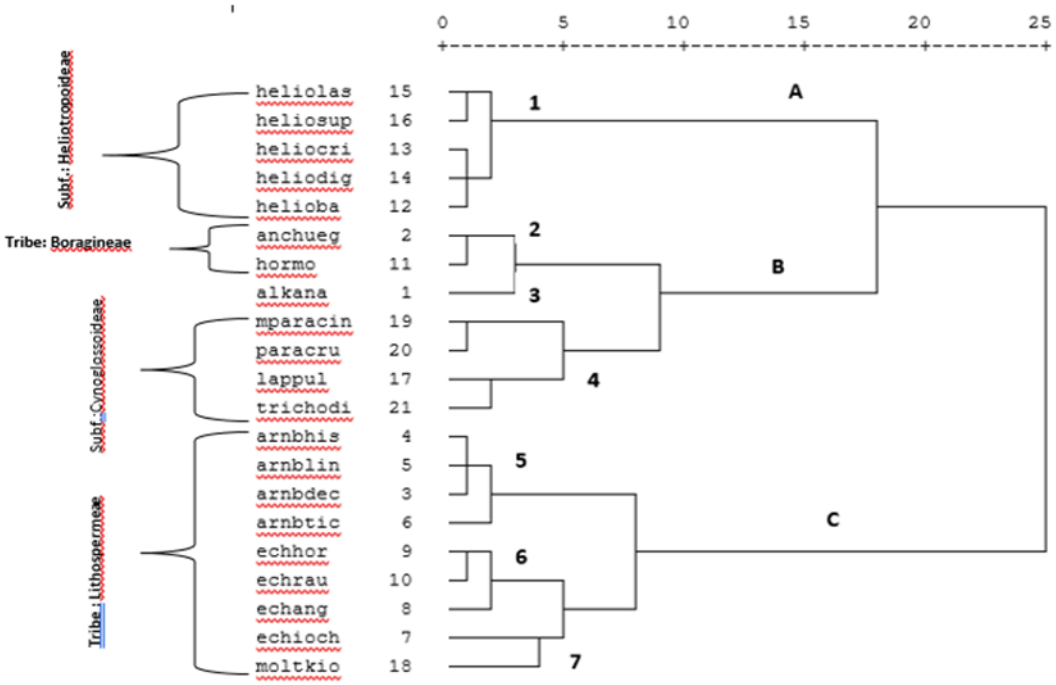

Fig.7.Dendogram derived from the hierarchial cluster analysis of morphological characters

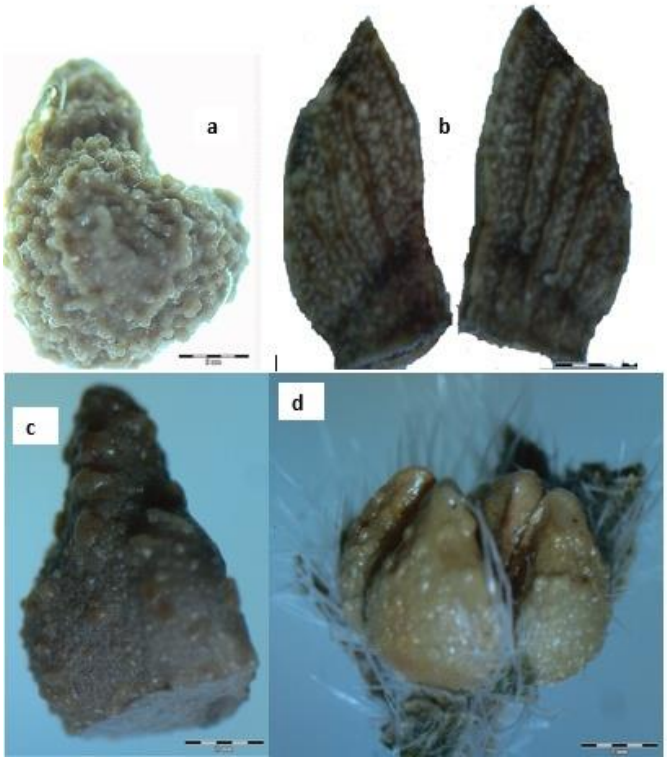

Fig.8. Nutlets of studied taxa **a**- *Alkana orientalis* ,**b**- *Anchusa egyptiaca* nulets showing basal areole, concave surrounded by a thickened basal ring . **c** -*Arnebia linearifolia* (areole basal flat) ,**d**- *Arnebia tinctoraia* nutlets

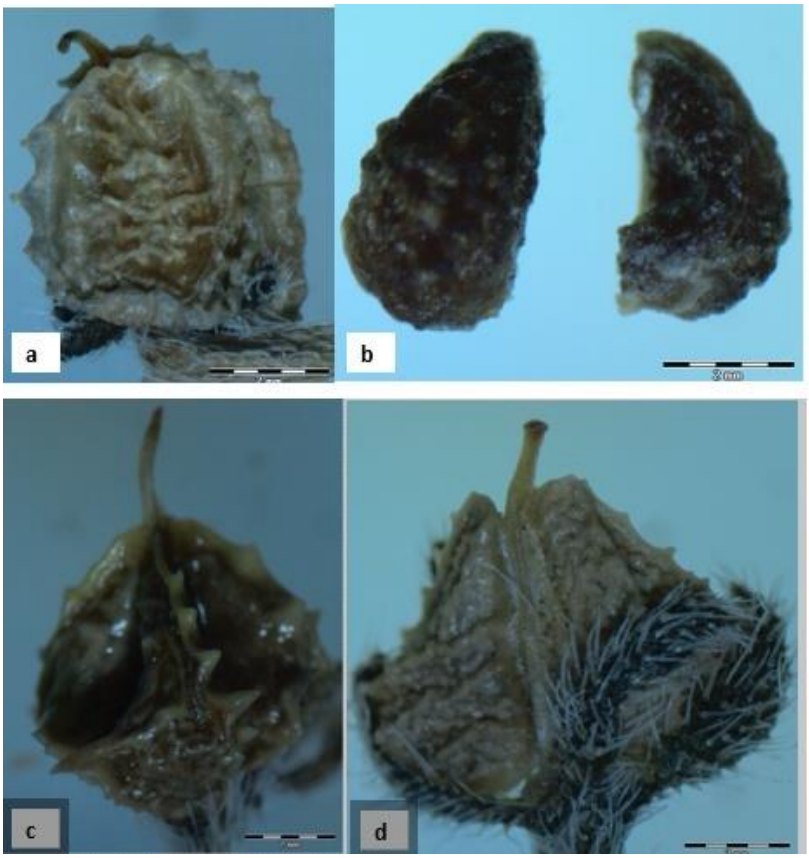

Fig.9. Shapes of nuts;**a** -*Echium rauwolfii* (areole basal flat) **b**- *heliotropium bacciferum* (ovoid triquetrous),**c**- *lappula spinocarpa* (nutlets attached to pyramidal gynobase),**d**- *Microparacaryum intermedia* (nutlet attached to the gynobase by their whole length)

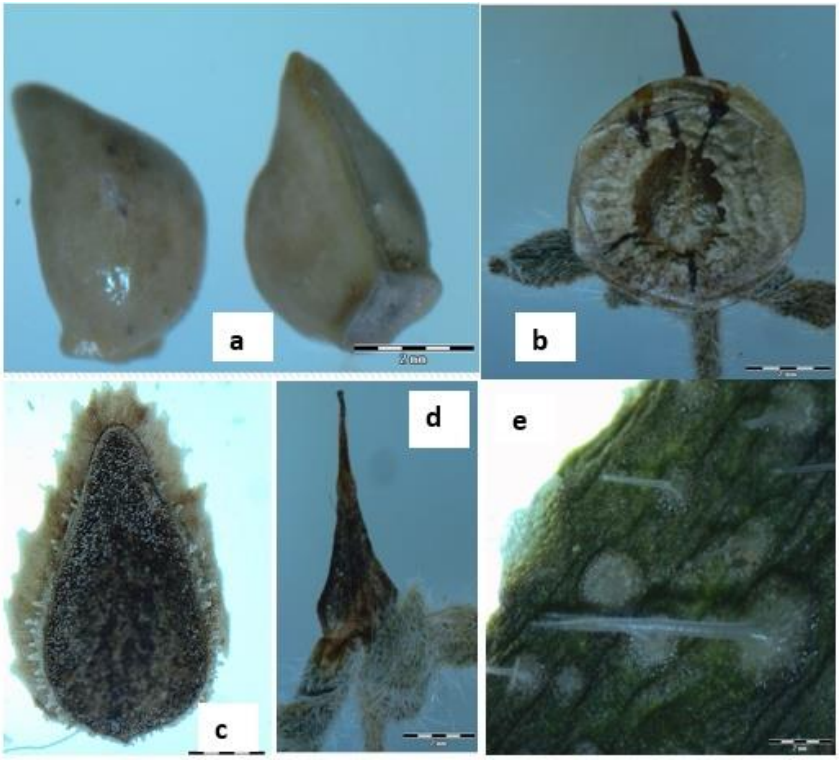

Fig. 10. **a**-*Moltkiopsis ciliata* (nutlets,keeled and sharp angled on ventral side);**b**- *nutlet of paracaryum rugulosum***c**-*Trichodesma ehrenbergi* (areole, ventral, extending nearly the whole length of the nutlet), **d**-pyramidal gynobase of *Paracaryum rugulosum***e**- bulbs hair

4. Discussion

The taxonomic limits of the tribe of sub family Boraginoideae have varied greatly between authors (Table: **3**) depending on the relevance attributed to some key characters such as morphology, type of attachment of mericarp to gynobase, and molecular data. Recent phylogeneticstudies indicate that the infrafamilial classification of the family Boraginaceae as currently used is highly obsolete(Chacón*el at*.,2016 and Luebert 2010). Moreover, the palynological terms and expressions used by different authors sometimes are making confusion.

The present study investigate mainly the pollen morphology of the 21 Taxa representing 12 genera collected from Saudi Arabia, belongsmainly to 2 subfamilies. (Heliotropoideae and 5 tribes of Boragonoideae (Echieae, Lithospermeae, Cynoglosseae, Boraginea and Eritricheae) according to Takhtajan (1997).

The definitions of eight pollen types in the present study on the basis of apertures and surface ornamentation, which correspond in general to taxonomic groups ensure the eurypalynous type of the family.

The results of both pollen and morphological data emphasize that , genus Heliotropium belongs to subfamily Heliotropioideae,It distinguished by its prolate shape, 6- heterocolpate aperture, regulate to psilate ornamentation, Not differentiated mesocolpium , no aperture membrane and psilate apocolium field.

The remaining studied genera belongs to the largest subfamily Boragonoideae which further divided into six tribes; where *Arnebia*, *Echium* ,and *Alkana* belongs to tribe Lithospermeae ; *Anchusa* and *Hormuzakia* belongs to tribe Boragineae; *Echiochilon*belongs to tribe Echiochileae (Långström and Chase, 2002), but according toLuebert (2010) andChacón*elat*., 2016, also*Echiochilon* was placed in anew subfamily: Echiochiloideae; Lappula was belonged to Eritrichieae(Takhtajan 1997) butbased on molecular data Chacón *el at*.(2016) place it in subfamily :Cynoglosoideae; Tribe:Rochelieae. As in Table (1) *Paracaryum* and *Microparacaryum* belongs to Cynoglossea and later was placed insubfamily Subf.:Cynoglossoideae ,Tribe: Cynoglosseae. Trichodesma belongs to tribes Cynogloseae ,but Luebert (2010) andChacón*elat*.,2016 raised it to Subf.:Cynoglosoideae Tribe: Trichodesmeae . As shown in table.2, the tribal classification systematic and the relationships occurring among studiedgenera varied greatly between authors.

The results of this study show thatpollen characteristics have great potential as a means of classification. The hierarchical cluster analysis and Dendogram of pollen data (Fig.6)and of morphological characters(Fig. 7), separatedall studied species Heliotropium in a single main

cluster . The previous result agree with Clarke(1977)in the fact that pollen of Heliotropum belongs to one of the most primitive pollen types in the Boraginaceae according to its size, ornamentation and polar-view outline ; thus confirm that the selected characters is reliable and agree with the different classification systems.

The taxa belong to the tribes of subfamily Boraginoideae vary between the two clusters of pollen and morphological characters. Based on the types of pollen apertures,Liu, Li *et al.* (2010) observed that *Echium* has classical 3-colporate pollen support that the genus *Echium* should remain in the tribe of Lithospermeae. There are two different points of view regarding the taxonomic position of *Echium* L. According to one of them, *Echium* L. is placed an individual tribe in the subfamily Boraginoideae (De Candolle, 1846 andPopov, 1953). By contrast, the other suggested *Echium* L. as a genus of the tribe Lithospermeae (Johnston, 1924and Riedl, 1967). Our result of cluster analysis of pollen data placedthe different species of*Echium* in one cluster with *alkana (Lithospermae)* ,while in cluster resulted frommorphological characters *Echium* was placed in one cluster with *Arnebia* (Lithospermeae), thus agree with the point of view of (Riedl 1967).

*Anchusa aegyptiaca*and *Hormuzakia aggregate* which belongs to Tribe Boragineae in all classification systems appeared adjacent in both clusters of morphological data and pollen data.

Alkana and *Anchusa* were previously placed in Tribe Boragineae by (Bentham and Hooker ,1873, Baillon, 1888), This agree with the resultsof cluster analysis of both pollen and morphological data but disagree with other authors (Takhtajan, 1997)who placed *Alkana* in subtribe Lithospermeae.

Echiochilon , *Moltkiopsis*were previously placed in different tribes (Echiochileae and Lithospermeae) as shown in Table (2) ,The clusters derived from both pollen and morphological data include both genera on the same clade.The result agree with Riedl (1997) who place *Echiochilon* in tribe Lithospermeae.

In morphological cluster ,*Lappula* , *Paracaryum* , *Microparacaryum* ,and *Trichodesma* were grouped in one clade , this assumption agree with the result of Chacón, Luebert *et al.*, (2016) who place the three genera in tribes of new Subfamily :Cynoglossoideae.

In pollen analysis only *Paracaryum* , *microparacaryum* and Lappula were included in one clade , while *Trichodesma ehrenbergi*form one clade with *Echium*spp and *Alkana orientalis*.

The presence of a marked equatorial constriction, ectocingulus in addition to morphological and nutlet characters are essential for identification and classification of *Lappula* ,*Paracarum* and *Microparacaryum* which support the placementof three genera in subfamily *Cynoglossoideae* . This result agreeswith Chacon, Luebert *et al.* (2016).

Our observations along with the data fromLiu *et al.*(2010)show that among the studied taxa all the species belong to genera *Echium ,Arnebia, Alkana, Echiochilon ,and Moltkiopsis* which belong to tribe Lithospermeae are the most diverse with distinct morphological characters of the pollen grains and apertures. This assumption interpret the presence of the taxa belong to tribe Lithospermeae in two different clades in the cluster of pollen data as in Fig.6. ; besides*Echium* Linn. is in the tribe Lithospermeae rather than in a novel tribe.

Thus as clearly shown in the present study, pollen morphology may be a usefuldiagnostic tool in Boraginaceae taxonomy.However;Groupings based only on pollen characteristics alone did not align completely with those based on morphological data.The palynological data ensure the eurypalynous type of the family and have an important role in separation between genera and sub familiallevel,but it become confusing at the tribal level due to the eurypalynous nature of the family. Sometimes, some tribes exhibit more than one pollen types.Also results show that groupings based only on pollen characteristics alone did not align completely with those based on morphological data.

References

Ahn, Y. and S. Lee (1986). "A palynotaxonomic study of the Korean Boraginaceae." Korean journal of Plant Taxonomy 16(3): 199-215.

Al Shehbaz, I. A. (1991). "The genera of Boraginaceae in the southeastern United States." Journal of the Arnold Arboretum, Suppl: 1-169.

APG II, A. (2003). "An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II." Botanical Journal of the Linnean Society 141(4): 399-436.

Baillon H. (1888) Boraginace´es. In: Baillon H. (ed.) Hist. Pl. (Baillon). Librairie Hachette & Cie, Paris, pp. 343–402.

Bentham G., Hooker J. D. (1873) Genera Plantarum (Bentham & Hooker). Lovell Reeve & Co, Williams & Norgate, Covent Garden, London, vol. 2.

Bigazzi, M. and F. Selvi (1998). "Pollen morphology in theBoragineae (Boraginaceae) in relation to the taxonomy of the tribe." Plant Systematics and Evolution 213(1-2): 121-151.

Böhle, U. and H. Hilger (1997). "Chloroplast DNA systematics of “Boraginaceae” and related families: a goodbye to the old familiar concept of 5 subfamilies." Scripta Botanica Belgica 15: 30.

Boulos, L. (2000). "Flora of Egypt, vol. 2." Cairo: Al Hadara Publishing: 352.-

Chacon J, Luebert F, Hilger H, Ovcinnikova S, Selvi F, Cecchi L, Guilliams CM, HasenstabLehman K, Sutory KK, Simpson MG, Weigend M (2016). A revised infrafamilial classification of the borage family (Boraginaceae s.str.) based on a molecular phylogeny with an emphasis on the placement of some enigmatic genera. Taxon 65(3):523-546.

Clarke, G. (1977). "Boraginaceae." Review of Palaeobotany and Palynology 24(2): A59-A101.-

Cohen, J. I. (2014). "A phylogenetic analysis of morphological and molecular characters of Boraginaceae: evolutionary relationships, taxonomy, and patterns of character evolution." Cladistics 30(2): 139-169.

Cronquist, A. (1981). An integrated system of classification of flowering plants, Columbia University Press.

De Candolle, A.P.(1844). Prodromus systematis naturalis regni vegetabilis, Verlag von J. Cramer.

De Candolle, A.P. (1846). Borrage. In: de Candolle A. (ed.) Prodr. (DC.). Fortin, Masson et sociorum, Paris, vol. 10: 1–178.

Díez, M. J. (1994). "A general survey of pollen types in Anchusa L.(Boraginaceae) in relation to taxonomy." Acta botanica gallica 141(2): 233-242.

Díez, M. J. and B. Valdés (1991). "Pollen morphology of the tribes Eritrichieae and Cynoglosseae (Boraginaceae) in the Iberian Peninsula and its taxonomic significance." Botanical journal of the Linnean Society 107(1): 49-66.

Erdtman, G. (1952). "Pollen morphology and plant taxonomy." GFF 74(4): 526-527.-

Frenguelli, G. (2003). "Pollen structure and morphology." Postêpy Dermatologii i Alergologii 20(4): 200.

-Gottschling M, Hilger HH. **(**2001). Phylogenetic analysis and character evolution of *Ehretia* and *Bourreria* (Ehretiaceae, Boraginales) and their allies based on ITS1 sequences. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 123: 249–268

Gurke, R. (1893). "Verbenaceae afneanae." Botanische Jahrbiicher **18**: 165-183.-

Hesse, M., et al. (2009). Pollen terminology: an illustrated handbook, Springer Science & Business Media.

Hilger, H, Selvi, F., Papini, A. & Bigazzi, M. 2004: Molecular systematics of Boraginaceae tribe Borageineae based on ITS1 and trnL sequences, with special reference to Anchusa s.l. Ann. Bot. 94: 201-212.

Hilger, H. H., Gottschling,M.Selvi,F.,Bigazzi,M.,Langstrom ,Zippel,E.,Diane,N.and Weigend,M (2005). "The Euro+ Med Treatment of Boraginaceae in Willdenowia 34: A Response." Willdenowia: 43-48.

Huysmans, S., et al. (1994). "Are the genera Hallea and Mitragyna (Rubiaceae." Blumea **39**(1/2): 321-340.

-Johnston, I. M. (1924). "STUDIES IN THE BORAGINACEAE,—II." Contributions from the Gray Herbarium of Harvard University: 3-61.

Johnston, I. M. (1957). "Studies in the Boraginaceae, XXIX. Echiochilon and Related Genera." Journal of the Arnold Arboretum 38(3): 255-293.

Khatamsaz, M. (2001). "Pollen morphology of Iranian Boraginaceae family and its taxonomic significance." Iranian Journal of Botany 9(1): 27-40.

Långström, E. and M. Chase (2002). "Tribes of Boraginoideae (Boraginaceae) and placement of Antiphytum, Echiochilon, Ogastemma and Sericostoma: a phylogenetic analysis based on atpB plastid DNA sequence data." Plant Systematics and Evolution 234(1-4): 137-153.

Liu, J.-X., Li, J.-Y., Zhang, Y.-L., Ning, J.-C., 2010. Pollen morphology of the tribe Lithospermeae of Boraginoideae in China and its taxonomic significance. Plant Syst. Evol. 290, 75– 83.

Luebert, F. (2010). Systematics, ecology and evolution of Heliotropium sect. Cochranea (Heliotropiaceae) and the biogeography of the Atacama Desert, Freie Uiversität Berlin.

Mabberley , D. J. (1997). The plant-book: a portable dictionary of the vascular plants, Cambridge university press.

Mabberley, D. J.(2008). Mabberley's Plant-Book: A Portable Dictionary of the Higher Plants, Their Classification and Uses. 3rd edition. Cambridge University Press, Cambridge

Mandaville, J. P. (1990). "Flora of Eastern Saudi Arabia." London: Kegan Paul Int.

Mansion, G., Selvi, F., Guggisberg, A., Conti, E. (2009). "Origin of Mediterranean insular endemics in the Boraginales: integrative evidence from molecular dating and ancestral area reconstruction." Journal of Biogeography 36(7): 1282-1296.

Migahid, A. M. (1978). "Flora of Saudi Arabia, Vol. 2." Riyadh: Riyadh University Publications 647.

Nazaire, M. and L. Hufford (2012). "A broad phylogenetic analysis of Boraginaceae: implications for the relationships of Mertensia." Systematic Botany 37(3): 758-78

Nowicke, J. W. and J. S. Miller (1989). "Pollen morphology and the relationships of Hoplestigmataceae." Taxon: 12-16.

-Nowicke, J. W. and J. S. Miller (1990). Pollen morphology of the Cordioideae (Boraginaceae): Auxemma, Cordia, and Patagonula. Morphology, development, and systematic relevance of pollen and spores, Springer**:** 103-121.

Popov, M. G. (1953). "Boraginaceae. In Flora URSS vol. 19." **19**(eds. B. K. Shishkin,--

and E. Bobrov. Moskva, Leningrad: Izdatel'stvo Akademii Nauk SSSR.).

Punt, W., Hoen, P. P., Blackmore, S., Nilsson, S. & Le Thomas, A.(2007). Glossary of pollen and spore terminology. Review of Palaeobotany and Palynology, 143, 1–81.

Reitsma, T. (1970). "Suggestions towards unification of descriptive terminology of angiosperm pollenn grains." Review of Palaeobotany and Palynology 10(1): 39-60.

Riedl, H. (1967). "Boraginaceae." Flora Malesiana Ser. I. I . 13(eds. P. Baas , R. W. J. M. van der Ham, R. Hegnauer , and N. Spitteler. Leiden : Publications Department Rijksherbarium.): 43 – 144.

Riedl, H. (1997). "Boraginaceae." Flora Malesiana-Series 1, Spermatophyta 13(1): 43-144.

Romeiras, M.M., Ascensão, L., Duarte, M.C., Diniz, M.A. & Pais, M.S.(2008). Taxonomy of Echium L. (Boraginaceae) species from Cape Verde Islands. Austral. Syst. Bot. 21: 26–38.-

Sahay, M. (1973). "Pollen morphology of Heliotropium." Journal of palynology.

Sauvage ,C., VINDT, J. (1954). Cerinthe L. Flore du Maroc. **2:** 146-149. Internationales, Tanger.

Selvi, F., Coppi, A., Cecchi, L.. (2011). "High epizoochorous specialization and low DNA sequence divergence in Mediterranean Cynoglossum (Boraginaceae): Evidence from fruit traits and ITS region." Taxon 60(4): 969-985.

Stevens, P. (2001). onwards. Angiosperm Phylogeny Website. Version 9, June 2008.

Takhtajan, A.L. (1987). Systema magnoliophytorum, Nauka.Leningrad.

Takhtajan, A. (1997). Diversity and classification of flowering plants. New York: Columbia University Press.

Verdcourt, B. (1991). Boraginaceae. In: R.M. Polhill (ed.), Fl. Trop. E. Afr. Balkema, Rotterdam & Brookfield.

Weigend, M. and H. H. Hilger (2014). "Codonaceae-a newly required family name in Boraginales. Phytotaxa 10(1): 26-30.