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Original Research

Exploring the Pollen Fertility Estimation of Selected Taxa of Boraginaceae from Pakistan

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Abstract

This research aimed to evaluate the pollen fertility and micromorphology of Boraginaceae species across Pakistan, with an emphasis on how population size influences reproduction. A total of 27 species of the Boraginaceae family were collected from Pakistan, and light microscopy was used to examine pollen parameters, including size, shape, exine thickness, and fertility percentage. The findings revealed a range of pollen shapes, including prolate, prolate-spheroidal, sub-prolate, oblate, sub-oblate, and oblate-spheroidal, with oblate and sub-oblate being the most frequent. Exine thickness varied significantly among species, with *Alkanna tinctoria* having the thickest exine (2.80 μm), while *Onosma hispida* exhibited the thinnest exine (0.75 μm). These variations in pollen shapes and exine thickness play a crucial role in species identification and may influence reproductive success, as thicker exines offer structural protection against UV radiation, desiccation, and microbial attack, enhancing pollen survival during pollination. Fertility rates also varied, with *Trichodesma indicum* exhibiting the highest pollen fertility rate (94.94%), whereas *Arnebia hispidissima* had the lowest (60.50%) with an average fertility of 78.83% across the species. Larger populations exhibited higher pollen viability, while small, isolated populations had reduced fertility, demonstrating the significant effect of population size on reproduction. This study uncovers previously undocumented pollen fertility and morphology

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differences among Boraginaceae species in Pakistan, adding valuable information to current knowledge and emphasizing elements that influence reproductive success.

Keywords: pollen fertility, LM, viability, boraginaceae, Pakistan

Introduction

Pollen grains refer to the male reproductive parts (covered by the sporal wall) present in seeded plants, showing a wide range of variations in size and shape [1-3]. Palynology is the scientific study of pollen, spores, and microfossils [4, 5]. In palynological investigations, we analyze new and old palynomorphs, including spores, pollen, dinoflagellate cysts, chitinozoans, acritarchs, and scolecodonts found with organic matter in different types of rocks [4]. Various authors studied the pollen morphology of Boraginaceae, as discussed in previous studies [2, 3, 6, 7]. The members of Boraginaceae are found in diverse habitats, and collections of various taxa are located primarily in temperate regions throughout the world, including about 130 genera and 2300 plant species, as discussed in previous studies [5, 8, 9], whereas Pakistan has 32 genera with 135 plant species. In many disciplines, including paleobotany, aeropalynology, pollen analysis, paleoecology, systematic botany, allergy, coal fields, medicopalynology, copropalynology, and mellittopalynology, the effective techniques of plant taxonomy and morphology of pollen can be employed. There are highly diverse variations among the palynology of angiosperms. The flora of Pakistan is rich in plant species, as discussed in previous studies [1, 4, 5, 10, 11]. Pakistan and Kashmir represent about 6,000 flowering plant species, and most of the plant diversity is found in Pakistan's Northwestern and Northern zones [2, 3, 12, 13].

The Boraginaceae family, commonly called the forget-me-not or borage family, is economically and ecologically important [14]. Species in this family play vital roles in various ecosystems. They frequently help to provide habitat and food for pollinators, stabilize soil, and enhance biodiversity by interacting with other animal and plant species, as discussed in previous studies [1, 5, 15]. Many Boraginaceae plants have useful commercial applications. *Borago officinalis* (borage) is farmed for its oil, which contains gamma-linolenic acid (GLA), an essential fatty acid with numerous health benefits [2, 7, 16]. In addition, many species are used in traditional medicine for their diuretic, anti-inflammatory, and wound-healing properties [17]. As recorded earlier, the family's ornamental plants serve esthetic and economic purposes in horticultural and floral arrangements [2, 5].

Evaluating the variety and fertility of Boraginaceae pollen is essential for appreciating its economic and ecological importance. The estimation of pollen fertility is an important component of pollen morphology because it provides information regarding plant reproductive success and viability, as recorded earlier [3, 7, 18]. This data is useful not just for agricultural approaches and conservation efforts but also has implications for taxonomic study. As discussed in previous studies, pollen fertility statistics can indicate a species hybridization potential, genetic stability, and plant tolerance to changing environmental conditions [1, 4, 7, 19]. Furthermore, enhanced pollen output may indicate specific ploidy levels, affecting certain species' evolutionary potential and economic value. The level of hybrid fertility may convey some information between parents regarding the degree of familial bonding. Generally, a hybrid formed between closely related taxa of the same genus (as indicated by ecology, morphology, etc.) will be less viable or sterile, whereas the result of hybridization within species with a stronger association taxonomically or intra-specific hybrids will be more fertile, as investigated already [3, 5, 20].

Particularly, both pollen and pollen-bearing parts in plants have a significant role in inviting and gathering floral visitors because they both notify and provide them with food [5, 21, 22]. Landowners, hybrid seed growers, and cultivators all stand to profit from an analytic comprehension of male fertility: it is an important variable for assessing production and fertility, having a major effect on reproductive gradients, selection, and expansion [1]. The fertility of pollen is extremely high in diploids as well as tetraploid conditions [23, 24]. Male gametes are carried via pollen, which has three separate categories with diverse morphological and chemical features and biological and physiological functions, as discussed in previous studies [3, 5, 25]. Intine, exine, and nucleus are the three main domains of pollen grains. In vitro, the pollen stability test is an invaluable method to evaluate pollen fertility, which is significant for flowering plants in generating fruits and seeds [26]. Therefore, plant breeders and growers ought to know about the viability of pollen for any plant species [27]. The development of pollen has two stages: (1) the stage of meiosis takes place in mother cells, and (2) the microspore development and release of pollen grains [1, 4, 28, 29]. The current study aims to investigate the pollen morphology and pollen fertility of selected taxa of Boraginaceae in Pakistan.

Material and Methods

Study Area

Pakistan is situated at 30°N and 70°E and has an overall area of 796,096 km^2 [1, 30, 31]. In terms of geography, plains constitute 39% of Pakistan, whereas

61% is made up of the mountainous regions [32]. The wide range of variations in temperature, elevation, rainfall, and other environmental factors have contributed to a rich flora with at least 5,700 flowering plant species, as discussed in previous studies [4, 33]. A large portion of Pakistan has both arid and semi-arid climates with seasonal and geographical variations. Monsoon rain is a major source supplying 59% of rainfall annually [34]. Fresh plant samples collected from their natural habitats in Pakistan were analyzed for LM to investigate the pollen fertility of selected plant taxa of Boraginaceae. The list of investigated plant taxa is shown in Table 1.

Light Microscopy

As investigated earlier, the pollen slide was prepared using glycerin jelly and safranine for light microscopy using the acetolysis technique [6, 35]. The prepared slide was then examined at several magnification levels. A microscope (MEIJI, Japan) was utilized for micrometric analysis of pollens. An infinity 1-5C-MEL (Canada) and a LEICA DM 1000 microscope were employed for micrographs.

Pollen Fertility Estimation

After slide preparation, based on staining via glycerin jelly, the pollen was observed at low magnification power, and then the percentage of pollen fertility was calculated. The number of viable and sterile pollens was assessed on each slide. Pollen that was damaged, broken, or improperly dyed was deemed sterile, whereas pollen that was effectively stained was assumed to be fertile.

The mathematical formula described by [3, 5, 6, 16] was used to determine the percentage of pollen fertility and pollen sterility.

$$\text{Fertility} = F/F + S \times 100$$

$$\text{Sterility} = S/S + F \times 100s$$

Results

The pollen micromorphology and pollen fertility estimation of 27 plant species of Boraginaceae were studied using light microscopy. This study was based on the existence of the plants in the flowering stage, which collected their flowers and performed their palynological analysis to determine their fertility and sterility rate. The LM photographs are shown in Figs. 1, 2, 3, and 4. The pollens of investigated plant species show a wide range of variations in shape, size, exine thickness, and pollen fertility. The highest fertility % was observed in *Trichodesma indicum* (94.94%), while the lowest was in *Arnebia hispidissima* (60.50%). The results of each taxon are represented as phytogeography, flower color, flowering period, and palynology. The results are summarized in Table 1.

Table 1. Distribution, morphological and palynological features of seleted taxa.

Alkanna tinctoria Tausch.
Phytogeography: It is found in Iran, Pakistan, Saudi Arabia, and Afghanistan.
Flower color and period: Blue & March-May
Palynology: Monad and tri-colporate pollen with psilate sculpturing. The shape of the pollen is oblate-spheroidal, and the exine thickness is 2.80 μm, with pollen fertility (85.80%).
Anchusa arvensis (L.) M. Bieb.
Phytogeography: Greece, Bulgaria, Hungary, Albania, Algeria, Egypt, Libya, Sudan, Iran, Iraq, Saudi Arabia, Israel, Malaysia, Indonesia, and Myanmar.
Palynology: Monad and tricolporate pollen with psilate sculpturing. The shape of the pollen is oblate, and the exine thickness is 1.25 μm, with pollen fertility (80.80%).
Arnebia benthamii (Wall. ex G.Don) I.M.Johnst.
Phytogeography: Pakistan, India to Nepal.
Flower color and period: Red, purple & June-July
Palynology: Monad and tetra-colporate pollen with psilate sculpturing. The shape of the pollen is sub-oblate, and the exine thickness is 1.50 μm, with pollen fertility (72.40%).
Arnebia decumbens Cross & Kralik.
Phytogeography: Algeria, Egypt, Sudan, Syria, Iraq, Iran, Afghanistan, Pakistan, and China.
Flower color and period: Yellow & April-May
Palynology: Monad and tricolporate pollen with psilate sculpturing. The shape of the pollen is oblate, and the exine thickness is 1.55 μm, with pollen fertility (65.20%).
Arnebia grandiflora (Trautv.) Popov.
Phytogeography: Pakistan, Iran, and Afghanistan.
Flower color and period: Yellow & May
Palynology: Monad and tetra-colporate pollen with psilate sculpturing. The shape of the pollen is oblate, and the exine thickness is 1.97 μm, with pollen fertility (65.60%).
Arnebia griffithii Boiss.
Phytogeography: Pakistan and Afghanistan.
Flower color and period: Yellow & March-May
Palynology: Monad and tetra-colporate pollen with psilate sculpturing. The shape of the pollen is sub-oblate, and the exine thickness is 2.10 μm with pollen fertility (78.20%).
Arnebia hispidissima DC.
Phytogeography: Pakistan, Saudi Arabia, Egypt, Sudan, and Kenya.
Flower color and period: Yellow & December-April
Palynology: Monad and 5-colporate pollen with psilate sculpturing. The shape of the pollen is prolate, and the exine thickness is 2.10 μm with pollen fertility (78.20%).

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Arnebia speciosa Aitch. & Hemsl.
Phytogeography: Endemic to eastern Afghanistan and Pakistan (Kurram).
Flower color and period: Yellow, red & May
Palynology: Monad and 6-colporate pollen with psilate sculpturing. The shape of the pollen is oblate-spheroidal, and the exine thickness is 2.70 µm, with pollen fertility (87.30%).
Asperugo procumbens L.
Phytogeography: Algeria, Egypt, Sudan, India, Pakistan, Sri Lanka, Afghanistan, Germany, Hungary, Switzerland, and the United Kingdom.
Flower color and period: Blue, purple & March-May
Palynology: Monad and tricolporate pollen with psilate sculpturing. The shape of the pollen is prolate-spheroidal, and the exine thickness is 1.13 µm, with pollen fertility (66.15%).
Buglossoides arvensis (L.) I.M. Johnst.
Phytogeography: Spain, Italy, Iraq, Iran, Afghanistan, Pakistan, India, and Japan.
Flower color and period: Blue, white & March-April
Palynology: Monad and 6-colporate pollen with psilate sculpturing. The shape of the pollen is prolate-spheroidal, and the exine thickness is 0.84 µm, with pollen fertility (84.30%).
Caccinia macranthera Brand.
Phytogeography: Iran, Afghanistan, and Pakistan.
Flower color and period: Purple & March-April
Palynology: Monad and 3-colporate pollen with psilate sculpturing. The shape of the pollen is oblate, and the exine thickness is 1.00 µm with pollen fertility (74.60%).
Cynoglossum lanceolatum Forssk.
Phytogeography: Algeria, Egypt, Libya, Saudi Arabia, Pakistan, India, Sri Lanka, Burma, and Nepal.
Flower color and period: Pale blue & June-August
Palynology: Monad and tetra-colporate pollen with psilate sculpturing. The shape of the pollen is per-prolate, and the exine thickness is 1.41 µm, with pollen (fertility 81.10%).
Ehretia serrata Roxb. Fl. Ind. ed. (Car. & Wall.)
Phytogeography: Pakistan, India, Nepal, and Afghanistan.
Flower color and period: White & March-May
Palynology: Monad and 3-colporate pollen with psilate sculpturing. The shape of the pollen is spherical, and the exine thickness is 0.9 µm, with pollen fertility (64.55%).
Euploca strigosa (Wild.) Diane & Hilger.
Phytogeography: Pakistan, Algeria, Angola, Cameroon, and India.
Flower color and period: White with boas & January-October
Palynology: Monad and 8-colporate pollen with foveolate sculpturing. The shape of the pollen is prolate-spheroidal, and the exine thickness is 1.45 µm, with pollen fertility (86.72%).
Gastrocotyle hispida (Forssk.) Bunge.
Phytogeography: Algeria, Egypt, Libya, Syria, Iraq, Iran, Afghanistan, Pakistan, India.

Flower color and period: Purplish-blue & March-May.
Palynology: Monad and 6-colporate pollen with foveolate sculpturing. The shape of the pollen is prolate-spheroidal, and the exine thickness is 1.02 µm with pollen fertility (83.95%).
Heliotropium bacciferum Forssk.
Phytogeography: Algeria, Egypt, Libya, Saudi Arabia, Pakistan.
Flower color and period: White & December-April.
Palynology: Monad and heterocolporate pollen with psilate sculpturing. The shape of the pollen is sub-oblate, and the exine thickness is 1.10 µm, with pollen fertility (88.28%).
Heliotropium europaeum L.
Phytogeography: Austria, Belgium, France, Germany, India, and Pakistan.
Flower color and period: White & July-September
Palynology: Monad and heterocolporate pollen with psilate sculpturing. The shape of the pollen is sub-oblate, and the exine thickness is 2.12 µm, with pollen fertility (79.95%).
Heliotropium supinum L.
Phytogeography: Austria, Belgium, France, Germany, Algeria, Egypt, Libya, India, and Pakistan.
Flower color and period: White & December-January
Palynology: Monad and 6-colporate pollen with sub-psilate sculpturing. The shape of the pollen is sub-oblate, and the exine thickness is 1.98 µm, with pollen fertility (74.53%).
Heliotropium curassavicum L.
Phytogeography: Canada, Mexico, Austria, Belgium, France, Germany, Algeria, Egypt, Libya, Pakistan, India, and Australia.
Flower color and period: White & March-April
Palynology: Monad and heterocolporate pollen with psilate sculpturing. The shape of the pollen is sub-oblate, and the exine thickness is 1.99 µm, with pollen fertility (86.13%).
Lappula squarrosa (Retz.) Dumort.
Phytogeography: Austria, Belgium, France, Germany, Iran, Afghanistan, Pakistan.
Flower color and period: White to pale blue & May-September
Palynology: Monad and 3-colporate pollen with psilate sculpturing. The shape of the pollen is per-oblate, and the exine thickness is 1.20 µm, with pollen fertility (76.20%).
Nonea caspica G.Don.
Phytogeography: Russia, Iraq, Iran, Turkestan, Afghanistan, Pakistan.
Flower color and period: Red to deep blue & March-April
Palynology: Monad and 4-colporate pollen with reticulate sculpturing. The shape of the pollen is sub-oblate, and the exine thickness is 1.90 µm, with pollen fertility (79.09%).
Nonea edgeworthii A.DC.
Phytogeography: Pakistan and India.
Flower color and period: White & March-April

Palynology: Monad and 4-colporate pollen with reticulate sculpturing. The shape of the pollen is oblate-spheroidal, and the exine thickness is 1.85 µm, with pollen fertility (85.85%).
Onosma bracteosa Hausskn. & Bornm.
Phytogeography: Pakistan, Iran, Iraq, and Turkey.
Flower color and period: Yellow & June-July
Palynology: Monad and 3-colporate pollen with psilate sculpturing. The shape of the pollen is oblate-spheroidal, and the exine thickness is 1.20 µm, with pollen fertility (73.42%).
Onosma dichroantha Boiss.
Phytogeography: Turkestan, Afghanistan, Pakistan.
Flower color and period: Yellow and red & March-May
Palynology: Monad and 3-colporate pollen with sub-psilate sculpturing. The shape of the pollen is sub-prolate, and the exine thickness is 1.30 µm, with pollen fertility (88.81%).
Onosma hispida Wall. & G.Don.
Phytogeography: Afghanistan, Pakistan, and India.
Flower color and period: White & May-June
Palynology: Monad and 3-colporate pollen with scabrate sculpturing. The shape of the pollen is sub-prolate, and the exine thickness is 0.75 µm, with pollen (fertility 94.14%).
Onosma limitaneum I.M. Johnst.
Phytogeography: Iran, Afghanistan, Pakistan.
Flower color and period: Yellow and red & May
Palynology: Monad and 3-colporate pollen with sub-psilate sculpturing. The shape of the pollen is prolate, and the exine thickness is 1.75 µm, with pollen fertility (70.82%).
Trichodesma indicum (L.) Lehm.
Phytogeography: Afghanistan, Pakistan, India, Philippines, Mauritius.
Flower color and period: White & March-August
Palynology: Monad and 3-colporate pollen with psilate sculpturing. The shape of the pollen is prolate-spheroidal, and the exine thickness is 2.1 µm, with pollen fertility (94.94%).

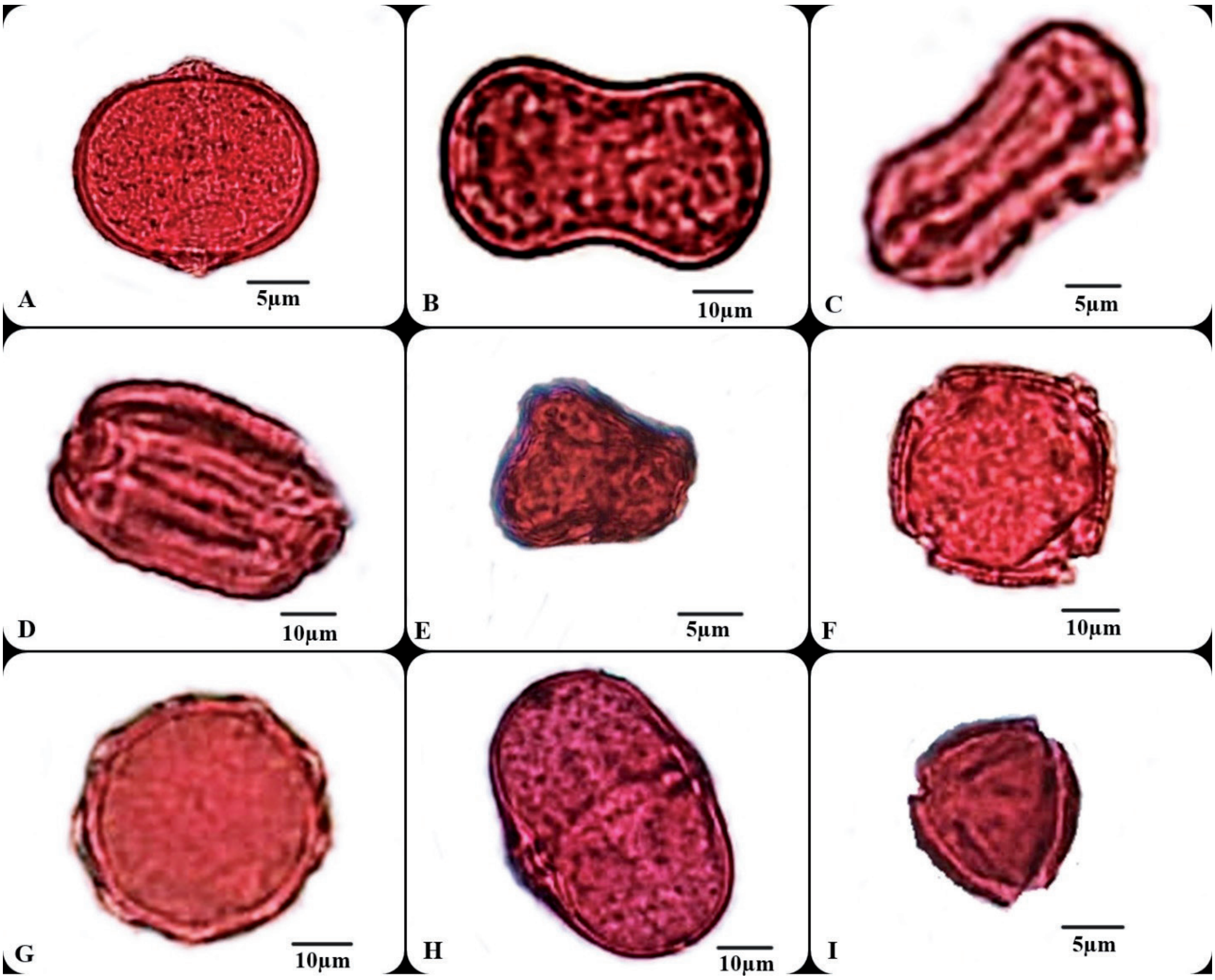

Fig 1. Light microscope micrograph: A. *Alkanna tinctoria;* B. *Anchusa arvensis;* C. *Arnebia benthamii;* D. *Arnebia decumbens;* E. *Arnebia grandiflora;* F. *Arnebia griffithii;* G. *Arnebia hispidissima;* H. *Arnebia speciosa;* I. *Asperugo procumbens*.

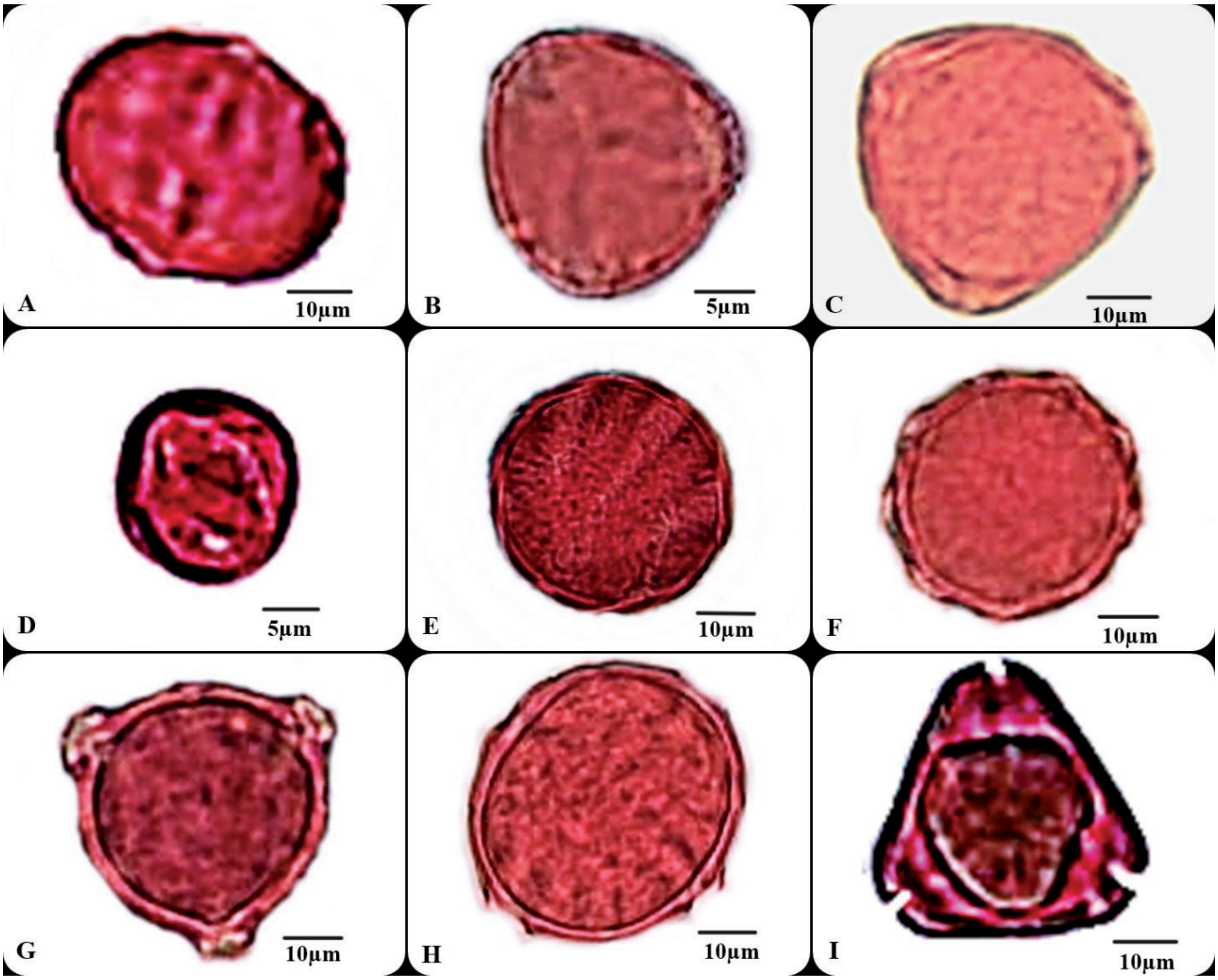

Fig 2. Light microscope micrograph: A. *Buglossoides arvensis;* B. *Caccinia macranthera;* C. *Cynoglossum lanceolatum;* D. *Ehretia serrata ;* E. *Euploca strigosa;* F. *Gastrocotyle hispida;* G. *Heliotropium bacciferum;* H. *Heliotropium europaeum;* I. *Heliotropium supinum.*

Discussion

In the current study, LM was employed to investigate the pollen fertility estimation and palynological aspects of 27 species of Boraginaceae collected from various parts of Pakistan. The pollen morphological features were evaluated using light microscopy techniques and are correctly compiled in quantitative and qualitative forms (Table 1). Pollen morphological changes and fertility percentages were observed during the research and recorded before this study [4-6, 36, 37]. Taxonomists rely on pollen fertility to differentiate probable hybrids from their parent plants and assess the level of fertility in plants established in adverse conditions. The pollen grains within this family are almost monad, very small, and show shapes such as oblate, suboblate, oblate-spheroidal, prolate, perprolate, subprolate, and prolate-spheroidal. Generally, the pollen grains were monad, psilate, isopolar, or heteropolar, and from tricolporate to 8-colporate pollens were noted.

The highest fertility % was observed in *Trichodesma indicum* (94.94%), while the lowest was in *Arnebia hispidissima* (60.50%). [38] investigated the average fertility rates in Brassicaceae and Papillionaceae, reporting 91.82% and 88.79%, respectively. Similarly, [39] examined pollen viability in the genus Hypericum and found pollen fertility rates ranging from 82% to 90%. [40] researched Asteraceae, revealing pollen viability rates ranging from 74.19% to 94.5%. While a lot of work has been conducted on pollen viability in various plant families, unfortunately, there have been no comprehensive studies on the estimation of pollen fertility within the Boraginaceae family in previous studies [5]. The only significant research into this area was conducted by [41], who evaluated the fertility rate of three Boraginaceae species, i.e., *Heliotropium indicum, Trichodesma indicum,* and *T. zeylanicum,* in India and found that pollen viability ranged from 70%-100%. This viability range aligns closely with current studies suggesting that the Boraginaceae family has comparable reproductive potential [3, 4, 6].

Bochynek states that small, dispersed populations have lower pollen fertility. [3, 7, 42] examined the effect of population size on some angiosperms' reproduction

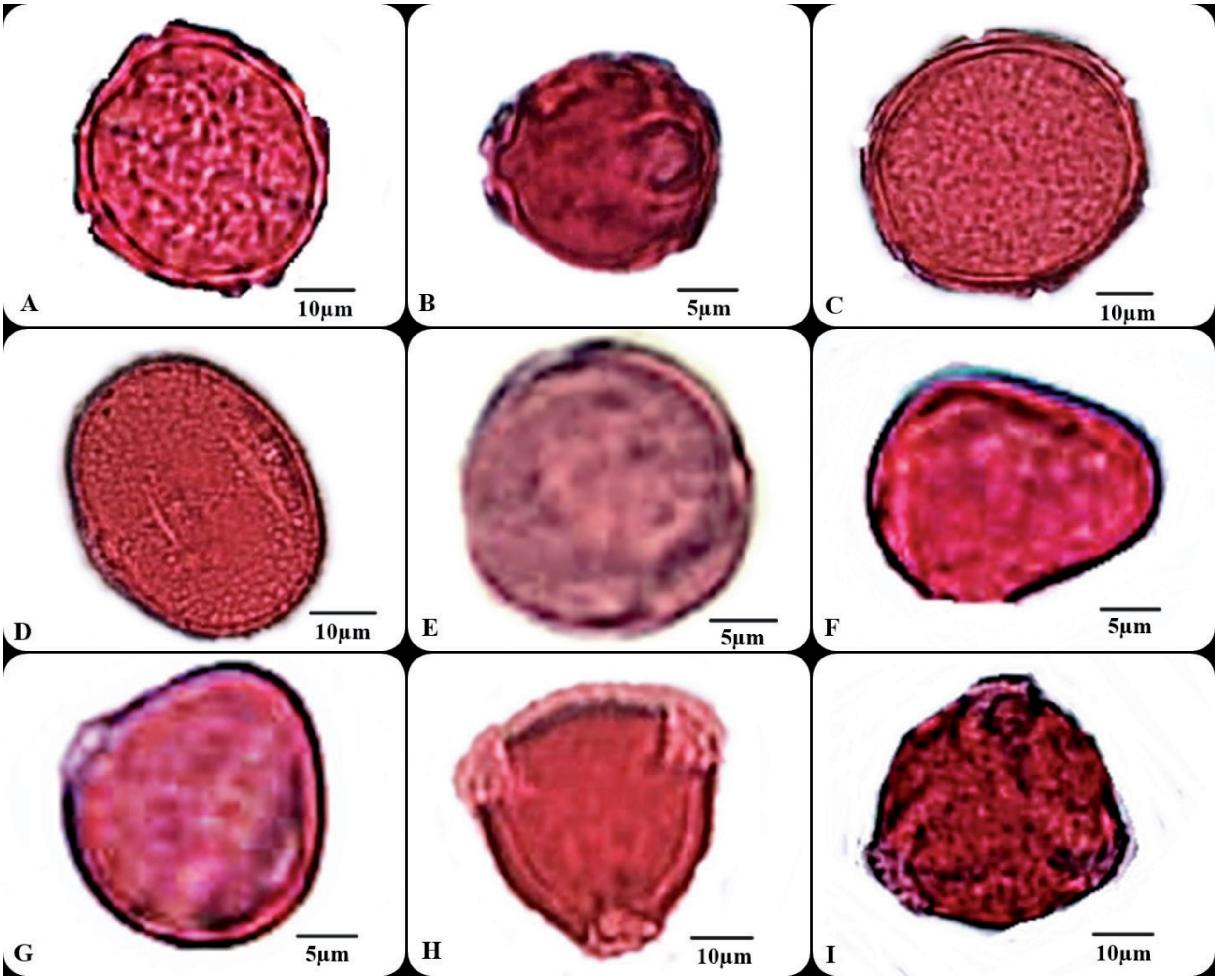

Fig 3. Light microscope micrograph: A. *Heliotropium curassavicum;* B. *Lappula squarrosa;* C. *Nonea caspica;* D. *Nonea edgeworthii;* E. *Onosma bracteosa;* F. *Onosma dichroantha;* G. *Onosma hispida;* H. *Onosma limitaneum;* I. *Trichodesma indicum.*

ability. When seed production per fruit per plant in tiny populations of plants was reduced, they discovered a significant reduction in reproduction. The researchers concluded that a potential cause of the decline in fertility in tiny populations could be the insufficient quantity and quality of pollen.

Menges [2, 5, 43] similarly reported low pollen fertility and reduced seed germination rates in very small, isolated populations. Environmental or physical parameters, such as the maturation of late-season pollen grains, may be the reason for lower fertility. It was found that several pollen levels of fertility displayed by species within a single family were significant [2, 4, 44]. Nazish investigated the pollen fertility status of coastal species that are significant to the economy and ecosystem. Scientists believe pollen productivity is a key factor in determining the successful adaptation of plant species. Pollen fertility studies have been adjusted or used to identify the large range of variation seen within species and differentiate between plant species and genera. [1, 4, 5, 7, 45]. There is also a clear decline in pollen fertility in tiny, scattered populations. Reproduction of various angiosperms was shown to be significantly inhibited in tiny populations, where plants generated fewer seeds per fruit per plant, according to research done by other researchers.

Conclusion

This study provides significant insights into pollen fertility and micromorphology of 27 species of Boraginaceae. This study helps us understand species' genetic diversity and stability in this area. The reported species show variations in their shape, size, exine thickness, and fertility rate, with prolate to spheroidal being abundant in many species of this family. The fertility rate varied from 60.50% in Arnebia *hispidissima* to 94.94% in *Trichodesma indicum.* The observed variations demonstrate the role of these traits in species identification. To gain a better understanding, future studies should focus on a more in-depth examination of the environmental factors that influence pollen morphology and fertility in different places.

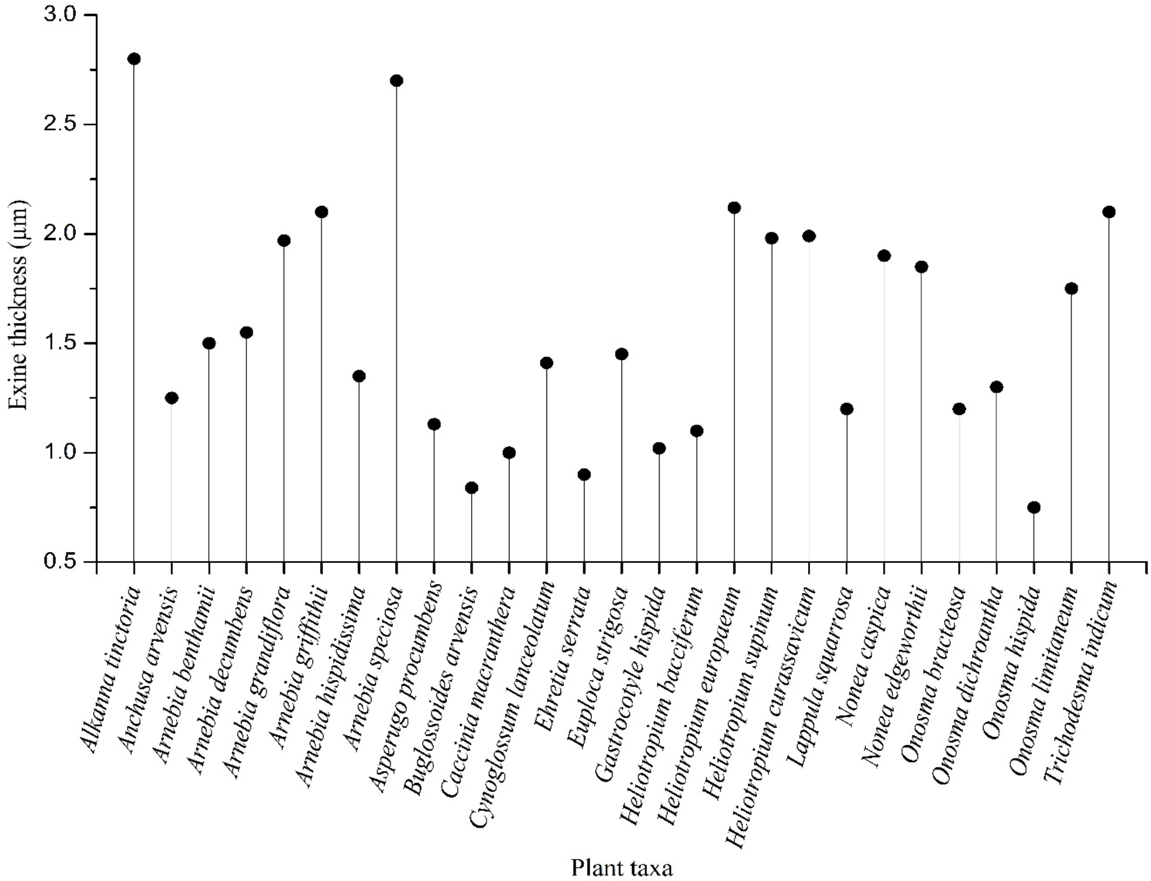

Fig. 4. Graphical representation of exine thickness of investigated taxa.

Conflict of Interest

The authors of the manuscript have no conflict of interest to declare.

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