

Pollen Morphology and Pollen Fertility Estimation of Three Medicinal Plant Species of *Hypericum* L. from Kaghan Valley, Northern Pakistan

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Abstract

Pollen morphology & pollen fertility estimation of three medicinal species belonging to genus *Hypericum* L. from flora of Kaghan valley, Northern Pakistan was investigated by light and scanning electron microscopy for the first time. The species are *Hypericum elodeoides* Choisy in, *Hypericum oblongifolium* Choisy, and *Hypericum perforatum* L. Pollen morphological characteristics with considerable variation are provided for these taxa. Among the studied taxa, the pollen grains were 3-4 colpi type and the largest pollen size was found in *H. oblongifolium* in polar view 23.0 µm and in equatorial view was 25.35 µm. The smallest pollen was of *H. elodeoides* in polar view which was 19.91 µm and in equatorial view was observed 12.53 µm in *H. perforatum*. The shape of the all pollen grain was circular to semi-angular in polar view, however in equatorial view the shape of the grains was oblate-spheroidal, in *H. elodeoides*, and *H. oblongifolium* while prolate in *H. perforatum*. Exine sculpturing was observed by scanning electron microscopy which showed foveolate sculpturing in *H. elodeoides*, reticulate in *H. oblongifolium* and scabrate in *H. perforatum*. Pollen fertility estimation ranged from 82.66% to 90.90% which shows that flora of selected species is well established.

Keywords

Hypericum, Pollen Fertility, Kaghan Valley, Pollen Morphology, SEM

1. Introduction

The flowering plant genus; *Hypericum* L. is one of the 100 large angiosperm genera and the largest genus of nine genera of the family Hypericaceae with about

469 species occurring naturally and have been introduced to, every continent except Antarctica. Approximately 80% of the diversity of the family is within *Hypericum* [1] [2] [3]. Family Hypericaceae belongs to order Malpighiales and contains well-known medicinally valuable species [2] [4]. The genus *Hypericum* is known worldwide for its traditional and modern uses. There are variety of bioactive compounds found in many species of *Hypericum*, which are used pharmacologically as well as to treat many diseases like tumors, cancer, AIDS, mild depression and kidney failure etc. [2] [5] [6]. The most well-known species of the genus *Hypericum* is St. John's wort *Hypericum perforatum* which is economically important crop plant with antiviral, anticancer, antibacterial and antidepressant properties [7] [8]. Flowers of this plant are known to use for relieving body pains and tea preparation in Kaghan Valley [9].

Genus *Hypericum* L has been studied by various researchers for its taxonomic, chemotaxonomic, pharmacological, karyological, ethnobotanical aspects. However a recent phylogenetic study based on morphological characters was carried out (Nurk and Blattner, [10] while based on phylogenetic characters was studied by (Nurk [11] and Rhufel *et al.* [12]. Palynology of the *Hypericum* recently studied by Otaghvari *et al.* and previously have been studied by Khan, Thomas, Aytug *et al.*, Charpin (studied regarding allergic pollen), Yi, Clarke, Barros and Ramos, Martonfi *et al.*, Meseguer and Sanmartin and Ocak *et al.*, [6] [13]-[24] but the pollen morphology of *Hypericum* from Pakistan is still not known. However it has been largely studied for its medicinal properties, ethnobotanical uses, traditional knowledge and antioxidant capacity [9] [25] [26].

The knowledge of pollen morphology and taxonomy can be used as an instrument of multiple scientific researches in systematic botany, paleobotany, paleoecology, pollen analysis, aeropalynology, criminology, allergy, stratigraphic correlation of oil-bearing rocks and coal fields, drugs in the field of medicopalynology and improvement of honey in the field of mellittopalynology and copropalynology [27]. Application of pollen morphology in plant taxonomy is best evidence in the flowering plants, especially in the angiosperms. The largest variety of pollen morphology occurs among the angiospermic plants [28]. Like other disciplines pollen grains have an important part in modern issue of plant taxonomy [29].

The present investigation is based on pollen morphology and pollen fertility estimation of three medicinally important plant species of genus *Hypericum* studied for the first time from Pakistan.

2. Materials & Methods

Study Area—The Kaghan valley covering an area of 19790.4 hectare spreads on either bank of river Kunhar, situated in the district Mansehra, Hazara division, Northern Pakistan (Figure 1). It is located between 34° - 14' and 35° - 11' north latitudes and 72° - 49' and 74° - 08' east longitude. It is bounded in the north by Batagram and Kohistan districts, in the east by Muzafarabad district of Azad

KAGHAN VALLEY

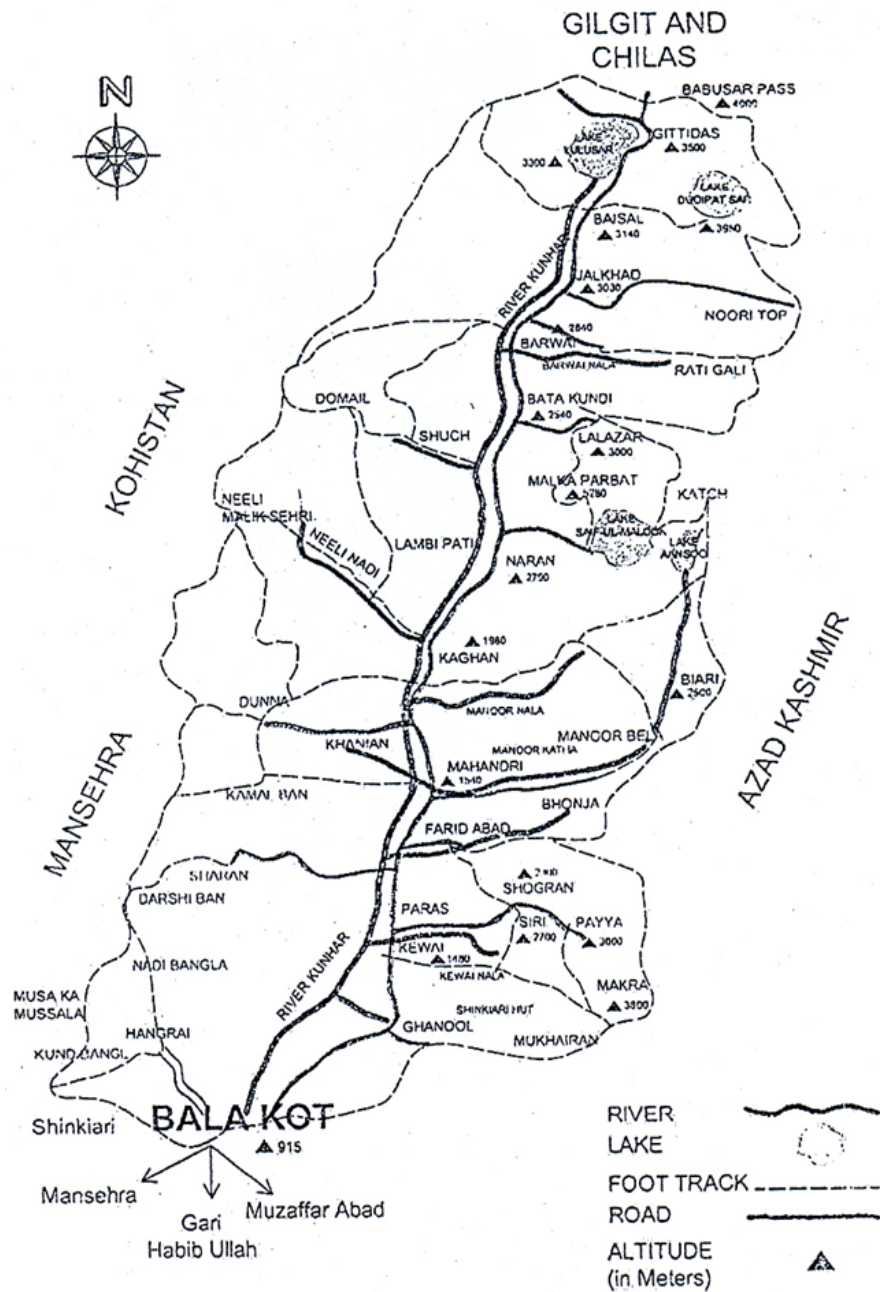


Figure 1. Map of the study area.

Jamu and Kashmir, in the south by Abbottabad and Haripur districts and in the west by Swat district [30]. The climate of the the Kaghan valley is cold in summer owing to snow clad mountains and very cold in winter Temperature ranges from 2°C - 30°C in the district The general vegetation is of the scrub type which includes the shrubs or medium size trees [31]. There is a great variety of medicinal plants growing throughout the area which are commonly being used by the local people.

Three medicinally important plant species of genus *Hypericum* L. were observed for their pollen morphology and pollen fertility estimation in the Plant Taxonomy Laboratory, Herbarium of Quaid-i-Azam University, Islamabad & Centralized Science Laboratory University of Karachi. Pollen materials of fresh specimens were collected from flora of Kaghan Valley and used for LM and SEM.

3. Light Microscopy (LM)

For light microscopy slides were prepared by using glycerin jelly and safranin according to modified method of Mazari *et al.* [32]. The slides were prepared and observed at different magnification. The micro-graphs of these mounted materials were taken using a digital camera (Meiji CCD Model 00179048, Canada) fitted on the Lieca light microscope (DM 1000, Germany). All photographs were taken on different object lenses by using oil emulsion.

4. Scanning Electron Microscopy (SEM)

The pollen grains were prepared for scanning electron microscopy (SEM). Pollen grains suspended in a drop of water were directly transferred with a fine pipette to a metallic stub using double sided cello tape and coated with gold in a sputtering chamber (Ion-sputter JFC-1100). Coating was restricted to 150A. The S.E.M examination was carried out on a Jeol microscope JSM-2.

5. Pollen Fertility Estimation

Pollen fertility percentage was calculated on the basis of staining by Glycerin jelly. The prepared slides were observed at low magnification. Glycerin jelly was used in pollen staining and prepared according to modified method of Mazari *et al.* [33]. Percentage of pollen fertility was calculated on the basis of staining by Glycerin jelly. The number of stained and unstained pollen grains was counted. Fully stained pollens were considered fertile while the lightly stained or unstained and deformed pollens were considered sterile. Percentage of pollen was determined by the formula: No. of total pollen/ No. of fertile pollen = Percentage Pollen fertility.

The descriptive terms for pollen morphology are used according to terminology of Erdtman), Kremp, Faegri & Iversen, Punt *et al.*, and Walker & Doyle [34]-[39].

6. Results

The pollen morphology and pollen fertility estimation of the three selected species of *Hypericum* was carried out by using light and scanning electron microscopy. Micrographs of pollen grains are presented in **Figure 2** and **Figure 3**. Findings of each species are represented in an order of local name, English name, phytogeography, flower color and season and palynomorph features. These results are summarized in **Table 1**.

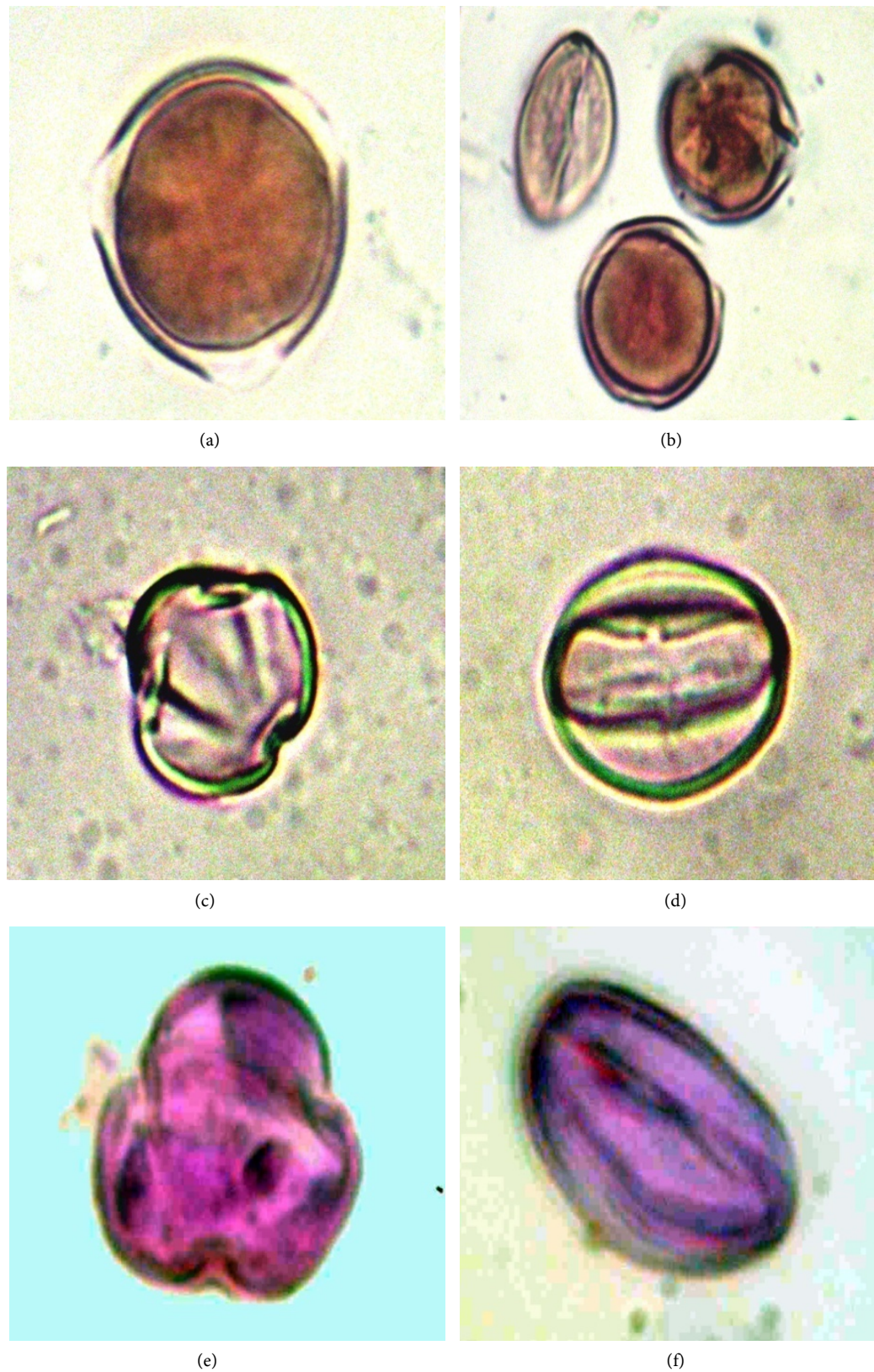


Figure 2. Light micrographs of pollen grains of *Hypericum* L. (a), (b): *Hypericum elodeoides*: Polar view, (a) (b) equatorial and polar view; (c), (d): *Hypericum oblongifolium*: Polar view, (c) equatorial view, (d), (e), (f): *Hypericum perforatum*: Polar view, (e) equatorial view, (f) (1000 \times).

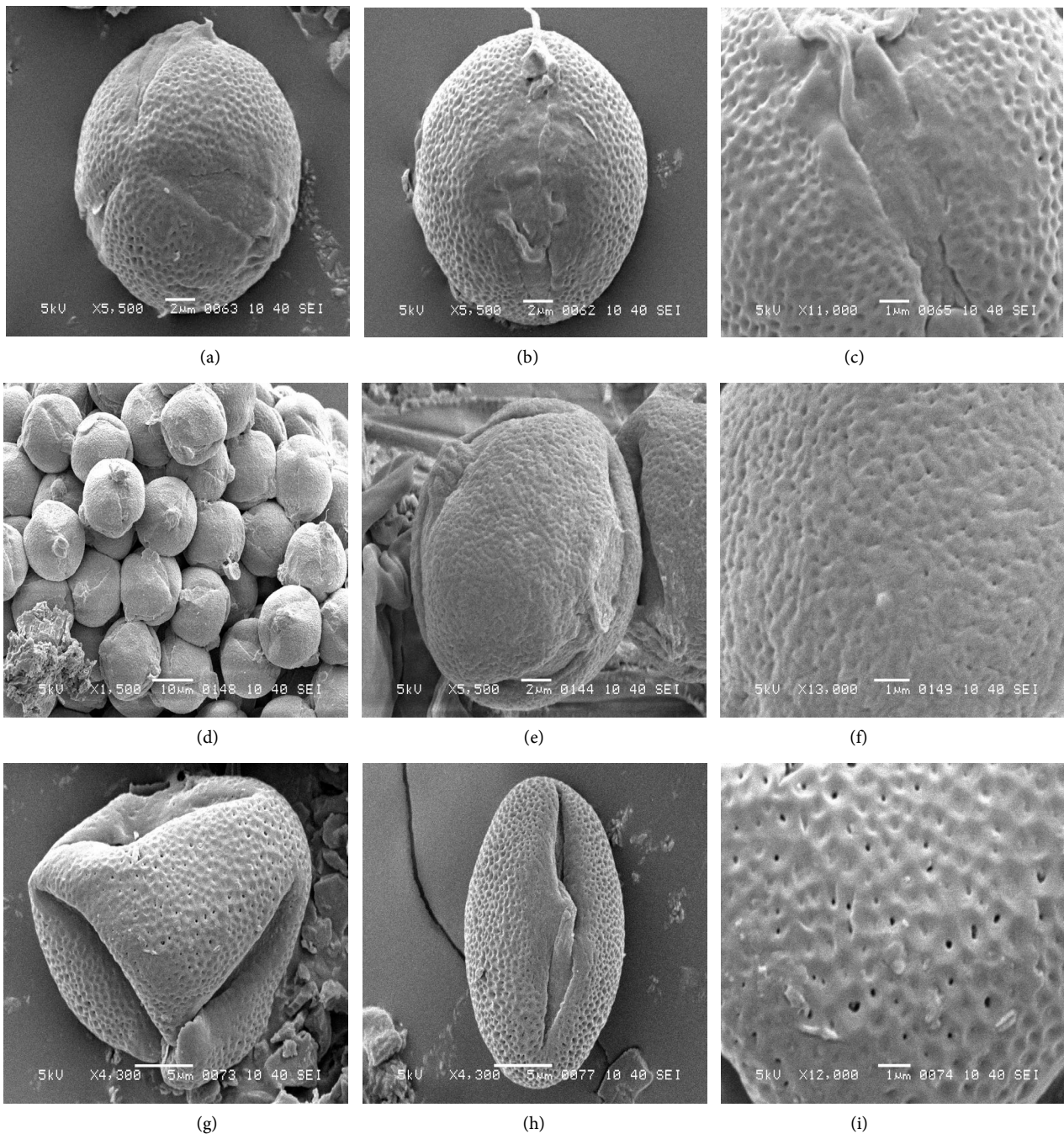


Figure 3. Scanning electron micrographs of pollen grains of *Hypericum* L. (a)-(c): *Hypericum elodeoides*: Polar view, (a) equatorial view, (b) exine sculpturing, (c) (d)-(f): *Hypericum oblongifolium*: Polar view in bunches, (d) equatorial view, (e) sculpturing, (f) (g)-(i): *Hypericum perforatum*: Polar view, (g). Equatorial view (h). Sculpturing, (i).

6.1. *Hypericum elodeoides* Choisy

Local Name: Kasni.

English Name: Marsh Stewart John's wort.

Phytogeography: It is found from Kashmir to Yunnan and Burma.

In Pakistan it is found in Basaoli, Budrawur, Gulmarg, Kashmir & Northern areas.

Flower color & period: Yellow & July-September.

Palynology: The pollen is monad, diad and tri to tetra-colporate. The shape of pollen in polar view is circular to semi-angular and in equatorial view is oblate-spheroidal (**Figure 2(a), Figure 2(b)**). The polar axis (P) is 19.91 μm (17.5 - 22.5 μm) and equatorial diameter is 20.6 μm (19 - 22.5 μm). P/E ratio is 0.96 μm . Exine thickness is 1.41 μm (1.25 - 1.5 μm) (**Table 1**).

Sculpturing: Foveolate with depressions less than 1 μm in diameter (**Figure 3(c)**).

6.2. *Hypericum oblongifolium* Choisy

Syn: *H. cernuum* Roxb., nomen

Local Name: Pinli

English Name: St. John's wort.

Phytogeography: It is found in Western Himalayas from Kurram to Nepal.

In Pakistan it is found in Kurram, lower Swat, Hazara, Malakandi, Kaghan, Nathiagali, Muree, Kashmir, Poonch.

Flower color & period: Yellow & March-August.

Palynology: The pollen is monad and tri-colporate. The shape of pollen in polar view is circular to semi-angular and in equatorial view is oblate-spheroidal (**Figure 2(c), Figure 2(d)**). The polar axis (P) is 23.0 μm (20 - 27.5 μm) and equatorial diameter is 25.35 μm (23.75 - 27.5 μm). P/E ratio is 0.90 μm . Exine thickness is 1.58 μm (1 - 2 μm) (**Table 1**).

Sculpturing: Lovely reticulate with diffuse lumina and muri (**Table 1** and **Figure 3(f)**).

6.3. *Hypericum perforatum* L.

Local Name: Bilsana.

English Name: Goat weed /Tipton's weed/ Klamathweed /St. John's wort.

Phytogeography: Europe and N. Africa to W. China and N.W., India. Introduced to East Asia, America. Australasia and South Africa.

In Pakistan it is found in Kurram, Chitral, Swat, Astor, Gilgit, Balistan, Hazara, Muree Hills, Poonch & Kashmir.

Flower color & period: Yellow & June to September.

Table 1. Summary of qualitative and quantitative characters of pollen of *Hypericum* taxa.

S. No.	Species	Vouchers	Type	Shape in polar view	Shape in equatorial view	P	E	P/E	Exine Thickness	Exine Ornamentation	Pollen fertility %
1.	<i>Hypericum elodoides</i> Choisy	P08	3-4colporate	circular to semi-angular	Oblate-spheroidal	19.91	20.6	0.96	1.41	Foveolate	82.66%
2.	<i>Hypericum oblongifolium</i> Choisy	P09	Monad and 3-colporate	circular to semi-angular	Oblate-spheroidal and spheroidal	23.0	25.35	0.90	1.58	Reticulate	90.69%
3.	<i>Hypericum perforatum</i> L.	P10	monad, trilete 3-colporate	circular to semi-angular	Prolate and rhomboidal	20.78	12.53	1.65	1.35	Scabrate	90.90%

Note: All the measurements are in μm . (P = Polar Diameter, E = Equatorial Diameter, P/E Polar diameter divided by Equatorial diameter).

Palynology: The pollen is monad and tricolporate. The shape of pollen in polar view is circular to semi-angular and in equatorial view is oblate, prolate and rhomboidal (**Figure 2(e)**, **Figure 2(f)**). The polar axis (P) is 20.78 μm (17.5 - 21 μm) and equatorial diameter is 12.53 μm (17.5 - 38 μm). P/E ratio is 1.65 μm . Length of colpi is 4 μm (5.75 - 8.25 μm) and width of colpi is 2.37 μm (2.25 - 2.5 μm). Exine thickness is 1.35 μm (1 - 2 μm) (**Table 1**).

Sculpturing: Scabrate, sculpturing elements less than 1 μm (**Table 1** and **Figure 3(i)**).

Pollen fertility: Pollen fertility is valuable for the taxonomists in attempting to distinguish putative hybrids from the parent plants and is also useful to determine the degree of fertility in those plants that are grown under unfavorable conditions [40]. The pollen fertility studies have been proved helpful for recognizing wide range of variation existing within plant species and differentiating plant species within genera. Many workers have tried to correlate in vitro pollen fertility with a survival of a plant under its natural habitat [41]. Matin *et al.* [42] studied pollen fertility of 50 medicinal plants of Shogran Valley. Fertility ratio of the plants ranged from 98% - 100%, which indicated that flora of Shogran was well established and stable. In the present study among the 3 species of *Hypericum*; pollen fertility percentage ranged from 82.66% to 90.90%. It indicates that *Hypericum* species from Kaghan valley is well established and the species are stable in the area.

7. Discussion

In recent studies on palynology of *Hypericum* by Otaghvari *et al.*, Martonfi *et al.* and Ocak, *et al.* [6] [22] [24] the regular and irregular pollen grains have been discussed. In the present study pollen examination of selected *Hypericum* taxa was conducted from Pakistan for the first time. Interesting findings were found regarding pollen characters of selected species. The pollen fertility estimation of the species was also investigated. Generally pollen grains were small, monad and isopolar. The type of pollen grains 3 - 4 colporate in *H. elodeoides* while 3-colporate in other two species. In polar view in all species pollen shape was circular to semi-angular (**Figure 2(a)**, **Figure 2(c)** and **Figure 2(e)**). But in equatorial view the pollen grains were oblate-spheroidal in *H. elodeoides* and *H. oblongifolium* but prolate *H. perforatum* (**Figure 2(b)**, **Figure 2(d)** and **Figure 2(f)**). The present study showed palynological variation among these three species of *Hypericum* especially in exine sculpturing of pollen grain which was foveolate sculpturing in *Hypericum elodeoides*, reticulate in *H. oblongifolium* and scabrate in *H. perforatum*. Some of our findings regarding shapes and small to medium size of pollen grains are in accordance with [6] pollen grains but in contrast with regard of exine sculpturing which is different in all species studied. Similarly palynological analysis of seven *Hypericum* taxa were studied by [31], by light and scanning electron microscopy. According to them there were no essential differences in the exine structure of seven *Hypericum* species. The

present showed much variation in regard of pollen shape in equatorial view and sculpturing pattern of exine which was different in all species studied. Pollen grains are mainly classified on the basis of their shape, size, aperture, polarity, symmetry and exine sculpturing. Along with these characters; exine sculpturing pattern is of great importance from phylogenetic and evolutionary point of view [39]. The present study showed that the studied palynological characters would be more useful for further palynological, karyological and taxonomic studies of *Hypericum* species.

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References

- [1] Crockett, S.L. and Robson, N.K.B. (2011) Taxonomy and Chemotaxonomy of the Genus *Hypericum*. *Medicinal and Aromatic Plant Science and Biotechnology*, **5**, 1-13.
- [2] Crockett, S.L. (2010) Essential Oil and Volatile Components of the Genus *Hypericum* (Hypericaceae) *Natural Product Communications*, **5**, 1493-1506.
- [3] Scotland, R.W. (2000) Taxic Homology and Three-Taxon Statement Analysis. *Systematic Biology*, **49**, 480-500. <https://doi.org/10.1080/10635159950127358>
- [4] APG III (2009) An Update of the Angiosperm Phylogeny Group Classification for the Orders and Families of Flowering Plants.
- [5] Ernst, E. (1995) St. John's Wort, an Anti-Depressant? A Systematic, Criteria-Based Review. *Phytomedicine*, **2**, 67-71. [https://doi.org/10.1016/S0944-7113\(11\)80051-6](https://doi.org/10.1016/S0944-7113(11)80051-6)
- [6] Otaghvari, A.M., Omrani, K.A. and Fasaee, F. (2015) The Pollen Morphology Study on Some Species of *Hypericum* L. Genus in the Northern Iran. *International Journal of Biology, Pharmacy and Allied Science*, **4**, 957-967.
- [7] Matzk, F., Meister, A., Brutovska, R. and Schubert, I. (2001) Reconstruction of Reproductive Diversity in *Hypericum perforatum* L. Opens Novel Strategies to Manage Apomixis. *The Plant Journal*, **26**, 275-282. <https://doi.org/10.1046/j.1365-313X.2001.01026.x>
- [8] Barnes, J., Anderson, L.A. and Phillip, J.D. (2001) St John's Wort (*Hypericum perforatum* L.): A Review of Its Chemistry, Pharmacology and Clinical Properties. *Journal of Pharmacy and Pharmacology*, **53**, 583-600. <https://doi.org/10.1211/0022357011775910>
- [9] Awan, M.R., Iqbal, Z., Muqarab S., Jamal, Z.S., Jan G., Afzal, M., Majid, A. and Gul, A. (2011) Studies on Traditional Knowledge of Economically Important Plants of Kaghan Valley, Mansehra District, Pakistan. *Journal of Medicinal Plants Research*, **5**, 3958-3967.
- [10] Nurk, N.M. and Blattner, F.R. (2010) Cladistic Analysis of Morphological Characters in *Hypericum* (Hypericaceae). *Taxon*, **59**, 1495-1507.
- [11] Nurk, N.M. and Crockett, S.L. (2011) Morphological and Phytochemical Diversity among *Hypericum* Species of the Mediterranean Basin. *Medicinal and Aromatic*

Plant Science and Biotechnology, **5**, 14-28.

- [12] Ruhfel, B.R., Bittrich, V., Bove, C.P., Gustafsson, M.H.G., Philbrick, C.T., Ruti-shauser, R., Xi, Z. and Davis, C.C. (2011) Phylogeny of the Clusioid Clade (Malpig-hiales): Evidence from the Plastid and Mitochondrial Genomes. *American Journal of Botany*, **98**, 306-325. <https://doi.org/10.3732/ajb.1000354>
- [13] Khan, A.H. (1969) Pollen Morphology of Indian Hypericaceae. *Palynology*, **5**, 97-99.
- [14] Thomas, J.L. (1970) Haploid and Diploid Pollen in *Hypericum patulum*. *Journal of Arnold Arboretum*, **51**, 247-250.
- [15] Aytug, B., Aykut, S., Merev, N. and Edis, G. (1971) Pollen Atlas of Plants in and around Istanbul. University of Istanbul, No. 1650, Fac For No:174.
- [16] Charpin, J., Surinyach, R. and Frankland, A.W. (1974) Atlas of European Allergenic Pollens. *Journal of Linnaean Society*, **161**, 105-121.
- [17] Yi, X.-Z. (1979) Pollen Morphology of Guttiferae in China. *Acta Botanica Sinica*, **21**, 36-41. (In Chinese)
- [18] Clarke, G.C.S. (1975) Irregular Pollen Grains in Some *Hypericum* Species. *Grana*, **15**, 117-125. <https://doi.org/10.1080/00173134.1975.11864626>
- [19] Clarke, G.C.S. (1976) The Northwest European Pollen Flora, 7 Guttiferae. *Review of Paleobotany and Palynology*, **21**, 125-142. [https://doi.org/10.1016/0034-6667\(76\)90051-8](https://doi.org/10.1016/0034-6667(76)90051-8)
- [20] Clarke, G.C.S. (1981) Pollen Morphology. pp. 115-118. In: Robson, N.K.B., Ed., *Studies in the Genus Hypericum L. (Guttiferae)*. 2. *Characters of the Genus*. Bulletin of the British Museum of Natural History (Botany), 8: 55-226.
- [21] Barros, M. and Ramos, A. (1984) Study of the Pollen of the Species of *Hypericum* sect. *Hirtella* Stef. in the Iberian Peninsula and the Balearic Islands. *5th Symposium of Palynology. APLE. Abstracts (Cordoba Estudio del polen de las especies de Hypericum sect. Hirtella Stef. en la Península Ibérica y Baleares. V Simposio de Palinología. APLE. Resúmenes. Córdoba)*.
- [22] Martonfi, P., Janikova, M. and Zezula, I. (2002) Palynological Analysis of Seven *Hypericum* Taxa. *Biologia, Bratislava*, **571**, 455-460.
- [23] Meseguer, A.S. and Sanmartín, I. (2012) Paleobiology of the Genus *Hypericum* (Hypericaceae): A Survey of the Fossil Record and Its Palaeogeographic Implications. *Anales del Jardín Botánico de Madrid*, **69**, 97-106.
- [24] Ocaik, A., Erkara, P.I., Koyuncu, O., Osoydan, K., Yaylaci, O.K., Özgüşi, K. and Kurt, F. (2013) Palynological Investigations on Some *Hypericum* taxa (Hypericaceae) Growing Naturally in Turkey. *Plant Systematics and Evolution*, **299**, 379-388. <https://doi.org/10.1007/s00606-012-0728-z>
- [25] Jan, S., Khan, M.A., Din, S., Murad, W., Hussain, M. and Ghani, A. (2008) Herbal Remedies Used for Gastrointestinal Disorders in Kaghan Valley, NWFP, Pakistan. *Pakistan Journal of Weed Science Research*, **14**, 169-200.
- [26] Raziq, N., Naveed, M., Chishti, K.A., Saeed, M., Rahman, S. and Khan, H. (2011) Correlation of the Antioxidant Capacity with the Phenolic Contents of *Hypericummonogynum* and *Hypericum perforatum*. *African Journal of Pharmacy and Pharmacology*, **5**, 1872-1876.
- [27] Meo, A.A. (2005) Palynological Studies of Selected Genera of the Tribes of Astera-ceae from Pakistan. Ph.D. Thesis, Quaid i Azam University, Islamabad, Pakistan.
- [28] Nair, P.K. (1964) Advances in Palynology. National Botanical Garden, Lukhnow.

- [29] Bashir, S. and Khan, M.A. (2003) Pollen Morphology as an Aid to the Identification of Medicinal Plants: *Trianthema portulacastrum* L., *Boerhaavia procumbens* Banks ex Roxb., and *Alternanthera pungens* Kunth. *Hamdard Medicus*, **XLVI**, 7-9.
- [30] Anonymous (1998) District Census Report of Mansehra. Population Census Organization Statistical Division, Government of Pakistan.
- [31] Mustafa, G. (2003) An Introduction to Mansehra District. Science-Religion Dialogue. <http://www.hssrd.org/journal/spring2003/mansehra.htm>
- [32] Mazari, P., Khan, M.A., Ali, B., Mangi, J.D., Bux, H., Khan, K.Y., Mughal, S., Ahmad, M., Zafar M. and Akhter, A. (2012a) Palynological Diversity in Selected Medicinal Plant Species of Asteraceae (Compositae) from Flora of Kaghan Valley. *Journal of Medicinal Plants Research*, **6**, 2747-2753.
- [33] Mazari, P., Khan, M.A., Ahmad, M., Khan, K.Y., Zafar M., Ali, B. and Niamat, R. (2012b) Pollen Fertility Estimation of Selected Taxa of Kaghan Valley, Pakistan. *Research in Plant Biology*, **2**, 16-21.
- [34] Erdtman, G. (1952) Pollen Morphology and Plant Taxonomy. Angiosperms. Chron. Bot. Co., Waltham.
- [35] Erdtmann, G. (1954) An Introduction to Pollen Analysis. Chron. Bot. Co., Waltham.
- [36] Kremp, G.O.W. (1965) Encyclopedia of Pollen Morphology. The University of Arizona Press, Tuscon.
- [37] Faegri, K. and Iversen, J. (1964) Text book of Pollen Analysis. 2nd Edition, Munksgaard, Copenhagen.
- [38] Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S. and Thomas, A.L. (2007) Glossary of Pollen and Spore Terminology. *Review of Palaeobotany and Palynology*, **143**, 1-81. <https://doi.org/10.1016/j.revpalbo.2006.06.008>
- [39] Walker, J.W. and Doyle, J.A. (1975) The Bases of Angiosperm Phylogeny: Palynology. *Annals of the Missouri Botanical Garden*, **62**, 664-723. <https://doi.org/10.2307/2395271>
- [40] Lawrence, G.H.M. (1969) Taxonomy of Vascular Plants. MacMillan Co., New York, 737-775.
- [41] Daiz, Z.I. and Lifante, Z.D. (1991) *Asphodelus cirerae*, a Forgotten Species of *Asphodelus* sect. *Verinea* (Liliaceae), Morphological, Palynological, Karyological and Ecogeographical Characterization. *Flora Mediterranea*, **71**, 87-109.
- [42] Matin, A., Khan, M.A., Aasraf, M. and Qureshi, R.A. (2002) Pollen Fertility Investigation of the Himalayan Region of Shogran Valley District Mansehra (Hazara), Pakistan. *Hamdard Medicus*, **XLV**, 78-81.