

POLLEN MORPHOLOGY OF *AJUGA* L., *LAMIUM* L. AND *PHLOMIS* L. (LAMIACEAE) FROM DISTRICT ABBOTTABAD PAKISTAN

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Abstract

Pollen morphology of six wild species belonging to three genera, (two species each), of the family Lamiaceae, (*Ajuga* L., *Lamium* L. and *Phlomis* L.) from the District Abbottabad (Pakistan), has been studied. The overall objective of this study was to assess the Lamiaceous Flora of District Abbottabad on palynological basis. Pollen grains were examined under light and scanning electron microscopy and photomicrographs of pollen under light microscope (LM) as well as Scanning Electron Microscope (SEM) were incorporated to show the diagnostic features. Pollen grains are free, radially symmetrical, isopolar, trizonocolpate; prolate to subprolate to spheroidal, sexine is thicker than nexine. Tectum is fossulate and reticulate. The shape of pollen and pollen aperture are found to be important at generic level while exine thickness and its ornamentation are found to be more significant characters at specific level and could prove to be a diagnostic feature for species identification.

Key words: Pollen, *Ajuga* L., *Lamium* L., *Phlomis* L. and Lamiaceae.

Introduction

The Lamiaceae, or mint family, is the seventh largest flowering plant family in the world with about 7,000 species organized into more than 250 genera (Thorne, 1992), including herbs, shrubs and even woody climbers of cosmopolitan distribution. Pakistan is represented by 60 genera and 202 species of this family (Hedge, 1990).

The plants belonging to the genus *Ajuga* L. are evergreen, clump-forming rhizomatous annual or perennial herbs, with most plants native to Europe, Asia, and Africa, but also growing in Australia and North America. There are at least 301 species of the genus *Ajuga* with many variations, (Israili & Lyoussi, 2009). Some species of *Ajuga* plants are used in traditional medicine. The genus *Lamium* L. including almost 40 species has been described as perennial and annual herbs, native to Europe, Asia, and North Africa (Yalcin & Kaya, 2006). The genus *Phlomis* L. comprises over 100 species including herbs, shrubs and sub-shrubs (Albaladejo *et al.*, 2005). This genus is distributed in north temperate regions, mainly in Europe and Asia. It is closely related to the genus *Eremostachys*.

In Pakistan, *Ajuga* and *Lamium* are represented by two species each while, *Phlomis* is represented by six species (Hedge, 1990). The science of palynology (study of pollen grains) has steadily grown over the last sixty years. It is a fascinating science with a multidimensional approach covering almost all branches of botanical sciences, like 'Palynotaxonomy' is helpful in taxonomic and evolutionary studies of plant kingdom. Pollen morphology of family Lamiaceae has been first examined by Erdtman *et al.*, (1963). Huang (1972), in his contribution to the Pollen Flora of Taiwan has described the pollen morphology of some species belonging to the Lamiaceae family. Azizian & Moore (1982) studied pollen of the genus *Phlomis* (Lamiaceae)

and compared them with their close relative *Eremostachys* and *Paraphlomis*. Gill & Chinnapa (1982) have studied pollen morphology of "West Himalayan Labiatae". Abu-Asab & Cantino (1993) conducted a palynological survey of the tribe *Ajugeae* (Labiatae) and found an exceptional amount of variations in structural features of pollens in the tribe. Abu-Asab & Cantino (1994) studied the pollen morphology of 57 genera belonging to the subfamily *Lamioideae* and sub-family *Pogostemonoideae* of family Lamiaceae and segregated different genera on the basis of pollen structure, including segregation of *Lamiophlomis*, *Lagopsis*, and *Phlomidioschema* from *Phlomis*, *Marrubium*, and *Stachys*, respectively, in the subfamily *Lamioideae*. In Pakistan, Perveen & Qaiser (2003) studied pollen morphology of 79 species representing 27 genera of the family using light-transmitted and scanning electron microscopy. Zafar *et al.*, (2006) also described the pollen morphology of three species, i.e., *Ajuga bracteosa*, *Eremostachys superba* and *Salvia moorcroftiana* belonging to three genera of the family Lamiaceae from the flora of Rawalpindi. Erkara *et al.*, (2011) described pollen morphological structures of eight Turkish species of genus *Ajuga*, with the help of light and scanning electron microscope.

The present investigations are based on the pollen morphology of six species belonging to three genera, namely *Ajuga* L., *Lamium* L. and *Phlomis* L., with combined help of light-transmitted and scanning electron microscopy. The main objective of present study is to record baseline pollen information of these genera, growing in the district Abbottabad.

Materials and Methods

Plant specimens with mature flowers were collected during April 2008 to August 2008, properly pressed, dried and mounted on standard herbarium sheets.

Polleniferous material was obtained and pollen grains were isolated by the standard method recommended by Bhutta (1973) and mounted in glycerine jelly stained with 1% Safranin, for light-transmitted microscopy (LM). The observations like, pollen diameter, polar axis (P) and equatorial diameter (E), aperture size and exine and intine thickness were measured with a Nikon S. E. No. 874451 research binocular under (E45, 0.65) and oil immersion (E100, 1.25), using 10X eye piece at the Botany Department, Hazara University. The measurements were based on 40-60 readings for each specimen. Photomicrography was made in the

Department of Genetics under “Leica DM 1000 photomicroscope using 7.3-29.2 mm Canon 8.0 Megapixels, Power Shot A630, digital camera. The SEM examination was carried out on a 30KV Scanning Electron Microscope, JSM 5910, JEOL, Japan, at Peshawar University.

Voucher specimen and permanent slides were deposited in the herbarium of Hazara University, Mansehra. The terminology used is in accordance with Punt *et al.*, (2007). A list of plants studied along with their localities is given in Table 1 below:

Table 1. List of species collected by Shamila Firdous from Abbottabad.

Plant Genus	Plant Species	Voucher No	Date of Collection	Locality
<i>Ajuga</i> L.	<i>Ajuga bracteosa</i> Wall.	HUP-3487	28.04.08	Havelian
	<i>Ajugaparviflora</i> Benth.	HUP-3488	17.05.08	Kokal-Barsin
<i>Lamium</i> L.	<i>Lamium album</i> L.	HUP-3497	29.05.08	Thandiani
	<i>Lamium amplexicaule</i> L.	HUP-3498	05.05.08	Bilal Town
<i>Phlomis</i> L.	<i>Phlomis bracteosa</i> Royle.	HUP-3519	29.06.08	Thandiani
	<i>Phlomis spectabilis</i> Falc.	HUP-3520	28.08.08	Jhaffar

Results

General pollen characters of the genus *Ajuga*, *Lamium* and *Phlomis*: Pollen grains in all species are usually radially symmetrical, isopolar, monad, amb circular in polar view and trizonocolpate. Pollen shapes vary from prolate-subprolate-spheroidal in these species, colpi may be acute or blunt, pollen shapes and the size range of pollen grains are given in Table 2, while exine sculpturing and pollen apertures are given in Table 3. Pollen images of scanning electron microscope and light microscope are shown in plates I, II and III, respectively. A graph is given at the end showing comparison of exine thickness among different species.

Pollen morphology of *Ajuga* L.: In both species of *Ajuga*, pollen grains are prolate having acute colpi and fossulate tectum. Exine is twice as thick as intine. Colpi are almost as long as broad in case of *Ajuga bracteosa*,

while they are twice as long as broad in case of *Ajuga parviflora*.

Pollen morphology of *Lamium* L.: In both species of *Lamium*, pollen grains have acute colpi and these are twice as long as broad. Tectum is fossulate and exine is twice as thick as intine in *Lamium album*, while tectum is microreticulate, with lumina $\pm 1.0 \mu\text{m}$ in diameter in *Lamium amplexicaule* and exine is almost three times thicker than intine. Exine is also thickened at two poles.

Pollen morphology of *Phlomis* L.: In *Phlomis*, pollen grains have colpi which are twice as long as broad and acute at their ends in case of *Phlomis bracteosa*. Tectum here is reticulate-punctate and exine is twice as thick as, while in *Phlomis spectabilis* colpi are three times longer than broad with blunt ends. Tectum is microreticulate, with lumina $1.0 \mu\text{m}$ in diameter and muri $0.4 \mu\text{m}$ wide and exine is thicker than intine.

Key to the species

1. Pollen grains with fossulate tectum 2
 Pollen grains with reticulate tectum 4
2. Pollen grains prolate with colpi as long as broad *Ajuga bracteosa*
 Pollen grains prolate with colpi twice as long as broad 3
3. Exine twice as thick as intine *Ajuga parviflora*
 Exine three times thicker than intine *Lamium album*
4. Pollen grains with reticulate-punctate tectum *Phlomis bracteosa*
 Pollen grains with microreticulate tectum 5
5. Pollen grains with colpi blunt at ends *Phlomis spectabilis*
 Pollen grains with colpi acuteat ends *Lamium amplexicaule*

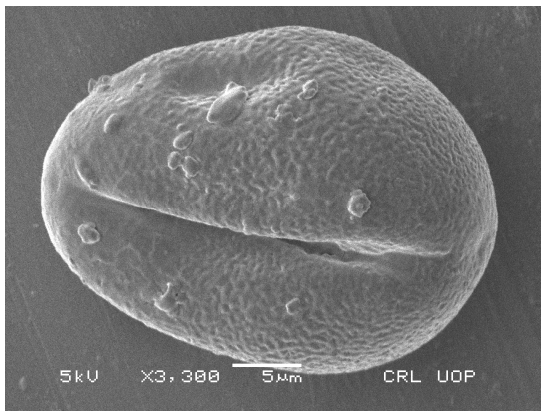


Fig. 1a.

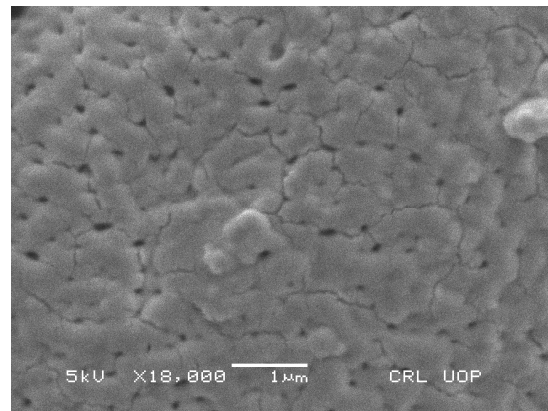


Fig. 1b.

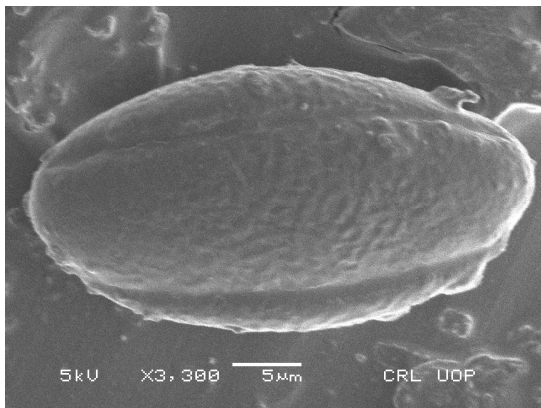


Fig. 2a.

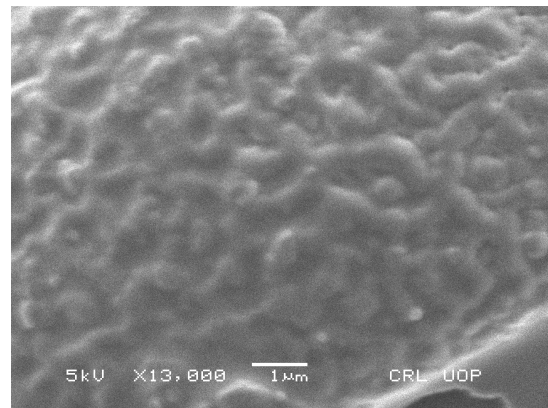


Fig. 2b.

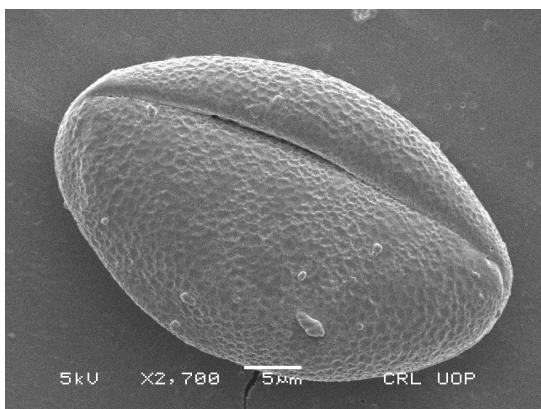


Fig. 3a.

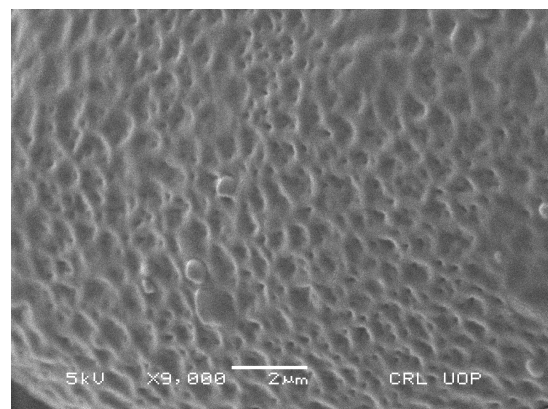


Fig. 3b.

Plate I (SEM Images): *Ajuga bracteosa*, Fig.1-a; pollen grain (equatorial view), Fig. 1-b; exine pattern, *Ajuga parviflora*, Fig. 2-a; pollen grain (equatorial view), Fig. 2-b; exine pattern, *Lamium amplexicaule*, Fig. 3-a pollen grain (equatorial view), Fig. 3-b; exine pattern.

Table 2. Pollen classes and pollen shapes of different species.

Name of specimen	Pollen class	Polar axis (µm)		Equatorial axis (µm)		P/E Ratio		Pollen shape
		Mean	Range	Mean	Range	Mean	Range	
<i>Ajuga bracteosa</i>	Monad Tricolpate	26.74±0.73	(15-35)	26.838±0.77	(17.5-37.5)	1.339±0.07	(1.0-1.62)	Prolate
<i>Ajuga parviflora</i>	Monad Tricolpate	21.8±0.64	(15-30)	17.321±0.45	(12.5-25)	1.349±0.17	(1.07-1.85)	Prolate
<i>Lamium album</i>	Monad Tricolpate	23.52±0.22	(20-27.5)	22.59±0.23	(17.5-27.5)	1.389±0.12	(1.12-1.82)	Prolate
<i>Lamium amplexicaule</i>	Monad Tricolpate	27.11±0.44	(17.5-32.5)	26.382±0.46	(32.5-17)	1.295±0.07	(1.0-1.43)	Subprolate
<i>Phlomis bracteosa</i>	Monad Tricolpate	22.29±0.34	(17.5-32.5)	21.289±0.27	(17.5-25)	1.0±0.05	(1.0-1.03)	Spheroidal
<i>Phlomis spectabilis</i>	Monad Tricolpate	24.44±0.36	(15-32.5)	25.72 ± 0.35	(20-33)	1.279±0.07	(1.07-1.5)	Subprolate

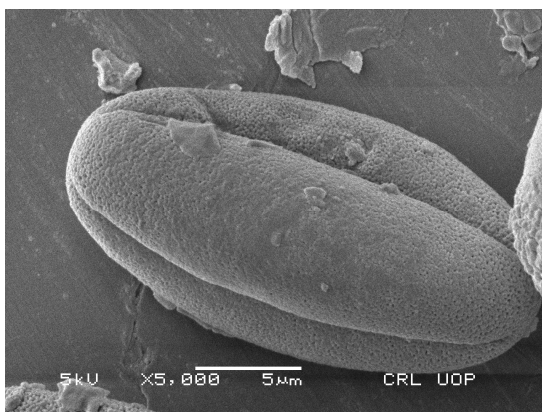


Fig. 4a.

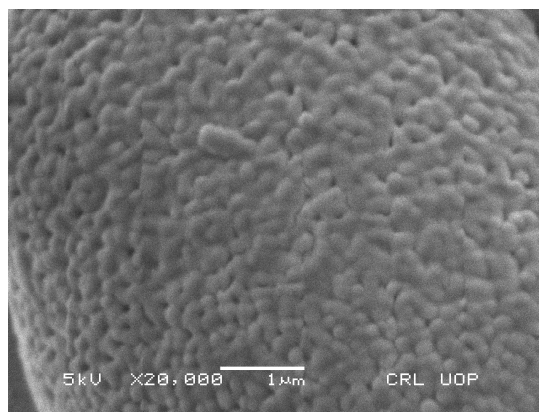


Fig. 4b.

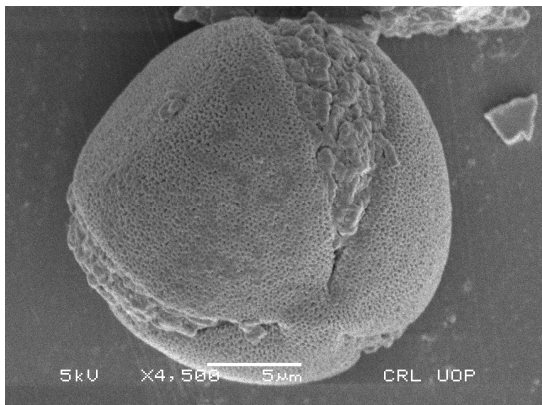


Fig. 5a.

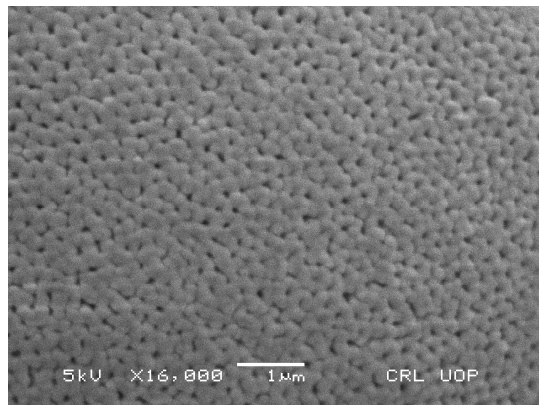


Fig. 5b.

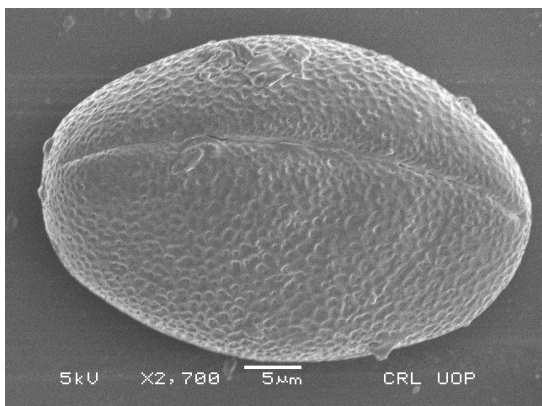


Fig. 6a.

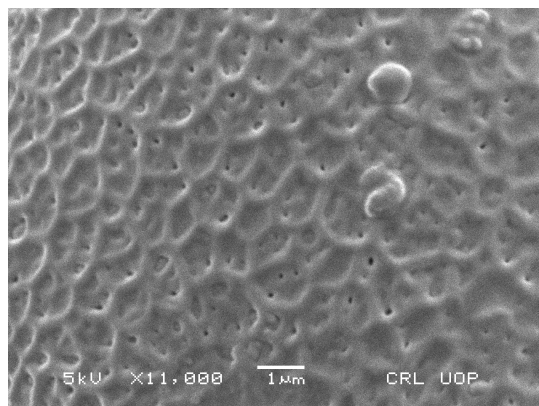


Fig. 6b.

Plate-II (SEM Images): *Lamium album*, Fig. 4-a; pollen grain (equatorial view), Fig. 4-b; exine pattern, *Phlomis bracteosa*, Fig. 5-a; pollen grain (polar view), Fig. 5-b; exine pattern, *Phlomis spectabilis*, Fig. 6-a; pollen grain (equatorial view), Fig. 6-b; exine pattern.

Table 3. Pollen apertures and pollen sculpturing of different species.

Name of specimen	No. of colpi	Colpus (µm)		Thickness (µm)				Intercolpium width (µm)	Sculpturing
		Length	Width	Exine	Sexine	Nexine	Intine		
<i>Ajuga bracteosa</i>	3	17.0±2.12	19.66±1.45	1.26±0.08	0.728±0.03	0.392±0.03	0.56	15.4±0.54	Fossulate
<i>Ajuga parviflora</i>	3	17.0±0.95	09.0± 0.58	1.206±0.37	0.708±0.02	0.472±0.01	0.59	12.525±0.48	Fossulate
<i>Lamium album</i>	3	17.667±1.03	8.167±0.74	1.120±0.04	0.728±0.03	0.392±0.03	-	13.44±0.49	Fossulate
<i>Lamium amplexicaule</i>	3	21.5±2.02	11.75±0.43	1.163±0.03	0.672±0.04	0.448±0.05	0.56	19.622±0.32	Microreticulate
<i>Phlomis bracteosa</i>	3	18.0±0.55	8.833±1.26	1.297±0.11	0.672±0.06	0.470±0.04	0.56	10.826±0.26	Reticulate
<i>Phlomis spectabilis</i>	3	22.33±0.94	8.889±0.35	1.418±0.08	0.970±0.09	0.448±0.09	0.784	14.878±0.34	Reticulate

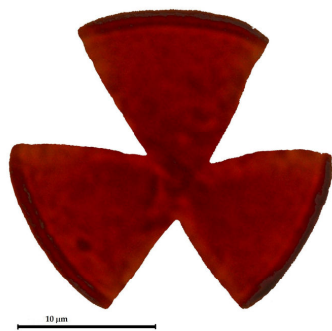


Fig. 1.



Fig. 2.

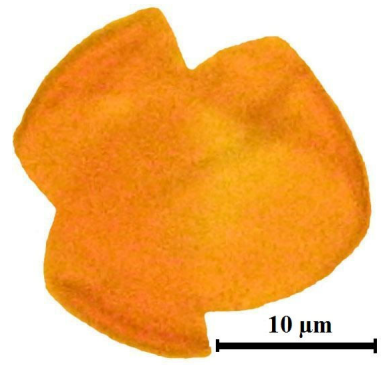


Fig. 3.

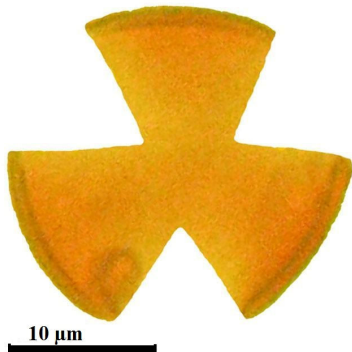


Fig. 4.

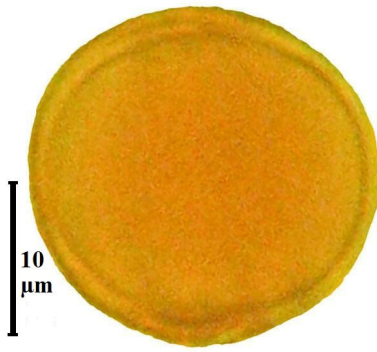


Fig. 5.

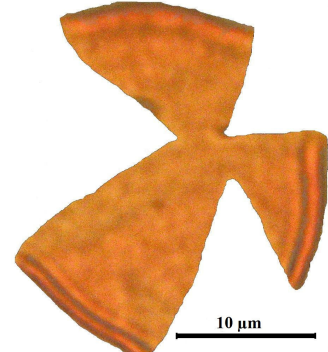


Fig. 6.

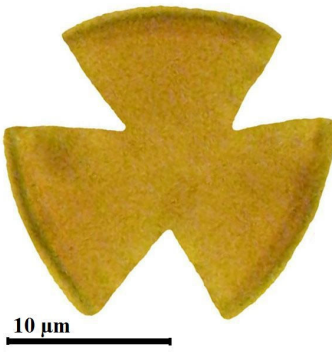


Fig. 7.

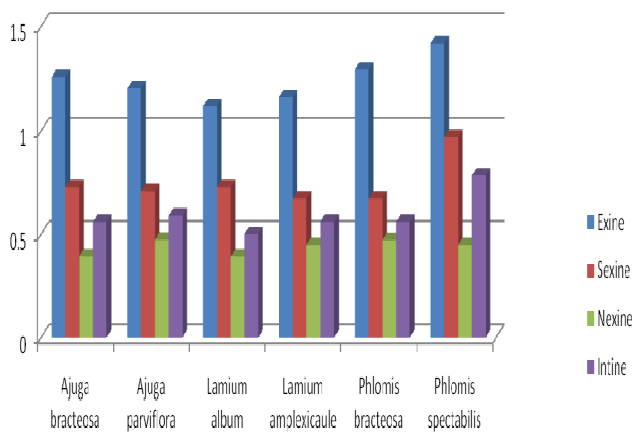


Fig. 8.



Fig. 9.

Plate III (LM Images): *Ajuga bracteosa*, Fig. 1. Polar view, Fig. 2. Equatorial view; *Ajuga parviflora*, Fig. 3. Polar view; *Lamium album*, Fig. 4. Polar view, Fig. 5. Equatorial view; *Lamium amplexicaule*, Fig. 6. Polar view; *Phlomis bracteosa*, Fig. 7. Polar view; *Phlomis spectabilis*, Fig. 8. Polar view; Fig. 9. Equatorial view.



Graph showing thickness of exine and intine in various species investigated.

Discussion

Pollen grains of both species of *Ajuga* are tricolpate and prolate in equatorial view. The shape of pollen is the same as observed by Huang (1972), Perveen & Qaiser (2003) and Zafar *et al.*, (2006). Pollen size shows great variation as 15-35 µm x 17.5-37.5 µm for *A. bracteosa* and 15-30 µm x 12.5-25 µm for *A. parviflora*, which was also observed by previous researchers, Huang (1972); Gill & Chinnapa (1982); Perveen & Qaiser (2003); Zafar *et al.*, (2006). Tectum observed here is fossulate for both species while Huang (1972), described as reticulate tectum for *A. bracteosa* whereas Perveen & Qaiser (2003) described as reticulate tectum for both species. Pollen grains observed here are isopolar for

both species, while Gill & Chinnapa (1982) described *A. bracteosa*. being heteropolar.

Pollen grains of both species of *Lamium* are tricolpate and subprolate (*L. amplexicaule*) to polate (*L. album*) having fossulate (*L. album*) to microreticulate (*L. amplexicaule*) tectum. Pollen size shows variation as 20-27.5µm x 17.5-27.5 µm for *L. album* and 17.5-32.5 µm x 17-32.5 µm for *L. amplexicaule*. Abu-Asab & Cantino (1994), described pollen of *L. album* as tricolpate, prolate-spheroidal having granulate tectum with pollen size range as 22-29 µm x 22-26 µm. Gill & Chinnapa (1982) also found tricolpate pollens in *L. album* with smooth sexine. No previous record exists in the published literature for pollen morphology of *Lamium* in Pakistan; particularly for *L. amplexicaule* neither on national or international level.

Pollen grains of *Phlomis* species are tricolpate and spheroidal (*P. bracteosa*) to sub-prolate (*P. spectabilis*) having a reticulate tectum. Pollen size shows variation as 17.5-32.5 µm x 17.5-25 µm for *P. bracteosa* and 15-32.5 µm x 20-33 µm for *P. spectabilis*. The shape of pollen, pollen class and tectum lie close to the observations of Perveen & Qaiser (2003) as they observed trizonocolpate, sub-prolate pollens with finely reticulate tectum for *P. bracteosa* and size range is 22.5-37.5 µm x 20-32.5 µm, while *P. spectabilis* has tricolpate pollens with prolate-spheroidal shape and coarsely reticulate tectum with size range as 26.25-32.5 µm x 23.5-32.5 µm.

Conclusion

Pollen grains are isopolar, trizonocolpate, having prolate to subprolate to spheroidal shapes with amb circular, while tectum varies from fossulate to reticulate. Pollen apertures are proved to be helpful in distinguishing the family Lamiaceae from closely related families like Verbenaceae, which possess tricolporate pollen. Reticulate tectum, which is a common feature in the Lamiaceae family, shows variations here as from microreticulate to reticulate-punctate. Although pollen characters of different species exhibit comparatively a slight range of variations, yet these are helpful in distinguishing the plants from one another, as pollen aperture and exine sculpturing is quite a significant character at specific level. Within genera like *Lamium* and *Phlomis* the exine sculpturing is proved to be the diagnostic feature that can be used to distinguish species. Similarly in case of *Ajuga*, the pollen aperture proved to be a significant character. The study has resulted in the

development of basic pollen reference database of Lamiaceous plants growing in the district Abbottabad, including catalogue highlighting morphotaxonomic traits.

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