Pak. J. Bot., 38(3): 511-520, 2006.

PALYNOLOGICAL OBSERVATIONS ON THE GENUS *CALENDULA* (CALENDULEAE-COMPOSITAE) FROM PAKISTAN

AKBAR ALI MEO AND MIR AJAB KHAN*

Department of Biology,
Government College, Satellite Town, Rawalpindi, Pakistan

Abstract

The pollen morphology of *Calendula* species was investigated by light and scanning electron microscopy. Pollen grains are prolate-spheroidal in equatorial view and semi-angular in polar view, tri. to tetrazoncolporate, caveate and echinate. Size of polar axis and equatorial diameter, exine thickness, spine length are presented. Variation exists at species level i.e. exine thickness is higher in *C.arvensis* L. (6.3 µm) as compared to *C.officinalis* L. (3.3 µm). Existence of simple and closely placed pair of spines are highly diagnostic in *Calendula*. In *C. officinalis* spines are mostly paired and in *C. arvensis* mostly simple but closely placed pairs of spines also exist.

Introduction

Compositae (Astesaceaee) is one of the largest plant families consisting of herbaceous plants with only few exceptions. It is cosmopolitan in distribution, occurring in all continents except Antarctica (Hicking & King, 1997). The family is most abundant in mountain sub-tropical latitude. It is highly evolved family among angiosperms and is generally regarded as occupying the highest position in plant kingdom due to its great preponderance and cosmopolitan range (Chopra, 1970). The family comprises ± 1535 genera and over c.2300 species distributed in 3 subfamilies and 17 tribes. It is also the largest plant family in Pakistan, represented by over 650 species distributed in 15 tribes (Ghafoor, 2002).

Compositae is an eurypalynous family and possess zonocolporate pollen grains (Erdtman, 1952, Sachdeva & Malik, 1986) Pollen grains of Astereae subtribe Solidaginae are helianthoid, spherical, tricolporate, echinate with abnormalities in size and colpus number (Wodehouse, 1926, 1935). Clark *et al*., (1980) examined that pollen grains of *Haplopappus* are tricolporate and echinate. Pollen size, spine length and number of spine rows between colpi can help in systematic studies in Astereae. There are several reports on palynology of Compositae viz., Heslop-Harrison (1969), Stanley & Linsken (1974), Reitsman (1970), Manten (1970), Mascarenhas (1975), Ferguson & Muller (1976), Vincent & Norris (1989), Feuer & Tomb (1977), Tomb *et al*., (1974), Cilliers (1991), Pinar & Donmez (2000). Malik *et al*., (1964), Zahur *et al*., (1978), Meo *et al*., (1988 a, b, 1989) Meo (1999), Nasreen & Khan (1998), Dawar *et al*., (2002) have sporadically reported palynology of different families in Pakistan. The Calenduleae occur throughout Africa. They are also found in South and central Europe and in west and southwest Asia, but seem to be concentrated in the Mediterranean area and South Africa. There are no native members of tribe calenduleae but several *Calendula* spp., occur as garden escapes or casuals (Clapham, *et al*., 1962).

*Department of Biological Sciences, Quaid-I-Azam University, Islamabad Pakistan

There are only two species of Calendula viz., *C. officinalis* and *C. arvensis* in Pakistan (Stewart, 1972). *Calendula officinalis* is native of S.Europe and cultivated in every flowery garden. *C. arvensis* is distributed in South Europe, Caucasus, Iran, Afghanistan and Pakistan (Nasir & Rafiq, 1995). *Calendula arvensis* is abundant, spring, annual, weed and is doubtfully native. It is found as gregarious in open places, wastelands, road side, graveyard and field margins. *Calendula* spp., is a repeated medical plant in homeopathic system of medicine. Seeds, flowers and leafs of *Calendula arvensis* are medicinally important. Calendulin, salicylic acid, oleanic acid, faradial and arvidiol chemicals are obtained from *Calendula arvensis* and its flowers are locally used in making an ointment for skin (Khalid, 1995).

Palynologically, the tribe is rather stenopalynous. However, certain pollen morphological features, recorded in the genera and species of the tribe, are undoubtedly interesting for taxonomic purposes. The most important pollen morphological studies of the taxa included in the Calenduleae were previously made by Stix (1960), Dimon (1971), Norlindh (1978) and Skvarla *et al*., (1978). Praglowski & Grafstrom (1980) viewed that a brief palynological investigation of the tribe would be useful. The tribe Calenduleae consists of 113 species and large number of subspecies (Norlindh, 1978). The present report give an account of the palonological observations on the genus *Calendula*.

Materials and Methods

Pollens were removed from dry herbarium material from Quaid-i-Azam University Herbarium (ISL), Islamabad. A list of the specimens used in this study and the Herbarium data are presented in the Appendix 1. Pollen grains were prepared according to the acetolysis method as described by Erdtman (1966). Light microscopical work was carried out using a Nikon Labophot microscope (1000 X) under oil immersion. Florets were treated in acetic acid for 5 minutes. Pollen grains were mounted on slides in glycerine jelly. For scanning electron microscopy (SEM), pollen was air dried on specimen stubs and then coated with gold for 3 minutes for using JEE-420, Jeol Vacuum evaporator. The specimens were examined and photographed with a Jeol JSM- 5910 scanning electron microscope using Mono-Crom film.

Polar axis (P), Equatorial diameters (E), P/E ratio, Exine thickness, shape in polar view, shape in equatorial view, spine length, number of spine rows between colpi and aperture types were recorded. The data was statistically analyzed (Table 1). Terminology as used by Erdtman (1952), Huang (1972), Walker & Doyle (1975), and Punt. *et al*., (1994) has been followed. The permanent slides of *Calendula* species have been placed in the pollen reference collection of the Department of Biological Sciences, Quaid-i-Azam, University, Islamabad.

Results

The pollen morphology of genus *Calendula* is generally radially symmetrical, usually prolate-spheroidal, polar axis 38.9-39.7µm, equatorial diameter 32.1-36.4 µm, trizonocolporate to tetrazonocolporate, caveate and echinate.

Table 1 summarizes the measurements of pollen grains from the taxa examined. Light microscopic micrographs of *Calendula* species are presented in Fig. 1 and scanning electron micrographs are presented in Figs. 2-3.

Appendix 1. Source of pollen material used in this study.

Taxon	Locality	District	Collected by	Voucher No.	Date of collection
Calendula officinalis L.	Kolpur	Quetta	Manzoor Hussain & M. Arif.	352	11/4/1978
	Attock	Attock	Muqarrab shah & Ayaz Abassi	414	8/4/1977
	Samangli	Quetta	Manzoor & Arif	944	26/4/1978
Calendula arvensis L.	National park	Rawalpindi	M. Arif, Maqsood & Sarfaraz	178	9/3/1977
	Odegram	Swat	Muqarrab Shah & Dilawar Khan	70	20/4/1976
	Mathakis (Tribal area)	Dir	Ghulam Farooq	15	22/3/1976

Table 1. Summary of pollen measurements, shape and sculpturing features in *Calendula* (Calenduleae) species.

Taxon	Equatorial view (µm)	Polar view (µm)	P/E ratio	Exine thickness (µm)	Number of spine rows between colpi	Spine length (µm)	Shape in equatorial view	Shape in polar view	Class	Aperture type	Sculpturing
Calendula officinalis L.	*36.4 ± 1.46 (31-40.5)	38.9 ± 1.19 (34-41.5)	1.07	3.3 ± 0.30 (2.5-4)	5-9	6.5 ± 0.42 (5-7.5)	Prolate-spheroidal	Semi-angular	Tri & Tetrazono colporate	Non-lacunate	Echinate
Calendula arvensis L.	32.1 ± 0.30 (31-33)	39.7 ± 1.12 (36-42.5)	1.24	6.3 ± 0.85 (4-8.5)	4-8	7.1 ± 0.48 (5-7.5)	Prolate-spheroidal	Semi-angular	Tri & Tetrazono colporate	Lacunate	Echinate

*Mean values followed by min-max in parentheses. P=Polar, E=Equatorial, ±=Standard error

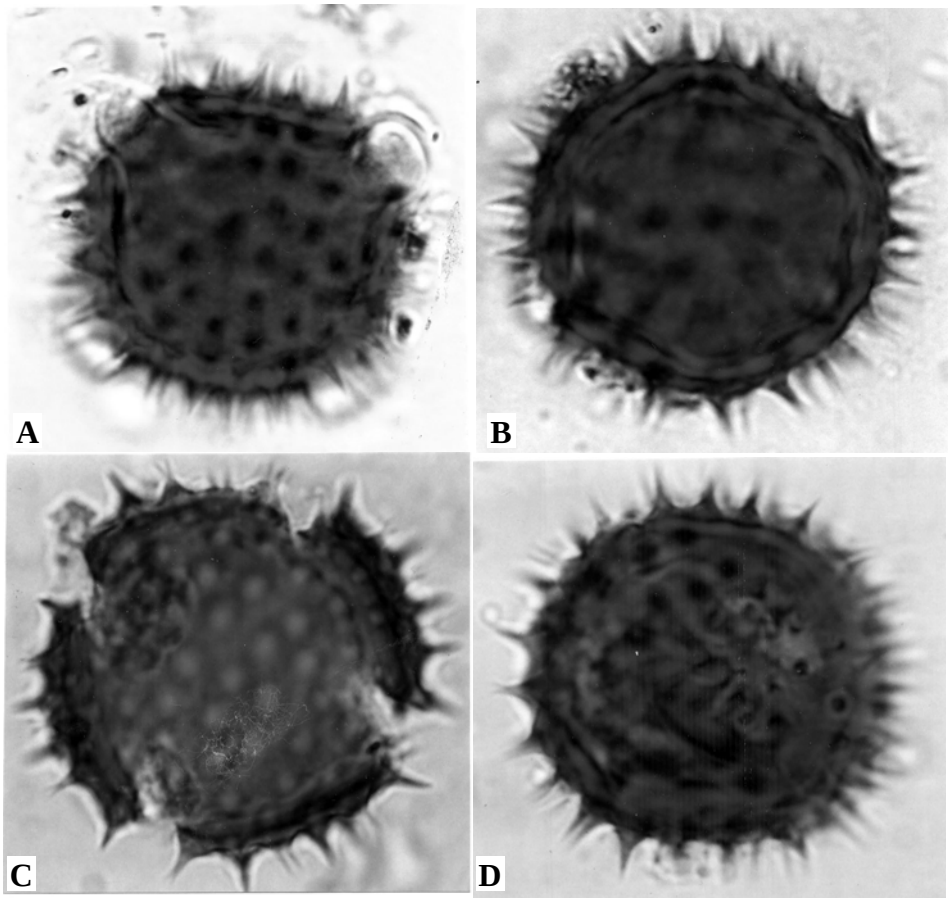

Fig. 1. Light micrographs of pollen grains of *Calendula* species (X1000) *Calendula officinalis* L. A. Polar view; B. Equatorial view. *Calendula arvensis* L. C. Polar view; D. Equatorial view

Size: The size of the pollen grain (Polar axis-equatorial diameter excluding spines) of *Calendula* species ranges from 38.9/36.4µm to 39.7/32.1µm. There is a little variation in the size of the pollen grains. *Calendula officinalis* and *Calendula arvensis* have almost similar sized pollen and P/E ratio is 1.09 in *C. officinalis* and 1.24 in *C. arvensis*.

Symmetry and shape: The pollen grains are radially symmetrical, isopolar and isodiametric Pollen shape is prolate-spheroidal in equatorial view and semi-angular in polar view.

Aperture: The pollen grains are trizonocolporate and tetrazonocolporate in both of the species. The Aperture type is non-lacunate in *Calendula officinalis* and lacunate in *C.arvensis*. Apertural membrane is spinate. Colpi is long in *C. arvensis* and short in *C.officinalis*.

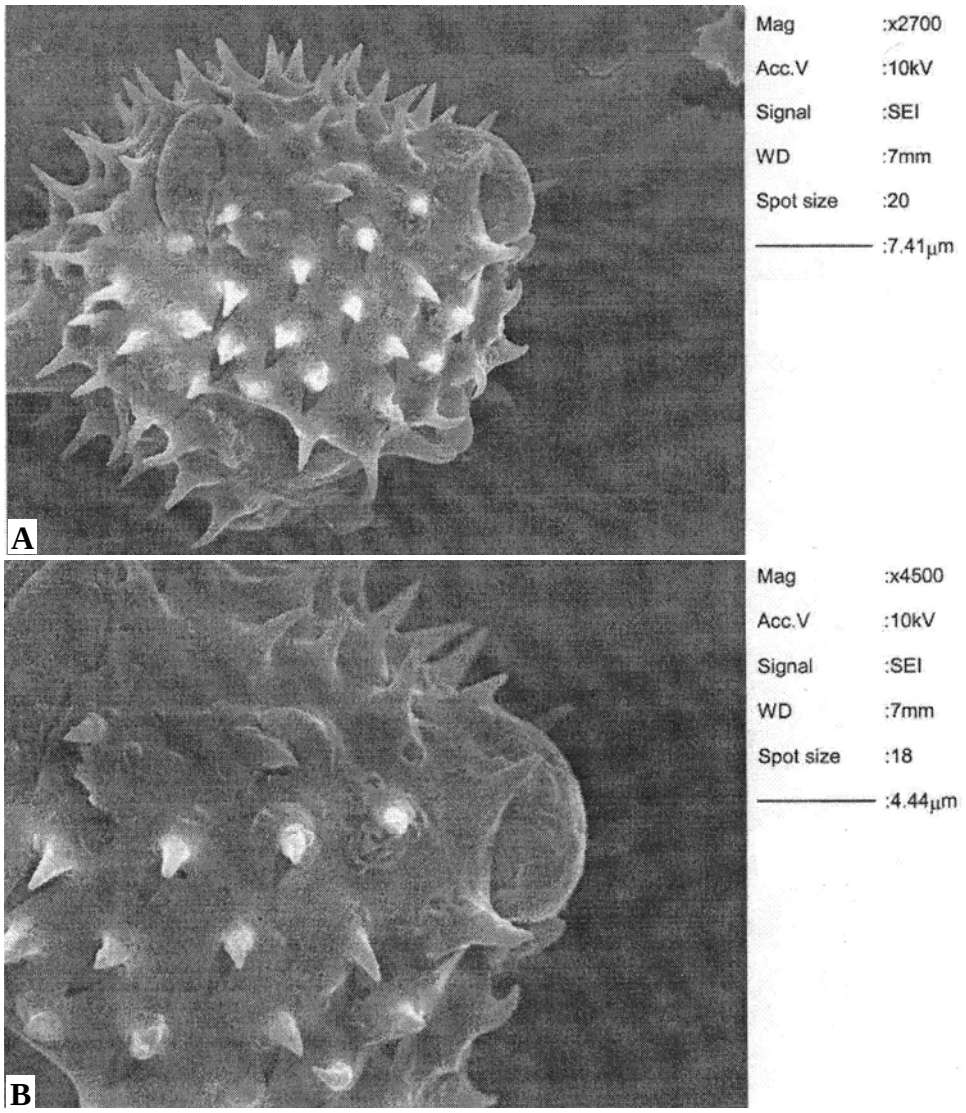

Fig. 2. Scanning electron micrographs of pollen grains *Calendula officinalis* L. A. Polar view, B. Exine pattern.

Spine: The spine length varies from 6.5μm in *C. officinalis* to 7.1 μm in *C. arvensis*. The number of spine rows between colpi varies from 5-9 in *C. officinalis* and 4-8 in *C. arvensis*. Existence of thin paired spines is taxonomically a highly distinguishing character in the genus *Calendula*. The pairs of closely placed thin spines appear as bifurcated in both the species.

Exine: Exine thickness varies from 3.3μm in *C. officinalis* to 6.3μm in *C. arvensis*. Surface view of pollen sculpturing is echinate in both the species.

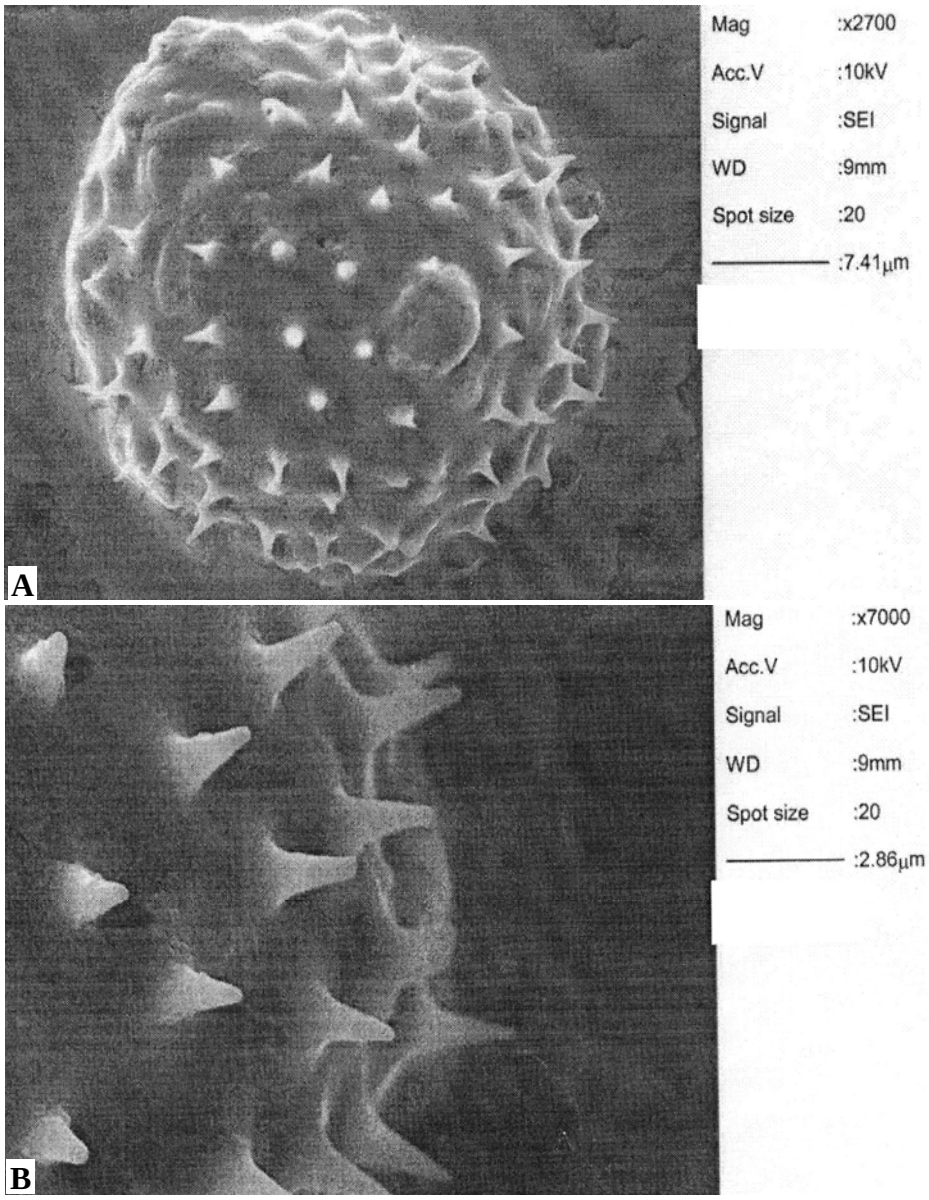

Fig. 3. Scanning electron micrograph of pollen grains *Calendula arvensis* L. A. Polar view, B. Exine pattern.

Ornamentation: Pollen grains were examined through scanning electron microscope. Pollen grains in both the species of *Calendula arvensis* and *C. officinalis* are caveate, Columella weakly developed and the foot layer is vestigeal or lacking. Sculpturing is echinate with pointed end, sometimes curved. The feature of spines appear to be the most diagnostic to distinguish *Calendula arvensis* from *C. officinalis* (Figs. 2&3). The spines are short and thin in *Calendula officinalis* as compared to *C.arvensis.*

Discussion

The family Asteraceae has not yet been published in Flora of Pakistan, neither flora of pollen is completed. Palynological studies not only provide additional information but also provide taxonomic characters, which proved to be helpful to improve the systematic position of taxa within their respective classification. The most significant pollen morphological studies of the taxa included in *Calendula* were previously made by Stix (1960), Dimon (1971), Praglowski (in Norlindh 1978), Skvarla *et al.,* (1977) and Praglowski & Grafstron (1980). Pollen morphology of *Calendula* is helpful at the specific level. Praglowski & Grafstron (1980) felt that a brief palynological investigation of tribe *Calenduleae* is helpful for taxonomic purposes. Existence of paired spines i.e., two closely placed spines and simple spines of pollen grain is diagnostic feature of the genus *Calendula* within the tribe *Calenduleae* has been discovered in the present investigation. However, the spines mostly appear paired in *Calendula officinalis* and mostly simple and some paired spines are found in *C. arvensis.* Such paired spines might be helpful in the delimitation of species and the classification of the tribe Calenduleae in its respective position in Asteraceae. Among the other characters exine thickness is a diagnostic character in *Calendula officinalis* (3.3μm) and *C. arvensis* (6.3μm) in the present study (Table 1). Praglowski & Grafstrom (1980) viewed that structural exine characteristic in this tribe support a consanguinity with Heliantheae and Senecioneae.

Nair (1961) reported 3-zonocolporate pollen in *Calendula*. However in the present investigation both the species have 4-zonocolporate (tetrazonocolporate) pollen as mentioned by Malik, *et al*, (1964) only in *Calendula arvensis.* The presence of trizonocolporate and tetrazonocolporate pollen in *C. officinalis* and *C. arvensis* is an evolutionary trend which would be helpful to establish a phylogenetic relationship of species within the genus *Calendula.* Praglowski & Grafstrom (1980) studied pollen morphology of 8 genera of the tribe Calenduleae and all the taxa were trizonocolporate. The emergence of tetrazonocolporate condition which exists in *Calendula arvensis* and *Calendula officinalis* has been reported here for the first time. Praglowski & Grafstrom (1980) consider the pollen of Calenduleae to show affinity with Astereae, Senecioneae and Heliantheae. They consider the consanguinity of Calenduleae with Senecioineae as expressed by Small (1919), Norlindh (1946) and Cronquist (1955b).

Praglowski & Grafstrom (1980) concluded that Calenduleae should be considered as offshoot of the Senecioneae rather than a direct derivative of Heliantheae. *Calendula arvensis* can be distinguished due to higher spine length (7.1μm) from *Calendula officinalis* (6.5 μm). Number of spine rows between colpi is usually a useful taxonomic character in the family Compositae. In C.arvensis the number of spine rows between colpi is 4-8 and 5-9 in *C. officinalis*. Praglowski & Grafstrom (1980) reported numerous slender spines (100-130) on the pollen of *Calendula*-type. Tomsovic (1997) studied the pollen characters of Echinops and investigated that in the genus Echinops, the species *E. strigosus* is very different and should be classified as a separate genus Psectra (Endllcher) including one species *Psectra strigosa* (L.). Tomsovic (1997), Hall (1928) and Clark *et al*., (1980) studied the Asteraceae and distinguished some genera on the basis of pollen features. There is little variation in size of the pollen grains of *Calendula* species in the present studies as both species have almost similar sized pollen ranged from 38.9-36.4μm to 39.7-32.1μm. Praglowski & Grafstrom (1980) pointed out with reference to taxonomy that pollen grains of *Calendula* (the type-genus for the tribe), possess some

morphological characteristics of this genus. These features include size of the pollen grains, which is the largest within the tribe (equatorial diameter upto 50μm) with long solid spines and presence of comparatively thick endexine. *Calendula* represents a characteristic pollen, morphologically well delimited and easily recognizable group, which can hardly be confused with any other representative of the tribe (Meo, 2005). Malik *et al*., (1964) studied pollen morphology of some Pakistani medicinal plants, which include *Calendula arvensis.* However, *C. officinalis and C. arvensis* can be distinguished on the basis of exine thickness, spine length, pair of closely placed spines, numbers of spine rows between colpi and the aperture type. Similarly, Pinar & Dönmez (2000) observed that spine cavities of pollen exine could be used as a diagnostic character in the genera of Compositae. On the basis of palynological studies it can be concluded that the pollen characters in the genus *Calendula* are helpful at the specific level. As revealed from scanning electron micrographs that spines appears to be the most diagnostic to distinguish *C. arvensis* from *C. officinalis*. The spines are short and thin in *C. officinalis* as compared with *C. arvensis* (Fig. 2). The light microscopic results also confirm the fact that spine length is higher in *C. arvensis* as compared to *C. officinalis* (Table 1). Key to the species of Calendula is presented for identification.

Key to the species of *Calendula*

1. Exine thickness 3.3μm, number of spine rows between colpi 5-9 …….. *C. officinalis*
1. Exine thickness 6.3μm, number of spine rows between colpi 4-8 ...……. *C. arvensis*

Acknowledgement

We would like to express our sincere thanks to the Department of Biological Sciences, Quaid-i-Azam University, Islamabad, for the use of Herbarium material of the *Calendula* species. We are grateful to Prof. Dr. Atta-ur-Rehman, Chairman HEC for providing financial grant for scanning electron micrographs. Sincerest thanks to Prof Dr. Muhammad Riaz Khan, Department of Physics, University of Peshawar, for the skillful preparation of the scanning electron micrographs. We thank Malik Duri-Iman for preparing the typescript.

References

Chopra, G.L. 1970. *Angiosperms* (*Systematic and life-cycle*). Unique Publishers, Lahore. 530 pp.

Cilliers, S.S. 1991. Pollen morphology and its taxonomic value in Bachylaena (Asteraceae) in South Africa. *S. Afr. J. Bot*., 57: 325-330

Clapham, A.R., T.G. Tutin and E.F. Warburg. 1962. *Flora of the British Isles*. Cambridge Univ. Press.

Clark, W.D., G.K. Brown and R.A. Mayes. 1980. Pollen morphology of *Haplopappus* and related genera (Compositae-Astereae). *Amer. J. Bot*., 67(9): 1391-1393.

Cronquist, A. 1955b. Phylogeny and taxonomy of the Compositae. *Am. Midl. Nat*., 53:478-551.

Dawar, R., M. Qaiser and A. Perveen. 2002. Pollen morphology of *Inula* L. (S.STR.) and its allied genera (Inuleae-Compositae) from Pakistan and Kashmir. *Pak. J. Bot*., 34(1): 9-22.

Dimon, M.Th. 1971. Problem generaux soul eves par letude pellinque de composes Mediterranean's. *Naturaqlia Monspel. Ser. Bot*., 22: 129-144.

Erdtman, G. 1952. Pollen morphology and plant taxonomy (*An introduction to Palynology-I, Angiosperms*). Almgvist and Wicksell, Stockholm.

Erdtman, G. 1966. Pollen morphology and plant taxonomy. *Angiosperms*. New York.

Ferguson, I.K. and J. Muller. 1976. *The evolutionary significance of the exine*. Linnean Soc. Symp. No.1. Academic Press, New York.

Feuer, S. and A.S. Tomb. 1977. Pollen morphology and detailed structure of family Compositae, Tribe Cichorieae II. Subtribe Microseridinae. *Amer. J. Bot*., 64(2): 230-245.

Ghafoor, A. 2002. Asteraceae–Anthemideae. In: *Flora of Pakistan*. (Edi): S.I. Ali & M. Qaiser. No.207. Jointly published by Department of Botany, University of Karachi and Missouri Botanical Press, Missouri Botanical Garden, St. Louis, Missouri, U.S.A.

Hall, M.H. 1928. *The genus Haplopappus, a phylogenetic study in Compositae*. Carnegic Inst. Wash. Publ. No. 389.

Heslop-Harrison, J. 1969. Scanning electron microscopic observations on the wall of pollen grain of *Cosmos bipinnatus*, Compositae. *Proc. Engis Stereoscan Colloq*., 9: 89-96.

Hickey, M and C. King. 1997. *Common families of flowering plants*. Cambridge University Press, U.K.

Huang, T.C. 1972. *Pollen flora of Taiwan*. National Taiwan Univ. Botany Dept. Press.

Khalid, S. 1995. *Weeds of Pakistan Compositae*. National Herbarium, National Agriculture Research Centre, Park Road, Islamabad.

Malik, N.A., S.A. Rehman and A.J. Ahmad. 1964. Pollen morphology of some Pakistani Medicinal plants. *Pak. J. Sci. Industrial Res*., 7: 130-136.

Manten, A. A. 1970. Ultra-violet and electron microscopy and their applications in palynology. *Rev. Plaeobot. Palynol*., 10:5-37

Mascarenhas. J. P. 1975. The biochemistry of angiosperms pollen development. *Bot. Rev*., 41: 259-314.

Meo, A.A., H.M.I. Hafiz, F. Baig and N.A. Baig. 1988a. Studies of pollen morphology of some Graminaceous (Poaceae) species. *Sarhad J. of Agric*., 4: 99-103.

Meo, A.A., H.M.I. Hafiz, F. Baig and N.A. Baig. 1988b. Pollen morphology and systematic relationships among Graminaceous (Poaceae) species. *J. Pure and Applied Sci*., 7(2): 15-20.

Meo, A.A., H.M.I. Hafiz, F. Baig and N.A. Baig. 1989. Pollen grain description of 13-non-allergic and 4-allergic Graminaeceous (Poaceae) species. *J. Pure and Applied Sci*., 8(2): 19-26.

Meo, A.A. 1999. Impact of pollen and inter-genetic crosses between Graminaceous (Poaceae) plants. *Pak. J. Biological Sci*., 2(3): 809-812.

Meo, A.A. 2005. Palynological studies of selected genera of the tribes of Asteraceae from Pakistan. Unpublished Ph.D. thesis, Quaid-i-Azam University, Islamabad.

Nair, P.K.K. 1961. Journal of Science & Industrial Research (New Delhi) 20C, No. 2.

Nasreen, U. and M.A. Khan. 1998. Palynological studies of *Matricaria chamomilla* (Babuna) and its related genera. *Hamdard*, 4: 94-97.

Nasir, Y.J. and R. Rafiq. 1995. *Wild Flowers of Pakistan*. (Ed.): T.J. Roberts. Oxford University Press.

Norlindh, T. 1978. Calenduleae–systematic review. In: *The Biology and Chemistry of the Compositae*. (Eds.): V.H. Heywood, J.B. Harborne and B.L. Turner. 2: 961-987, London, New York, San Francisco.

Norlindh, T. 1946. Studies in the Calenduleae II. Phytogeography and interrelation. *Bot. Notiser*., 81: 471-509.

Pinar, N.M. and E.O. Dönmez. 2000. Pollen morphology of some Turkish endemic *Helichrysum Gaertner* species (Compositae). *Pak. J. Bot*., 32(2): 295-301.

Praglowski, J.and E. Grafstrom. 1980. The pollen morphology of the tribe Calenduleae with reference to taxonomy. *Bot. Notiser*., 133:177-188.

Punt. W., S. Blackmore, S. Nilsson and le A. Thomas. 1994. *Glossary of Pollen and Spore terminology*, -LPP foundation, Utrecht, LPP contribution series No.1.

Reitsman, T.J. 1970. Suggestions towards unification of descriptive terminology of angiosperm pollen grains. *Rev. Palaeobot. Palynol*., 10: 39-60.

Sachdeva, S.K. and C.P. Malik. 1986. *Experimental plant taxonomy*. Kalyani Publications, New Delhi.

Skvarla, J.J., B.L. Turner, V.G. Patel and A. S. Tomb. 1978. Pollen morphology in Compositae and in morphologically related families. In: *The biology and chemistry of the Compositae*. (Eds.): V.H. Heywood, J.B. Harborne & B.L. Turner. pp. 141-248. Academic Press, London, New York.

Small, J. 1919. The origin and development of the Compositae. *New phytol*., 18: 201-234.

Stanley, R.G. and H.F. Linskens. 1974. *Pollen*. Springer-Verlag. New York.

Stewart, R.R. 1972. *An annotated catalogue of vascular plants of West Pakistan*. Fakhri Printing Press Karachi.

Stix, E.1960. Pollen morphologische Untersuchungen an Compositen, *Grana Palyn*., 2: 41-104.

Tomb, S.A., D.A. Larson and J.J. Skvarla. 1974. Pollen morphology and detailed structure of family Compositae, tribe Cichorieae. I. subtribe Stephanomeriinae. *Amer. J. Bot*., 61(5): 486-498.

Tomsovic, P. 1997. Some palynological observations on the genus *Echinops* (Asteraceae) and their taxonomic implications. *Preslia, Praha*, 69: 31-33.

Vincent. P.L.D. and G.F.M. Norris. 1989. An SEM study of the external pollen morphology in *Senecio* and some related genera in the subtribe Senecioninae (Asteraceae- Senecionineae). S. Afr. Tydskr. *Plantk*, 55: 304-309.

Walker, J.W. and J.A. Doyle. 1975. The basis of angiosperm phylogeny: Palynology. *Ann. Mo. Bot. Gard*., 62: 664-723.

Wodehouse, R.P. 1926. pollen grain morphology in the classification of Anthemideae. *Bull. Torrey Bot. Club*., 53: 479-485.

Wodehouse, R.P. 1935. *Pollen grains*. New York.

Zahur, M.S., A.A. Bhutta and M. Ashraf. 1978. (a) Palynological studies of the plants growing in Punjab. (b) Seasonal variation in the frequency of air-borne pollen and spores which cause allergies and asthma with special reference to "Central Punjab". P. S. P. Final Research Report.

(Received for publication 28 August 2003)