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Comparative Pollen Studies of the Genera *Lantana, Verbena* and *Vitex* of Family Verbenaceae from Pakistan

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ABSTRACT

Palynological characters not only provide the additional information but are also helpful to cater the systematic position of the species with its respective family. This paper reports the palynological information on nine species of family Verbenaceae from Pakistan. The species were *Lantana indica, L. camara, Verbena bonariensis*, *Ver. officinalis, Ver. bipinnatifida, Ver. tenuisecta, Ver. hybrida, Vitex trifolia* and *V. negundo.* In this context comparative pollen analysis was based on type of pollen, shape in polar and equatorial view, polar and equatorial diameter and polar: equatorial (P: E) ratio, length and width of colpi, exine thickness and sculpturing of pollen. The largest polar diameter among these three genera was observed in *Ver. hybrida* (44.75 µm) and smallest *in V. negundo* (16 µm). The largest equatorial diameter was noted in *Ver. hybrida* (39.75 µm) and smallest in *V. negundo* (18.5 µm). Largest P: E ratio was noted in *L. indica* (1.285 µm), while smallest in V*itex trifolia* (0.789 µm). Largest colpi length (7.5 µm) was observed in *Ver. bipinnatifida* and *Ver officinalis* and smallest (4.6 µm) in *L. camara.* Greater polar views were observed in the genera *Lantana* and *Verbena,* while equatorial views were greater in *Vitex*. Almost all species of these genera possessed differences in the shape of either equatorial or polar views. Pollen fertility estimation ranged from 56 - 98.58%, which showed that pollen flora of selected species is well established. These data provides useful information for taxonomists and other scientist working about the delineation of species belonging to *Lantana*, *Verbena* and *Vitex* genera.

Key Words: Pollen characteristics; *Lantana; Verbena; Vitex;* Verbenaceae

INTRODUCTION

Flora of Pakistan is highly diverse. Of nearly six thousand species of flowering plants reported to be occurring in Pakistan and Kashmir, a very large number is found in the Northern and North Western parts of Pakistan. Ten percent of the flora is Sino-Himalayan. As reported by Ali and Qaiser (1986) and Nasir and Rafiq (1995), there is no clear cut boundary and thus flora of each region intermingle. Major floristic zones are four, including Saharo-Sindian, Irano-Turanian, Sino-Japanese and Indian.

The family Verbenaceae is mainly distributed in the tropics and subtropics of the southern hemisphere. Genera *Lantana, Verbena and Vitex* are commonly found in Pakistan in the sub-Himalayan tract, plains of Baluchistan, North Waziristan, Sulaiman mountains, Kurram, NWFP, Kohat, Jehlum, Mirpur in Kashmir, lower Sawt, Rawalpindi and adjoining areas (Jafri & Ghafoor, 1972). In Pakistan the common species of these genera are *Lantana indica, L. camara, Verbena bonariensis*, *Ver. officinalis syn: Ver. tenuispicata, Ver. bipinnatifida, Ver. tenuisecta, Ver. hybrida, Vitex trifolia* and *V. negundo.* This family includes many ornamental and medicinal plants in addition to teak, which is highly prized for its wood (Jafri & Gafoor, 2005). *L. camara* is cultivated as ornamental plant and its decoction is given in tetnus, rheumatism and malaria. It is also used in atoxy of abdominal viscera. A strong decoction of the leaves is taken internally and a poultice of crushed leaves is applied to the wound. The secretion of *L. camara* leaves have remarkable ecological actions against herbivores and pathogens, as well as playing important role in attraction of pollinators and fruit and seed dispersal (Fahn, 1979; Gottlieb & Salatino, 1987; Gershenzon & Croteau, 1991; Pare & Tumlinson, 1999). The leaves of *L. alba* are used to cure the snake bite (Vashista, 1972). *Vitex* yields good timber many are cultivated as ornamentals such as *V. trifolia*, and *V. negundo*. The dried leaves of *V. negundo* are smoked like cigarette for relieving headache and the leaves are chewed to reduce toothache and to relieve labour pain (Farrukh *et al*., 2006). *Ver. hybrida* is commonly cultivated as garden ornamental. The *Ver. officinalis* is useful in nerve complaints, the root of this species is considered as a remedy for Scrofula. In present study an attempt was to further distinguish nine species of the family Verbenaceae based on palynological characters in order to strengthen the recognition.

MATERIALS AND METHODS

Sampling and pollen preparations. For pollen morphology, dried pollen material from herbarium specimens was first kept in acetic acid for softening and

then used to prepare slides by using glycerin-jelly mixed with 1% safranin. Glycerin-jelly was prepared by dissolving 120 g of gelatin in 500 mL of water. After this operation 1 g of thymol and 225 g of glycerol was added and mixed. At the end 50 mg of safranin carefully weighed was added. The volume of the solution was made to 1 L by adding more distilled water. The whole solution was boiled, while continuous stirring. The boiling was stopped when all the materials were properly dissolved. This fluid was used for staining the pollens (Zafar *et al.*, 2006).

Specimens from Quaid-i-Azam University (QAU) Herbarium Islamabad were taken and pollen preparations made by the acetolysis method from mature un-opened buds. For light microscope study, according to the procedure outlined by Erdtman (1966), with the help of dissecting needles the anthers were removed from the filaments and placed on a glass slide along with few drops of acetic acid then crushed to release pollen grains on the slide. Anther wall material was discarded, while excess of water was removed with filter paper. Pollen grains were stained with 1% safranine mixed in Glycerin jelly any bubbles formed was carefully removed by slightly heating the slide; cover slip was placed on it. When cooled the glass slide was labeled and the edges of the cover slip were sealed with transparent nail varnish. The prepared slides were studied under the light microscope. Their photographs were taken with the Nickon FX-35 Camera equipped with photomicrograph system (Japan).

Pollen fertility investigation. Inflorescence were taken along with anthers and dissected. Mature anther selected was squashed in a drop of acetocarmine. Debris was removed gently and a cover slip was placed over the stain. The slides were observed at low magnification. The number of stained and un-stained pollen grains was tabulated. Fully stained pollen grains were considered fertile, while deformed and un-stained ones considered sterile, the percentage of fertile pollen grains was calculated.

RESULTS

Palynological studies of the Genera *Lantana*, *Verbena* and *Vitex* have been carried out to find pollen morphological characters to be utilized for Taxonomic purpose. Pollen morphology of these genera were described. Pollen features and their fertility estimates for the genera *Lantana, Verbena* and *Vitex* are presented in Table I and II. Different views of pollen are given in Fig. A to S.

DISCUSSION

Like other disciplines pollen grains have an important part in the modern issue of plant taxonomy (Bashir & Khan, 2003). Pollen morphology can be useful in supporting the taxonomic suggestions (Clarke *et al.,* 1980). Tomsovic (1997) utilized pollen characters as additional information for systematic studies. Huang (1972) also used pollen characters for systematic purposes.

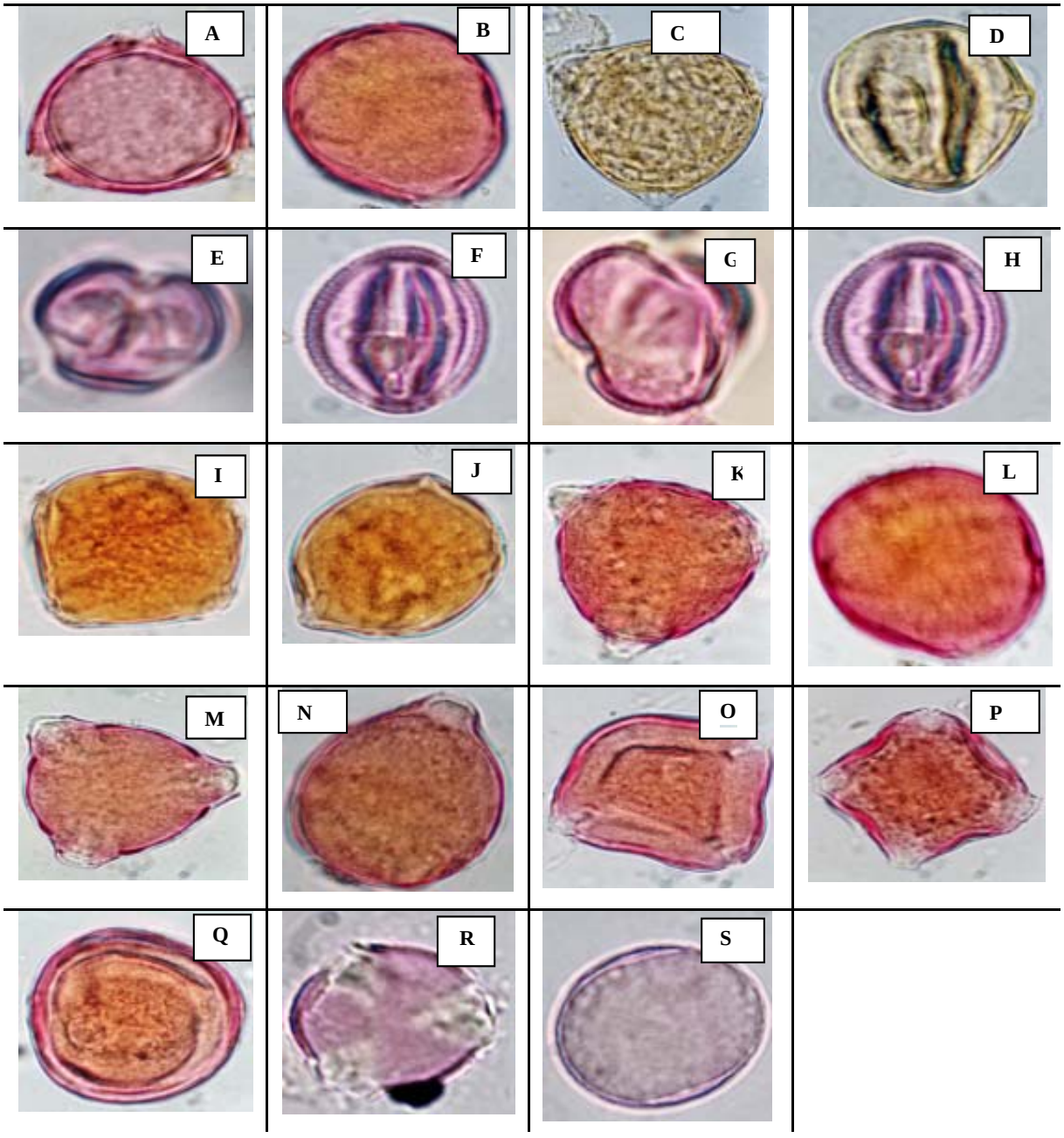
The palynological studies of *Lantana camara, L. indica*, *Verbena officinalis* and *V. tenuisecta* from Pakistan were carried out by Zafar *et al.* (2006). The study of pollen morphology has gained great significance in Plant taxonomy and the advancements in microscopy have led to the effective use of new pollen morphological parameters for the taxonomic purpose.

This research work was conducted to examine the value of palynology in the systematics of *Lantana*, *Verbena* and *Vitex*. The present account is based upon the light microscope techniques; therefore, the sculpturing observed in study was in *L. camara* smooth, in *L. indica* reticulate, in *Ver. bonariensis* reticulate, in *V. hybrida, V. officinalis* and *V. bipinnatifida* granular, in *V. negundo* smooth and in *V. trifolia* granular. For structures and pattern describing the different characters terminology followed was that of Erdtman (1969).

With the help of palynology, considering the entire characters, one can discriminate between morphologically same genera, (Uzma & Khan, 1998). Though *Verbena bipinnatifida* and *V. tenuisecta* have great similarity in their morphological characters but with the help of palynological studies the discrimination between the two was observed. The shape of *Verbena bipinnatifida* (Fig. M - N) was sub-angular and sub-prolate in polar and equatorial views respectively, while *V. tenuisecta* (Fig. R - S) was with Semi-angular and Sub-prolate shape in polar and equatorial views. There is a great difference even in size of both as *V. bipinnatifida* has 42 µm in polar and 33 µm in equatorial view, exine thickness was 3.75 µm and length and width of colpi was 7.5µm and 10 µm, respectively whereas in *V. tenuisecta* has 28.75 µm in polar and 23.4 in equatorial view, exine thickness was 1.75 µm, length and width of colpi were 13.5 µm and 8.5 m, respectively (Table I). The colpi and pores were prominent in *V. bipinnatifida* and surface is granular.

Interesting findings were observed in pollen morphology of family Verbenaceae. All the species comprised tricolporate pollen, while *V. officinalis* (Fig. O - Q) had tricolporate as well as tetracolporate pollen that distinguishes it from the rest of the species. This showed that evolutionary process of pore diversity is occurring in the genus. *V. bonariensis* (Fig. I - J) has all the tetracolporate pollen. The largest polar diameter among these three genera was observed in *Ver. hybrida* (Fig. K - L) i.e., 44.75 µm and smallest was *in V. negundo* (Fig. E - F) that was 16 µm. The largest equatorial diameter observed was 39.75 µm in *Ver. hybrida* and smallest was 18.5 µm in *V. negundo*. Larger P/E ratio was 1.285 µm in *L. indica* (Fig. C - D), smaller was 0.789 µm *V. trifolia* (Fig. G - H). Larger colpi length was 7.5 µm in *Ver. bipinnatifida* and *Ver. officinalis* (Table I) and smaller (4.6 µm) in *L. camara* during the study the polar views were observed more in *Lantana* and *Verbena,* while in *Vitex* equatorial views were observed frequently and polar views were somewhat rare.

Fig. A- *Lantana camara* (Polar view -1000x), B- *Lantana camara* (Equatorial view - 1000x), C- *Lantana indica* (Polar view – 1000x), D- *Lantana indica* (Equatorial view – 1000x), E- *Vitex negundo* (Polar view – 1000x), F- *Vitex negundo* (Euatoiral view – 1000x), G- *Vitex trifolia* (Polar view -1000x), H- *Vitex trifolia* (Equatorial view - 1000x), I- *Verbena bonariensis* (Polar view – 1000x), J- *Verbena bonariensis* (Equatorial view – 1000x), K- *Verbena hybrida* (Polar view – 100x), L- *Verbena hybrida* (Equatorial view – 1000x), M- *Verbena bipinnatifida* (Polar view -1000x), N- *Verbena bipinnatifida* (Equatorial view - 1000x), O-P *Verbena officinalis* (Polar view – 1000x), Q- *Verbena officinalis* (Equatoral view – 1000x), R- *Verbena tenuisecta* (Polar view – 1000x), S- *Verbena tenuisecta* (Equatorial view – 1000x)



Generally almost all the species of these three genera studied posses a difference either in the shape of equatorial or polar views.

The palynological characters not only provide the additional information but are also helpful to cater the systematic position of the species with its respective family. It can be concluded that not only the general morphology but also the pollen morphology is of significance in species delimitation (Wodehouse, 1935). Not only the general morphology but also pollen morphology was of significance in species delimitation and pollen characters are correlated with morphological features (Qureshi *et al.*, 2002).

Table I. Comparative pollen characteristics of three genera *Lantana, Verbena* and *Vitex* of family Verbenaceae

Species	Type	Shape in Polar view	Shape in Equatorial view	Polar diameter	Equatorial diameter	P/E ratio	Length of colpi	Width of colpi	Exine thickness	Sculpturing
Lantana camara	Tricolporate	Semi-angular	Spheroidal	34µm (30-40µm)	33µm (30-40µm)	1.030 µm	4.6 µm	6.75 µm	3.75µm	Smooth
L. indica	Tricolporate	Semi-angular	Sub-prolate	39.5µm (35-47.5µm)	30.75µm (27.5-33.75µm)	1.285 µm	5.625 µm	6.25 µm	2.5 µm	Reticulate
Vitex negundo	Tricolporate	Circular-lobate	Prolate spheroidal	16µm(12.5-20µm)	18.5µm (15-22.5µm)	0.865 µm	6.25 µm	4.375 µm	3.75 µm	Smooth
V. trifolia	Tricolporate	Circular-lobate	Perprolate	18.75µm (17.5-20µm)	23.75µm (22.5-25µm)	0.789 µm	-	-	1.25µm	Granular
Verbena bonariensis	Tricolporate, Tetra-colporate	Rect-angular, circular	Sub-prolate	37.5µm (32.5-40µm)	32µm (25-35µm)	1.172 µm	7.5 µm	7.5 µm	5µm	Reticulate
Ver. hybrida	Tri-colporate	Angular	Sub-prolate	44.75µm (40-52µm)	39.75µm (33.75-47.5µm)	1.112 µm	13.75 µm	6.25 µm	7.5µm	Granular
Ver. bipinnatifida	Tricolporate	Sub-angular	Sub-prolate	42µm (37.5-50µm)	33µm (26.25-40µm)	1.273 µm	7.5 µm	10 µm	3.75µm	Granular
Ver. officinalis	Tricolporate & tetra-colporate	Semi-angular & rectangular	Sub-prolate	36.75µm (30-42µm)	36.75µm (35-40µm)	1 µm	7.5 µm	5 µm	3.75µm	Granular
Ver. tenuisecta	Tricolporate	Semi-angular	Sub-prolate	28.75µm (25-30µm)	23.4µm (20-25µm)	1.229 µm	13.75 µm	7.5 µm	2.5µm	Reticulate

Table II. Percentage of pollen fertility estimation of *Lantana, Verbena* and *Vitex* (Verbenaceae)

No	Species	Fertile pollen	Sterile pollen	%fertility
1	*L. camara*	144	28	83.72
2	*L. indica*	172	56	98
3	*Ver. bonariensis*	52	14	78.79
4	*Ver. hybrida*	22	17	56
5	*Ver. bipinnatifida*	133	33	80.12
6	*Ver. officinalis*	134	52	72.04
7	*Ver. tenuisecta*	81	4	94
8	*V. negundo*	136	2	98.55
9	*V. trifolia*	489	7	98.58

Pollen fertility investigation is an important feature of palynology and shows the fertility status of plants, which indicates the viability of pollen grains to develop into male gametophyte to continue their generation through fertilization. It may indicate the adaptability of pollen grains to the environment and it is also suggested that their ploidy level may exist due to higher level of pollen fertility (Awan *et al.,* 2001) pollen fertility test are currently used to an advantage in the interpretation of cytological situation in the plants and are of particular importance to draw attention towards the fact that studies involving hybrids and their parents have provided interesting data (Ravi, 1979).

Pollen fertility rate observed in *L. camara* was 83.72, while it was 94.91 by Zafar *et al.* (2006) for *L. indica,* it was 98 same as that of Zafar *et al.* (2006) for *Ver. officinalis* it was 72.04, while that of Zafar *et al.* was 96.11, *V. tenuisecta* was 94, same as that of Zafar *et al.* (2006). The pollen fertility rate of *V. bipinnatifida* was 80.12, *V. hybrida* 56, *V. negundo* was 98.55 and *V. trifolia* was 98.58. These results showed that the well established flora among these three genera was *V. negundo* (Table II) with high pollen fertility rate in Pakistan. Pollen fertility is valuable for the taxonomists in attempting to distinguish putative hybrids from the parent plants and is also useful to determine the degree of fertility/stain ability in those plants that are grown under un-favorable conditions (Lawrence, 1969).

In conclusion, the application of pollen morphology to plant systematics is comparatively a recent trend. The pollen characters have often been in agreement with the conclusions drawn from other fields of study, particularly Anatomy, Morphology and Cytology. The taxonomic and evolutionary importance of pollen morphology may be at specific, generic or higher levels.

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