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Biosystematic Studies in *Heliotropium indicum, Trichodesma indicum and T. zeylanicum* of Boraginaceae

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A B S T R A C T

In the present observation biosystematics of three species of Boraginaceae viz., of *Heliotropium indicum, Trichodesma indicum* and *Trichodesma zeylanicum* was studied. All the three species showed a marked difference in leaf and inflorescence morphology, stomatal behaviour, epidermal trichome, pollen grain characteristics and stem anatomy, hypodromous and brochidodromous venation of their leaves. Leaves were landeolate to ovate. The inflorescence colour was pale blue to white. Length of inflorescence was found to be 4.0 to 6.5. The stomatal Index in these three species varied greatly. *T. indicum* and *T. zeylanicum* showed anomocytic stomata with 16mm in length and 12mm width and Stomatal Index was 34.88 and 44.68 respectively. *Heliotropium indicum* exhibited anisocytic stomata which were 12mm long and 10mm wide. The Stomatal Index was 32.65. All the three species showed the presence of unicellular glandular hairs which differed in forms. All the three species showed tricolpate pollen grains, but differed in their form and size. All the three species showed more or less similar stem anatomical features with differences only in their hypodermal layers. Numerical analysis with reference to morphological, stomatal, pollen grains, trichomes, stem anatomy and stomatal index was carried out for 31 characters. It was found that *Heliotropium indicum* showed similarity with *Trichodesma indicum* in only eight characters and with *Trichodesma zeylanica* in only nine charaters. *Trichodesma indicum* and *T. zeylanicum* showed resemblances in twenty two characters. On the basis of matching coefficient it was observed that *Heliotropium indicum* showed only 25.80% resemblance with *T. indicum*, and 29.03% with *T. zeylanicum.Trichodesma indicum* and *T. zeylanicum* showed 70.96% resemblances, and therefore might be considered as same species of the genus *Trichodesma*.

Introduction

The family Boraginaceae of Dicotyledons includes about 1800 species, and about 141 species are found in India. *Heliotropium indicum.* L, *Trichodesma indicum* (L.) Lehm and *Trichodesma zeylanicum* (Burm.f.) R.Br are common Indian species. *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.

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.

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. Three varieties have been recognized viz., *T. zeylanicum* var. *zeylanicum*, *T. zeylanicum* var. *grandi-florum*, *T. zeylanicum* var. *latisepalum*.

It is reportedly poisonous to stock in Australia (Watt and Breyer- Branwijk, 1962). *Trichodesma zeylanicum* is listed by Holm *et al.* (1979) as a "principal" weed in Kenya and Zimbabwe. Drummond (1984) describes it as a "serious late weed" in Zimbabwe. Wells *et al*. (1986) refers to *Trichodesma zeylanicum* competing and replacing vegetation and being unpalatable. It competes in the latter stages of crop growth. It can dominate river borders, fallow fields, and other disturbed ground and make land preparation difficult. *Trichodesma zeylanicum* is extremely unpleasant to handle because it has bulbous-based spiny hairs that break off in the skin when the plant is handled. It has the ability to establish and spread in the United States and would be a most unwelcome addition to the flora.

Biosystematics is an experimental taxonomic study largely concerned with morphological, anatomical, genetical, cytological, chemical and palynological aspects. This branch of taxonomy is the consideration of the natural relationships among taxa which includes the description, naming and classification of plants together

with studies of their evolution and phylogeny. The application of phytomorphological, anatomical, epidermal, palynological, cytological and biochemical studies in biosystematics can better be appreciated.

The *Heliotropium L.* of Heliotropiaceae Schrad (Boraginaceae Juss.) is considered as a paraphyletic taxon (Chase, 1993 and Ferguson, 1999). Now Heliotropiaceae deal as a separate family (Nadia Diane, *et al*., 2002). *Heliotropium* is selected for its importance and their strong resemblances on the morphological characters. The intrageneric classification of *Heliotropium* into sections has been a debatable matter. De Candolle (1845) divided *Heliotropium* into four sections and excluded the genus *Heliophytum*. Recently Asmaa Olwey (2014) described this genus and divided into seven Sections, Section-1 *Heliotropium*, Section-2 Orthostachys, Section-3 Pleurolasia, Section-4 Pseudocoelomae, Section-5 Pterotropium, Section-6 Ruditotheca and Section-7 Zeylanica. The genus *Heliotropium* comprises 10 species (Alfarhan *et al*. 2005 and Masrahi, 2012). Metcalfe and Chalk (1950) have studied the trichomes and distinguished the different genera and species of Boraginaceae. Leaf epidermal characters are useful in systematic and phylogeny of several plant taxa and can be employed as useful taxonomic characters in segregating the major groups of plants (Mbagwu *et al.*, 2007; Ayodele and Zhou, 2008 and Albert and Sherma, 2013). Leaf anatomy and distribution of foliar trichomes of four species of *Heliotropium* have been investigated by Mona Alwahibi and Najat Bukhary (2013). A comparison between the anatomical feature of leaves and stems of five species of genus *Heliotropium* were carried out by Hoyam Osman and Maha Kordofani (2012). Erdtman (1952) reported the occurrence of tricolpate pollen grain in *H. vellosum* and *H. indicum*. The aim of this study is to evaluate the systematic relationships among three closely related species of Boraginaceae, viz., *Heliotropium indicum*, *Trichodesma indicum* and *Trichodesma zeylanicum* on the basis of resemblances and differences compared to the modern findings of taxonomic relationship.

In the present observation biosystematics of three species of Boraginaceae viz., *Heliotropium indicum*, *Trichodesma indicum* and *Trichodesma zeylanicum* was studied with reference to leaf and inflorescence morphology, stomatal behavior, epidermal trichome, pollen grain characteristics and stem anatomy.

Materials and Methods

Five plant specimens of each of the three species of Boraginaceae, viz., *Heliotropium indicum*, *Trichodesma indicum* and *T. zeylanicum* were collected from different localities of District Gaya (Bihar). The plant specimens were identified following Alfarhan *et al*. (2005) and Masrahi (2012). The epidermis was removed from lower surface of the leaves from each of the sample and was fixed in 20% glycerin. The slides were examined under the light microscope at 10x and 40x.Various anatomical features, such as number and type of stomata, length and width of stomata, trichomes form and types were studied. The stomata size and hairs were recorded with the help of a calibrated eyepiece. The stomatal index (S.I.) was calculated using the formula adopted from Salisbury (1972) that is:

$$SI = \frac{S}{S + E} \times 100$$

Where S donates the number of stomata per unit area and E the number of epidermal cells in the same unit area.

Pollen grains from fresh plants of each of the three species were collected on a slide and acetolyzed. Acetolyzed pollen grains were subjected to microphotography for study. For anatomical studies stem cross sections of each of the three species were prepared using freshly-collected material or material fixed in ethanol with the help of a semirotative microtome to make semi-permanent and permanent slides.

Numerical Analysis

All the examined specimens were analyzed by means of Hierarchical Cluster analysis using Euclidean distance measuring similarity and dissimilarity percent (Matching coefficient). The relationships between the three closely related species of Boraginaceae, viz., *Heliotropium indicum*. *Trichodesma indicum* and *T. zeylanicum* are illustrated as (Matching coefficient).The experiments were carried out in replicates of five and the results obtained have been presented in Tables-1, 2, 3, 4, 5 and 6.

Results and Discussion

The morphological characters of leaves and inflorescence of the three species of Boraginaceae, viz., *Heliotropium indicum, Trichodesma indicum* and *Trichodesma zeylanicum* have been presented in Table-1. From the results it is evident that the shape of leaves was lanceolate in *H. indicum* and *T. indicum*, and ovate in *T. zeylanicum. T. indicum* and *T. zeylanicum* showed reticulate Brochidodromous venation in their leaves while *Heliotropium indicum* showed reticulate Hyphodromous venation. The length and width of leaves showed no significant difference in the three species of Boraginaceae. *Heliotropium indicum* showed Scorpioid coiled cyme where in *T. indicum* and *T. ze*ylanicum it was uncoiled Scorpioid cyme. The colour of inflorescence was pale blue to white in *H. indicum* and white in *T. indicum* and *T. zeylanicum* (Fig. 1, 2 and 3). The inflorescence axis ranged between 5.8 to 8.5 cm in H. indicum and between 4.0 to 8.5 cm in *T. indicum* and *T. zeylanicum*.

The stomatal behavior and stomatal indices of the three species of Boraginaceae have been presented in Table-2 and Fig. 4, 5 and 6. The number of epidermal cells/cm^2 was maximum in *Heliotropium indicum* (33/cm^2) followed by *T. indicum* (28/cm^2) and *T. zeylanicum* (26/cm^2). The number of stomata per square centimeter of leaves was maximum in *T. zeylanicum* (21), followed by *H. indicum* (16) and *T. indicum* (15). In *Heliotropium indicum* the stomata were of anisocytic type whereas in *T indicum* and *T. zeylanicum* the stomata were anomocytic type. The subsidiary cells were Tetracytic in *T. indicum* and *T. zeylanicum* and actinocytic in case of H. inicum. The stomatal Index (SI) was maximum in *T. zeylanicum* (44.68) followed by *T. indicum* (34.88) and *H. indicum* (32.65). The stomatal size showed no great difference between the three species of Boraginaceae.

The trichomes (epidermal hairs) of the three species of Boraginaceae have been depicted in Table-3 and Fig. 7, 8 and 9. In all the three species the trichomes were unicellular and glandular. In *Heliotropium indicum* the trichomes were dense, smooth walled, hollow with oval base. In *T. indicum* and *T. zeylanicum* the trichomes were less dense, rough, filled with globose base.

Pollen grain characteristic for three species of Boraginaceae have been presented in Table-4 and Fig. 10, 11 and 12. In all the

three species the pollen grains were tricolpate. These were elliptical in *H. indicum*, rounded in *T. indicum* and oval in *T. zeylanicum*. Length, width, apertures and P/E ratio showed no much difference between the three species of Boraginaceae.

Table.1 Leaf and Inflorescence Characters of Three Species of Boraginaceae

Characters analysed	Species of Boraginaceae		
	Heliotropium indicum	*Trichodesma indicum*	*Trichodesma zeylanicum*
Leaf shape	Lanceolate	Lanceolate	Ovate
Venation in leaves	Reticulate Hyphodromous	Reticulate Brachidodromous	Reticulate Brachidodromous
Length of leaves in cm	1.7 to 2.5	1.5 to 3.0	2.0 to 3.5
Width of leaves in cm	1.5 to 3.0	2.0 to 3.5	1.5 to 2.6
Inflorescence type	Scorpioid cyme, coiled	Scorpioid cyme, uncoiled	Scorpioid cyme uncoiled
Inflorescence colour	Pale blue to white	White	White
Inflorescence length in cm	5.8 to 8.5	4.5 to 8.5	4.0 to 6.5

Table.2 Stomatal Behavior and Stomatal Indices of Three Species of Boraginaceae

Characters analysed	Species of Boraginaceae		
	Heliotropium indicum	*Trichodesma indicum*	*Trichodesma zeylanicum*
Epidermal cells/cm^2	33	28	26
Number of Stomatal/cm^2	16	15	21
Stomatal type	Anisocytic	Anomocytic	Anomocytic
Subsidiary cells	Actinocytic	tetracytic	Tetracytic
Length of stomata in mm	10.6	12.5	9.0
Width of stomata in mm	5.7	10.3	6.5
Stomatal Index %	32.65	34.88	44.68

Table.3 Trichome Characters of Three Species of Boraginaceae

Characters analysed	Species of Boraginaceae		
	Heliotropium indicum	*Trichodesma indicum*	*Trichodesma zeylanicum*
Glandular hair	Present	Present	Present
Unicellular hair	Present	Present	Present
Hair density	Dense	Less dense	Less dense
Hair wall	Smooth	Rough	Rough
Hair vestibule	Hollow	Filled	Filled
Hair base	Oval	Globose	Globose

Table.4 Pollen Grain Characters of Three Species of Boraginaceae

Characters analysed	Species of Boraginaceae		
	Heliotropium indicum	*Trichodesma indicum*	*Trichodesma zeylanicum*
Length of Pollen in µm	26	22	28
Width of Pollen in µm	19	17	22
Pollen aperture in µm	0.7	0.6	0.5
P/E ratio	0.65	0.85	1.12
Shape of Pollen	Elliptical	Rounded	Oval
Pollen types	Tricolpate	Tricolpate	Tricolpate

Table.5 Stem Anatomical Characters of Three Species of Boraginaceae

Characters analysed	Species of Boraginaceae		
	Heliotropium indicum	*Trichodesma indicum*	*Trichodesma zeylanicum*
Epidermis	Single layered	Single layered	Single layered
Hypodermis	Two layered	Three layered	Three layered
Fascicular and Interfascicular cambium	Present	Present	Present
Cortex	Oval parenchymatous	Oval parenchymatous	Oval parenchymatous
Vessel clusters	Two groups	Three groups	Three groups
Wood porous	Diffuse	Diffuse	Diffuse
Pith cells	Rounded	Oval	Oval

Table.6 Numerical Analysis with Reference to Morphological, Anatomical and Palynological Features

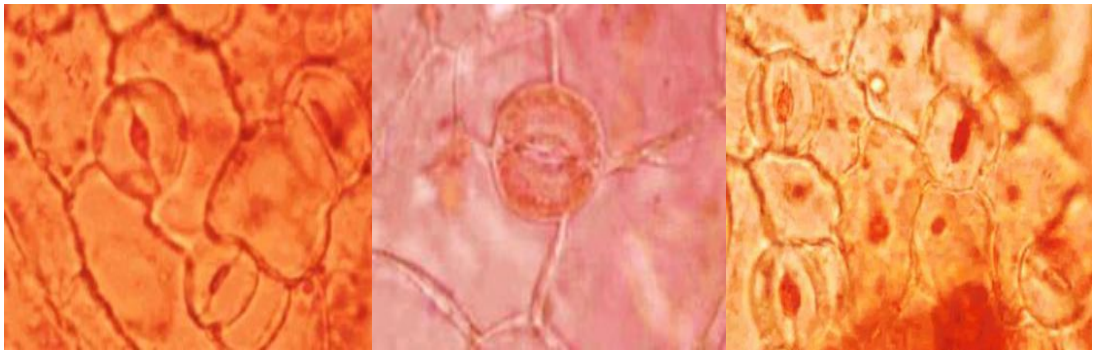
Characters analysed	Species of Boraginaceae		
	Heliotropium indicum	*Trichodesma indicum*	*Trichodesma zeylanicum*
Lanceolate leaf-1; Ovate leaf-2	1	1	2
Hyphodromous venation-1; Brochidodromous venation-2	1	2	2
Scorpioid cyme coiled-1; Scorpioid cyme non-coiled-2	1	2	2
Length of Inflorescence, <5.0cm-1; >5.0-2	2	1	1
Flower colour, White-1; Pale to blue white-2	1	2	2
Anomocytic stomata-1; Anisocytic stomata-2	1	2	2

Stomatal density, Dense-1; Less dense-2	1	2	2
Subsidiary cells, Actinocytic-1; Tetracytic-2	1	2	2
Stomatal length, 7to 10mm-1; >10mm-2	2	2	1
Epidermal cells, Wavy-1; Straight-2	1	1	2
Stomatal Index, 1- ranged from 28-37%; 2- ranged from 38-47%	1	1	2
Glandular hairs, Present-1; Absent-2	1	1	1
Unicellular hair, Present-1; Absent-2	1	1	1
Hair density, Dense-1; Less dense-2	1	2	2
Hair wall, Smooth-1; Rough-2	1	2	2
Hair vestibule, Hollow-1; Filled-2	1	2	2
Colpate pollen-1; Non colpate pollen-2	1	1	1
Shape of pollen, Elliptical-1; Rounded-2; Oval-3	1	2	3
Aperture on pollen, 0.5µm-1; >0.5µm-2	1	2	2
P/E ratio, 1. <1.0; 2. >1.0	1	1	2
Stem epidermis, one layered-1; two layered-2	1	1	1
Hypodermis, one layered-1; two layered-2; three layered-3	2	3	3
Fascicular cambium, present-1; absent-2	1	1	1
Interfascicular cambium, present-1; absent-2	1	1	1
Cortical cells, oval-1; polygonal-2	1	1	2
Vessel clusters, two groups-1; three groups-2	1	2	2
Wood porous, diffuse present-1; absent-2	1	1	1
Pith cells, rounded-1; oval-2	1	1	1

Fig.1 *Heliotropium indicum*, **Fig.2** *Trichodesma indicum* **Fig.3** *Trichodesma zeylanicum*

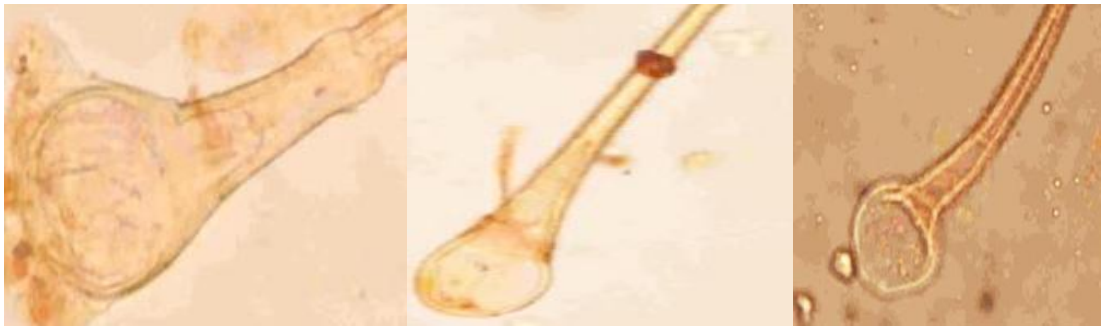
Floral twigs of three species of Boraginaceae

Fig.4 *H. indicum* **Fig.5** *T.indicum* **Fig.6** *T. zeylanicum*



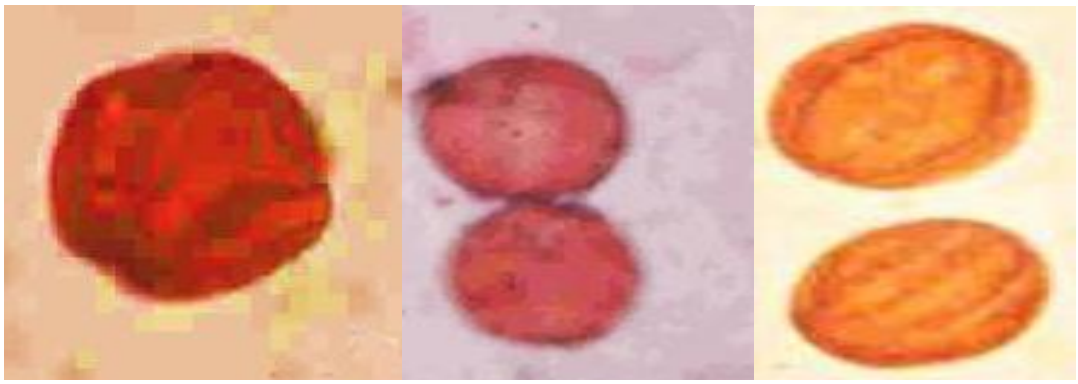
Stomata and Subsidiary cells (X400)

Fig.7 *H. indicum* **Fig.8** *T. indicum* **Fig.9** *T.zeylanicum*



Trichomes of the three species (X100)

Fig.10 *H. indicum* **Fig.11** *T. indicum* **Fig.12** *T. zeylanicum*



Pollengrains of the three species (X1000)

The anatomical features of stem of three species of Boraginaceae showed a typical dicot characters in having conjoint collateral and open vascular bundles (Table-5, Fig. 12, 13 and 14). Epidermis was one layered in all the three species. The hypodermis is two layered in *H. indicum* and three layered in *T. indicum* and *T. zeylanicum*. Fascicular and interfascicular cambia were observed in all the three species. In *H. indicum* vessel clusters were in two groups whereas in *T. indicum* and *T. zeylanicum* these were in groups of three. Wood porous was diffuse in all the three species of Boraginaceae.

Numerical analysis with reference to morphological, stomatal, pollen grains, trichomes, stem anatomy and stomatal index was carried out for 31 characters and has been presented in Table-6. It was found that *Heliotropium indicum* showed similarity with *Trichodesma indicum* in only eight characters and with *Trichodesma zeylanica* in only nine characters. *Trichodesma indicum* and *T. zeylanicum* showed resemblances in twenty two characters. On the basis of matching coefficient it was observed that *Heliotropium indicum* showed only 25.80% resemblance with *T. indicum*, and 29.03% with *T. zeylanicum*. *Trichodesma indicum* and *T. zeylanicum* showed 70.96% resemblances, and therefore might be considered as same species of the genus *Trichodesma*. The present findings gain support from the work Diane *et al*., 2002, Altaf *et al*., 2003, Qureshi, 1985, Wael taha and Kasem, 2015. Wael Taha and Kasem (2015) have studied the anatomical and morphological characters of seven species of *Heliotropium* and found more or less similar results.

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