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Palynological studies on some plants of Boraginaceae

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Abstract: *The family Boraginaceae includes about 148 genera and more than 2700 species. Plants of this family have alternately arranged leaves, or a combination of alternate and opposite leaves. The leaf blades usually have a narrow shape; many are linear or lance-shaped. They are smooth-edged or toothed, and some have petioles. Most species have bisexual flowers. Most species have coiled shaped inflorescence. The flower has usually five-lobed calyx. The corolla varies in shape from rotate to bell-shaped to tubular. There are five stamens and one style with one or two stigmas. The fruit is a drupe.*

In the present investigation the pollen grains of different morphovars of three species of Boraginaceae viz. Heliotropium indicum, Trichodesma indicum and T. zeylanicum collected from fifteen localities of Gaya (Bihar) were studied. The results revealed evident that the size of pollen grains of H. indicum and T. indicum were of more or less similar size (length 20µm to 26µm and width 15µm to 19µm). The pollen grains of Trichodesma zeylanicum in all the specimens were comparatively larger in size, with length ranged from 24µm to 29µm and width from 21µm to 24µm. P/E ratio was 0.63 to 0.76 in H. indicum; 0.83 to 0.88 in T. indicum and 0.93 to 1.16 in T. zeylanicum. The size of pollen grains was also more or less same in all the three species and ranged from 65µm to 125µm. The fertility of pollen grains was same in all the three species and varied from locality to locality. The viability of pollen grains was 70 to 100%.

Boraginaceae can serve as a model for the investigation of various morphological features including pollen shape, pore number and ornamentation. Future studies can focus further on the examination of the morphology, anatomy, development, genetics, and evolution of these and other variable characters within this diverse family for establishing phylogenetic relationship.

Key Words: *Boraginaceae, Heliotropium indicum, Trichodesma indicum, Trichodesma zeylanicum, morphovars, pollen grains, phylogeny*

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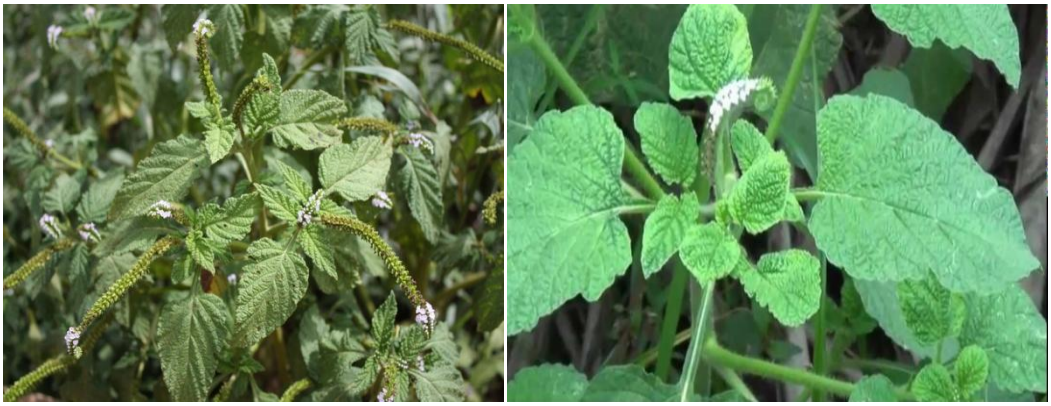
I. Introduction

Boraginaceae (borage or forget-me-not), the family of flowering plants (dicotyledonous angiosperm) includes about 148 genera and more than 2700 species. Plants of this family are frequently herbaceous and hairy and can be annuals or perennials. Some are vines or trees, and a few are obligate parasites. Plants have alternately arranged leaves, or a combination of alternate and opposite leaves. The leaf blades usually have a narrow shape; many are linear or lance-shaped. They are smooth-edged or toothed, and some have petioles. Most species have bisexual flowers, but some taxa are dioecious. Pollination mostly occurs by hymenopterans and bees. Most species have coiled shaped inflorescence. The flower has usually five-lobed calyx. The corolla varies in shape from rotate to bell-shaped to tubular, but it generally has five lobes. It can be green, white, yellow, orange, pink, purple, or blue. There are five stamens and one style with one or two stigmas. The fruit is a drupe.

Most members of this family have hairy leaves. The coarse character of the hairs is due to the presence of cystoliths of silicon dioxide and calcium carbonate. These hairs can induce an adverse skin reaction, including itching and rash in some individuals, particularly among people who handle the plants regularly, such as gardeners. In some species anthocyanins cause the flowers to change color from red to blue with age. This is may be a signal to pollinators.

The family includes a number of garden ornamentals, such as *Heliotropium* (heliotrope), *Martensia virginia* (bluebell), *Phacelia* (Scorpionweed), *Pulmonaria* (Lungwort), and *Myosotis* (forget-me-not).

***Heliotropium indicum.* L:** *Heliotropium indicum*, commonly known as Indian heliotrope**,** Syriari or Hathsura, is an annual, hirsute plant. It is a common weed in waste places and settled areas and is native to Asia. India heliotrope is an annual, erect, branched plant that can grow to a height of about 15–50 centimeters (5.9–19.7 in). It has a hairy stem, bearing alternating ovate to oblong-ovate leaves. It has small white, blue or violet flowers with a green calyx; five stamens borne on a corolla tube; a terminal style; and a four-lobed ovary. It is a upland species adapted to clay bottomland soils and also invade bare soil (Photograph-1 and 2).

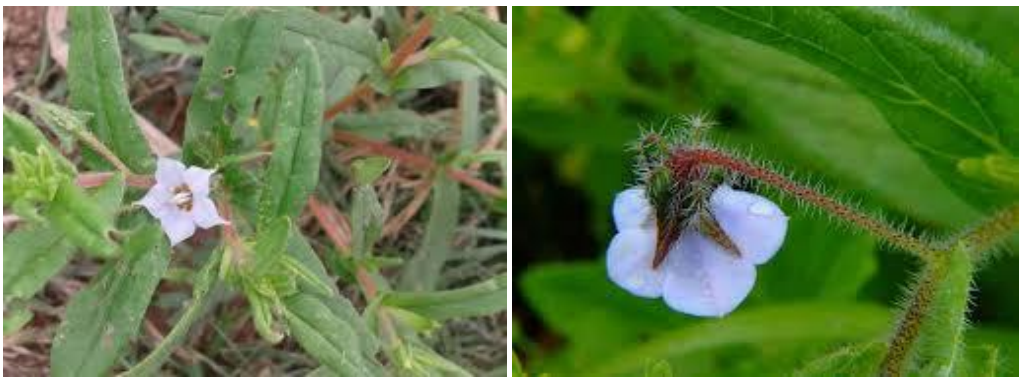


1. 2.

Photograpgs-1 and 2: Floral twigs of *Heliotropium indicum*

Trichodesma indicum (L.) Lemh: *Trichodesma indicum* (L.) Lemh is an erect, spreading, branched, annual herb, about 50cm. in height, with hairs springing from tubercles. The leaves are sessile, opposite, lanceolate, 2 to 8 cm. long, pointed at the tip, and heart shaped at the base. The flowers occur singly in the axils of leaves. The calyx tube is green, hairy, and 1 to 13 cm. long, with pointed lobes. The flower tube is pale blue, with the limb about 1.5 cm.in diameters, and petals pointed. The fruit is ellipsoid, and is enclosed by the calyx. The nutlets are about 5mm long, and rough on the inner surface. This species is found throughout India, on roadsides and stony dry wastelands, up to 1500m.

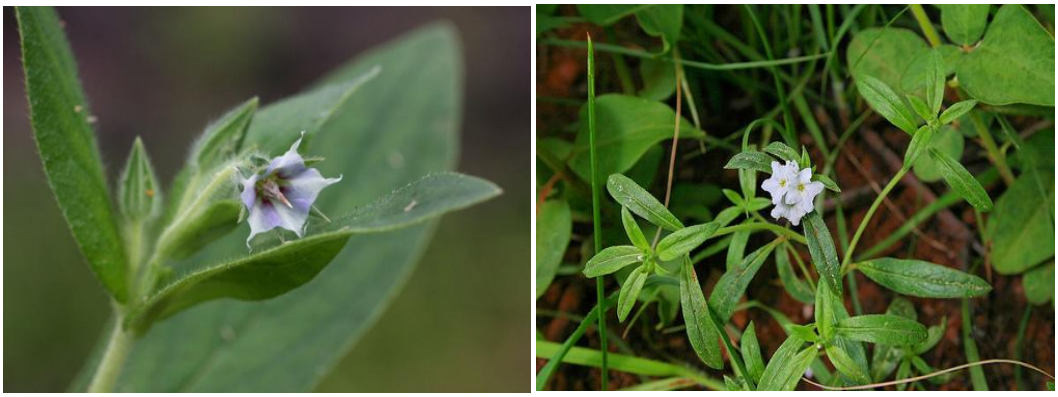
The plant is acrid, bitter in taste. It is a thermogenic, alexetric, anodyne, anti-inflammatory, carminative, constipating, diuretic, depurative, ophthalmic, febrifuge and pectoral. This herb is also used in arthralgia, inflammations, dyspepsia, diarrhea, dysentery, strangury, skin diseases and dysmenorrhoea (Photographs-3 and 4).



3. 4.

Photographs-3 and 4: Floral twigs of *Trichodesma indicum*

***Trichodesma zeylanicum* (Burm.f.) R.Br**: *Trichodesma zeylanicum* (Burm.f.) R.Br, commonly known as Camel Bush or Cattle Bush, is an erect annual herb or shrub up to two metres high, much branched, rough and hairy with unpleasant bulbous-based spiny hairs that break off in the skin when the plant is handled. Leaves are opposite below, becoming alternate higher on the plant, ovate to narrowly lanceolate up to 12 cm long, tapered to the base, with no distinct petiole, conspicuously veined below, also covered in spiny hairs. Flowers are single, hanging from long pedicels in the axils of upper leaves, pale blue with a white center, or all white, with 5 petals fused in a campanulate form, 12 mm across, the calyx also hairy with 5 acute lobes, just exceeding the length of the corolla. The calyx swells as the fruit develops, containing four 3-angled seeds, 4 mm long, rough on the inner surface, smooth and glossy on the back, gray-brown in color (Photograph-5 and 6).



5. 6.

Photograph-5 and 6: Floral twigs of *Trichodesma zeylanicum*

The family is characterized by a scorpioid cymose inflorescence (Buys and Hilger, 2003) [1], a gynobasic style, and a two-part ovary that breaks into four nutlets. This circumscription is equivalent to, and has been referred to as, Boraginaceae s.s. or Boraginoideae (Gottschling *et al*., 2001; Diane *et al*., 2002) [2, 3].

Biosystematics is an experimental taxonomic study from the stand point of evolutionary processes, and is largely concerned with morphological, anatomical, genetical, cytological, chemical and palynological aspects.

The size and shape of pollen, number and position of furrows, number and position of apertures and the details of sculpturing of the exine are of taxonomic value. The form, number, distribution and position of apertures are important palynological criteria in assessing the relationships and phylogeny of plants. According to the position, the aperture may be proximal, distal, and zonal. In terms of evolution, the proximal position is most primitive and zonal position as most advanced. Wael Taha Kasem (2015) [4] studied pollen grains types in seven different species of *Heliotropium* and significant differences were recorded. Tricolpate pollen grains type was noticed in all the studied taxa. Oval form found in *H.longiflorium.* circular found in *H. arbainense* and *H.petrocarpum.* elliptical form was noticed in *H. strigosium*, rounded form was recorded in *H. zeylanicum* also, triangular was noticed in *H.jizanense*. On the other hand, the pollen length, aperture size also varied between the taxa. The maximum pollen length and width (P⁄E) of 1.81 µm was recorded in *H.longiflorium* followed by 1.68 found in *H.strigosium.* The lowest P⁄E was noticed in *H. arbainense* (0.70 µm).

The present work is related to study the pollen grains of different varieties (morphovars) of three species of Boraginaceae viz. *Heliotropium indicum, Trichodesma indicum* and *T. zeylanicum* collected from fifteen localities of Gaya (Bihar).

II. Materials and Methods

The pollen grains of higher plants constitute the most vital unit of the flower with regard to their form and function. These one-celled microscopic haploid units represent the essential genetic bridge between generations. Description of the fundamental features of pollen morphology by early Botanists has demonstrated the potential value of palynology in phylogeny and plant taxonomy. Palynology has been valuable aid for taxonomical rearrangements. It has helped in identification and delimitation of taxa, elucidation of phylogenetic relationships, placing taxa of uncertain affinities. An understandmg of the ontogeny of the pollen grain and of the deposit ion and fundamental structure of sporopollenin are of significance for comparative study of pollen morphology.

Collection of plant Varieties: Twenty five plants of each of the three species of Boraginaceae, viz., *Heliotropium indicum, Trichodesma indicum* and *T. zeylanicum,* which differed from each other slightly in their morphological features were collected from fifteen localities of District Gaya (Bihar). The plant specimens were identified following the standard monograph 'The Botany of Bihar and Orissa" Volume-IV (Gamopetalae), H. H. Haines (1921) [5], Alfarhan *et al*. (2005) [6] and Masrahi (2012) [7].The localities from where the plants of each of the three species collected were as follows:

Delha (DLH) MU campus, Bodh Gaya (BG), Bramhyoni Pahari (BP), Gaya College Campus, Rampur, Gaya (GC), MahaBodhi College Campus, Bela Ganj, Gaya (MB), Manpur (MP), Fatehpur (FP), Mohanpur (MOP), Paraiya (PRA), Tekari (TK), Chekand (CK), Bara (BR), Tankappa (TKP), Panchanpur (PNP), Ramshila (RMS).

Pollen Fertility (Viability) Assessment: In plants of all the three species of Boraginaceae viz., *Heliotropium indicum, Trichodesma indicum* and *Trichodesma zeylanicum* fresh pollen was collected in the field from recently opened anther and brought into the laboratory. Depending on the amount of pollen per anther, one anther for each sample of pollen or all of the pollen from one flower was chosen. Pollen was extracted and

mixed on a microscope slide and then divided into three samples: (1) fresh pollen, (2) pollen heated to 80°C for 2 h (designated 2-h pollen), and (3) pollen heated to 80°C for 24 h (designated 24-h pollen).
Tests for viability (Fertility): Four methods for staining, along with in vitro pollen germination, were used to test pollen viability.

1. **Baker's procedure (Dafni, 1992) [8].** This test detects the presence of alcohol dehydrogenase. The test solution consisted of 7 mg phosphate buffer/l0 ml H_2O) (pH 7.3-7.5); nitroblue-tetrazolium just to give a slight yellow colour; 6 mg nicotinamide adenine dinucleotide and 0.5–1 ml of ethanol (35%). The pollen grain was considered viable if it turned violet or pink.

2. **X-Gal-test (Trognitz 1991) [9].** This test detects the presence of β-galactosidase. The test solution consisted of 1 mg X-Gal (5-bromo-4-chloro-3-indoyle-b-galactoside) dissolved in 50 µl N, N- methyl formamide and 1 ml acetate buffer (50 mmol, pH 4.8). The pollen grain was considered viable if it turned blue.

3. **MTT (Norton, 1966) [10].** This test detects the presence of dehydrogenase. The test solution consisted of a 1% concentration of the substrate 2, 5-diphenyl tetrazolium bromide (MTT or thiazolyl blue) in 5% sucrose. The pollen grain was considered viable if it turned deep pink or if it presented no colour but showed irregular black lines over its surface.

4. **p-Phenylenediamine.** This test detects the presence of myeloperoxidase. The test solution consisted of one vial peroxidase indicator reagent (Sigma 390-1), and 200 µl 3% hydrogen per- oxide (1:9, 30% hydrogen peroxide and phosphate buffered saline solution pH 7.4) added to 50 ml Trizmal 6.3 dilute buffer prewarmed to 37°C prepared by mixing Trizmal 6.3 buffer concentrate (Sigma 90-3 C) with deionized water 1:9. The solution can be kept in the refrigerator for about 15–20 days without loss of potential activity. If during this time the solution turned from light brown to very dark brown or black it was discarded. This solution was always kept and used in the dark. In order to stain pollen grains, a small amount of the solution was taken and warmed it at 37°C about 10–15 min. The pollen grains were considered viable if they turned totally black.

5. The in vitro germination test used the hanging drop method (Shivanna and Rangaswamy 1992) [11] with various sucrose solution concentrations (0%, 5%, 10%, 15%, 20%, 30%, 40% and 50%) with 2 $X10^{-3}$M H_3BO_3 and 6 X 10^{-3} M Ca $(NO_3)_2$ added. Dishes were left at room temperature (20°C) for a maximum of 24 h. Pollen grains were considered to have germinated when pollen tube length was greater than or equal to pollen diameter. For each species, germination at the optimal sucrose solution was recorded.

All pollen viability tests were conducted by incubating the pollen in the medium for 30 min at 37°C. The process of staining using both X-Gal and p-phenylenediamine, was conducted in the dark. Five replicas per sample were used, and five random groups of 100 pollen grains each per replica were assessed. A light microscope with either X160 or X400 magnification, depending on pollen size was used.

Palynological characters such as pollen type, aperture morphotype, exine ornamentation, spine type and length, grain size and shape, pollen fertility like features were observed. Mature pollen grains from mature anthers were dusted on a clean slide and stained with one percent acetocarmine. The acetolysed pollen grains were mounted in glycerine jelly and the slides were sealed with paraffin wax. Acetocarmine was found to be the most suitable stain for pollen grain studies in Boraginaceae. The size of the pollen grains was measured by using ocular micrometer. Twenty readings were taken in each case. The pollen grains were micro photographed to study the shape of pollengrains and the wall ornamentation. Photomicrographs were taken for all plant materials. Jetner – Biolux research microscope was used for micro photographing. Pollen size is calculated by taking measurements of polar axis and the maximum breadth in the equitorial view of the grain and applying the formula P/E x100.

Pollen sterility was examined by smearing mature anther in 1:1 mixture of glycerine and acetocarmine. The slides were kept 30 minutes for better staining and then examined under microscope. Fully stained pollen grains were counted as fertile and partially stained or unstained was counted as sterile. Pollen sterility was calculated by using the following formula:

$$\% \text{ of Pollen sterility} = \frac{\text{Number of sterile pollen grains}}{\text{Total number of pollen grains}} \times 100$$

The Qualitative and quantitative palynological features selected were as follows:

Sl. No.	Character	Category
1.	Pollen aperture morphotype	qualitative
2.	Pollen exine ornamentation	qualitative
3.	Spine type	qualitative
4.	Spine length(µm**)**	quantitative
5.	Pollen grain size range (µm)	quantitative
6.	Pollen grain shape	qualitative
7.	Pollen fertility **(**%)	quantitative

The results obtained have been presented in Table- 1, 2 and 3: Fig- 1, 2 and 3.

Table-1: Showing morphological features of Pollen grains of *Heliotropium indicum* collected from fifteen areas of Gaya.

Features	DLH	BG	BP	GC	MB	MP	FP	MOP	PRA	TK	CK	BR	TKP	PNP	RMS
Length of pollen in µm	21	23	20	19	24	25	26	26	27	24	23	23	26	26	25
Width of pollen in µm	17	16	17	19	19	18	17	17	16	15	19	19	19	18	17
Pollen aperture in µm	0.7	0.7	0.6	0.8	0.6	0.7	0.7	0.8	0.6	0.5	0.6	0.7	0.7	0.7	0.8
P/E ratio	0.65	0.67	0.65	0.66	0.65	0.65	0.66	0.70	0.74	0.75	0.75	0.76	0.68	0.67	0.63
Shape of pollen	Elliptical	Elliptical	Elliptical	Rounded	Rounded	Elliptical	Elliptical	Elliptical	Elliptical	Elliptical	Rounded	Rounded	Elliptical	Elliptical	Elliptical
Pollen types	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate
Aperture morphotype	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent
Exine ornamentation	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose
Spine type	Pointed	Pointed	Pointed	Pointed	Pointed	Pointed	blunt	blunt	Pointed	Pointed	Pointed	blunt	Pointed	Pointed	Pointed
Spine length in µm	7.5	7.6	7.5	7.5	7.7	9.1	9.5	9.4	9.7	9.4	10.5	10.7	11.5	11.4	11.3
Grain size µm	65	67	70	75	76	85	86	84	87	89	112	115	114	125	120
Pollen fertility %	70	72	75	76	78	85	87	86	88	85	91	95	100	98	97

Pent. = Pentoporate

Table-2: Showing morphological features of Pollen grains of *Trichodesma indicum* collected from fifteen areas of Gaya.

Features	DLH	BG	BP	GC	MB	MP	FP	MOP	PRA	TK	CK	BR	TKP	PNP	RMS
Length of pollen in µm	20	23	20	22	24	22	23	22	24	24	23	23	23	22	23
Width of pollen in µm	17	16	17	19	19	18	17	17	16	15	19	19	19	18	17
Pollen aperture in µm	0.6	0.6	0.6	0.6	0.6	0.7	0.7	0.8	0.6	0.5	0.6	0.7	0.7	0.7	0.6
P/E ratio	0.85	0.87	0.85	0.86	0.85	0.85	0.86	0.80	0.84	0.85	0.85	0.86	0.88	0.87	0.83
Shape of pollen	Rounded	Rounded	Elliptical	Rounded	Rounded	Elliptical	Rounded	Elliptical	Rounded	Elliptical	Rounded	Rounded	Elliptical	Rounded	Elliptical
Pollen types	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate
Aperture morphotype	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent
Exine ornamentation	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose
Spine type	Pointed	blunt	Pointed	blunt	Pointed	Pointed	blunt	blunt	Pointed	blunt	Pointed	blunt	Pointed	Pointed	Pointed
Spine length in µm	8.5	8.6	8.5	8.5	8.7	9.4	9.5	9.4	9.7	9.4	10.5	10.7	11.5	11.4	11.5
Grain size µm	75	77	75	75	76	85	86	84	87	89	112	115	114	125	120
Pollen fertility %	75	73	77	78	78	85	87	86	88	85	95	97	100	98	98

Pent. = Pentoporate

Table-3: Showing morphological features of Pollen grains of *Trichodesma zeylanica* collected from fifteen areas of Gaya.

Features	DLH	BG	BP	GC	MB	MP	FP	MOP	PRA	TK	CK	BR	TKP	PNP	RMS
Length of pollen in µm	28	28	27	27	28	26	27	28	29	24	26	26	26	27	28
Width of pollen in µm	22	21	22	23	23	24	23	22	21	21	22	23	23	22	24
Pollen aperture in µm	0.5	0.6	0.6	0.6	0.6	0.7	0.7	0.5	0.6	0.5	0.6	0.7	0.7	0.7	0.6
P/E ratio	1.15	1.14	1.16	1.15	1.16	1.14	1.16	0.89	0.89	0.95	0.95	0.96	0.98	0.97	0.93
Shape of pollen	Oval	Oval	Elliptical	Oval	Oval	Elliptical	Oval	Elliptical	Oval	Elliptical	Oval	Oval	Elliptical	Oval	Elliptical
Pollen types	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate	Tricolpate
Aperture	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent	Pent

morph otype															
Exine ornamentation	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose	Spinose
Spine type	blunt	blunt	Pointed	blunt	blunt	Pointed	blunt	blunt	Pointed	blunt	Pointed	blunt	Pointed	blunt	Pointed
Spine length in µm	8.6	8.6	8.6	8.5	8.7	9.4	9.5	9.4	9.7	9.4	10.5	10.7	11.5	11.4	11.5
Grain size µm	76	77	76	76	76	85	86	84	87	89	112	115	114	125	120
Pollen fertility %	76	75	77	78	78	86	87	86	88	85	96	97	100	98	98

Pent. = Pentoporate

Fig-1: *H. indicum*

Fig-2: *T. indicum*

Fig-3: *T. zeylanicum*

Pollen grains of the three species (X1000)

III. Results

The pollen grains of higher plants constitute the most vital unit of the flower with regard to their form and function. These one-celled microscopic haploid units represent the essential genetic bridge between generations. Description of the fundamental features of pollen morphology by early Botanists has demonstrated the potential value of palynology in phylogeny and plant taxonomy. In the present investigation the morphological features of pollen grains of the three species of Boraginaceae viz. *Heliotropium indicum*, *Trichodesma indicum* and *T. zeylanicum* in fifteen different localities have been studied (Table- 1, 2 and 3; Fig-1, 2 and 3). From the result it is evident that the size of pollen grains of *H. indicum* and *T. indicum* were of more or less similar size (length 20µm to 26µm and width 15µm to 19µm) (Table- 1 and 2). The pollen grains of *Trichodesma zeylanicum* in all the specimens were comparatively larger in size, with length ranged from 24µm to 29µm and width from 21µm to 24µm (Table-3). In specimens of all the three species the pollen aperture did not show great variation and was in the range of 0.5µm to 0.8µm. P/E ratio was 0.63 to 0.76 in *H. indicum*; 0.83 to 0.88 in *T. indicum* and 0.93 to 1.16 in *T. zeylanicum*. In most of the specimens of *H. indicum* elliptical shape of pollen grains was observed except in specimens from GC, MB, CK and BR where the shape of pollen was rounded. In *T. indicum* some specimens showed elliptical and some rounded shape. In the specimens of *T. zeylanicum* oval shape of pollen grains was observed in specimens from DLH, BG, GC, MB, FP, PRA, CK, BR and PNP while in rest of the specimens it was elliptical. In all the specimens of *H. indicum*, *T. indicum* and *T. zeylanicum* the pollen grains were Tricolpate with pentoporate aperture and spinose exine. The spine was pointed in most specimens of *H. indicum* and *T. indicum* and blunted in only a few specimens. In *T. zeylanicum* the spines were blunted in most specimens, although pointed spines also observed in a few cases. The length of spines showed no much difference between these three species and was in the range of 7.5µm to 11.5µm. The size of pollen grains was also more or less same in all the three species and was in the range of 65µm125µm. The fertility of pollen grains was same in all the three species and varied from locality to locality. In specimens of *H. indicum* the viability of pollen grains was 70 to 78% in localities of DLH, BG, BP, GC and MB; 85 to 88% in localities of MP, FP, MOP, PRA and TK and 91 to 100% in localities of CK, BR, TKP, PNP and RMS

(Table- 2). *T. indicum* and T. zeylanicum also showed a more or less similar pattern of pollen viability (Table- 2 and 3). The present findings gain support from the work of Wael Taha Kasem (2015) [4] who studied a more or less similar pollen characters in seven species of Boraginaceae. The present findings are also in accordance with Ashwini and Baidyanath (2016) [12] who studied a more or less similar pollen characters in three species of Boraginaceae viz. *Heliotropium indicum, Trichodesma indicum* and *T. zeylanicum*.

IV. Discussion

The advantage of pollen shape diversity within Boraginaceae could relate to a type of lock-and-key pollination (Ghorbel and Nabli, 1998; Biggazi and Selvi, 2000; Cohen, 2010) [13, 14, 15]. Biggazi and Selvi (2000) [14] provide evidence that pollen of a particular shape can be captured and retained between stigmatic papillae of a complementary shape. This lock-and-key pollination orients pollen of the correct shape while restricting access and retention of foreign pollen to the stigmatic surface. The extent of the interconnection between pollen and stigma papillae shape has been explored primarily in Borageineae (Ghorbel and Nabli, 1998; Biggazi and Selvi, 2000) [13, 14], but this type of pollination appears to be more widespread in the family, with Cohen (2010) [15] providing evidence of this relationship in Lithospermeae. By placing in a phylogenetic context the shapes of both pollen and stigmatic papillae, it would be possible to identify the number of origins of this lock-and-key pollination and to test if shifts in the shape of one are associated with changes in the other. As with pollen shape, pollen pore number is variable in Boraginaceae and this character is most evolutionarily labile within Borageineae and Lithospermeae. Within these tribes, seven and five transitions, respectively, are resolved for pollen pore number. In Borageineae, most of these transitions are in single species, but in Lithospermeae, shifts in pollen pore number tend to characterize larger clades. In general, pollen pore number in Boraginaceae has increased from three pores to six or greater, a trend observed in other groups as well, such as *Cuscuta* L. (Welsh *et al*., 2010) [16], *Dioscorea* L. and *Sanguisorbeae* (Chung *et al*., 2010). Dajoz *et al*., (1991) [17, 18] and Furness and Rudall (2004) [19] suggest that an increase in pollen pore number may be advantageous because a greater number of pores results in a greater number of germination sites, and therefore a greater probability that at least one of these sites will be in an area favourable for germination. This advantage provides an explanation for the trend of increasing pollen pore number in Boraginaceae. Despite this putative advantage, most species of the family, and many of the more speciose and geographically widespread genera such as *Anchusa* and *Onosma*, bear pollen with three to five functional pores. Dajoz *et al*., (1991) [18] provide evidence that, although pollen with fewer pores may not germinate as quickly as pollen with more pores, pollen with fewer pores is longer lived and tends to produce pollen tubes with an increased growth rate. This helps explain the small number of pores in most species of the family as well as the presence of heterocolpate pollen in Cynoglosseae.

V. Conclusions

The present study is the first to investigate the phylogenetic relationships among different morphological variants of three species Boraginaceae using palynological data. From these phylogenies, it is evident that additional species-level phylogenetic studies should be undertaken on specific clades in which large, widespread genera, such as *Myosotis*, *Cynoglossum*, *Eritrichium*, and *Anchusa*, are resolved as non-monophyletic. Further analyses of these genera and their relatives will help to determine the most appropriate manners in which to circumscribe genera. In future family-level studies of Boraginaceae, it will be important to include more Indian representatives of the family.

Many small genera are endemic to this region, but to date Indian members have been poorly sampled in evolutionary studies of Boraginaceae. Including species from this region will provide critical data on phylogenetic relationships and character evolution, and will allow for a comprehensive re evaluation of the taxonomy of the family, which is overdue. Given the morphological diversity, as well as the patterns of evolution of vegetative, floral, pollen, and fruit features, Boraginaceae can serve as a model for the investigation of various morphological features, including heterostyly, corolla shape and symmetry, inflorescence bracts, leaf venation, pollen shape and pore number, and fruit ornamentation. Future studies can focus further on the examination of the morphology, anatomy, development, genetics, and evolution of these and other variable characters within this diverse family.

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