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Pollen morphology of selected species of Scrophulariaceae of District Dir Upper, Pakistan

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The present investigation is carried out to illustrate and assess the taxonomic significance of Palynological features of 9 species belonging to 6 genera of the family Scrophulariaceae collected from District Dir Upper, Pakistan. Pollen grains are usually radially symmetrical, isopolar, oblate-spheroidal or prolate-spheroidal or sub-prolate, tricolporate and psilate, except *Pedicularis oederi* which has bisyncolpate pollen. Pollen characters such as size, shape, colpi and exine thickness, and P/E ratio are found considerably important for systematic utilization. Pollen fertility estimation ranged from 70 to 98%, which shows that pollen flora of selected species is well established.

Key words: Scrophulariaceae, palynology, Polar/Equatorial ratio (P/E), pollen fertility.

INTRODUCTION

Dir Upper is one of the districts of Khyber Pakhtunkhwa, Pakistan. The district lies between 35°-04' to 35°-46' North Latitudes and 71°-32' to 72°-22' East longitudes, taking its name from the village of Dir, the headquarter of the former rulers. It shares boundaries with Chitral District in North, with District Swat in East, with Lower Dir District in South while in the West it adjoins Bajaur agency. The total area of the district is 3,699 km² while the population according to the census of 1998 was 575,858. The district comprises of 2 main subdivisions; Dir and Wari (Anon, 1998).

Scrophulariaceae is a large cosmopolitan family (Jussieu, 1789) with about 222 genera and 4480 species (Willis, 1973) and are mainly distributed throughout the temperate areas of the northern hemisphere (Heywood, 1985). Members are mostly herbaceous with few shrubs and climbers. The characteristics of a typical scroph include; bilaterally symmetrical mostly tubular flowers, ovaries with axile placentation and numerous ovules, capsular fruits, and seeds with endosperm, each are shared with one or several related families (Richard et al., 2001).

Scrophulariaceae is an eurypalynous family (Erdtman, 1952). The pollen morphological characters of the family were previously examined by many researchers all over the world. A pollen-morphological study of the whole family Scrophulariaceae has been conducted by Varghese (1968). Saeidi and Zarrei (2006) studied the pollen morphology of 17 species of the genus *Veronica* (Scrophulariaceae) in Iran. The earlier works report data of pollen morphological characters of genus *Veronica* (Hong, 1984; Fernandez et al., 1997). The palynology of some *Pedicularis* species has already been investigated. Risch (1939), Belkina (1972), Dutta and Chanda (1979) and others have provided some palynological data from certain German, Russian and Indian members of the genus *Pedicularis* respectively. Tsoong and Chang (1965) studied the pollen morphology of 193 Chinese species and its systematic implications. Similarly, Wang et al. (2003) investigate the pollen morphology of Chinese species of *Pedicularis* (Scrophulariaceae). Scrophulariaceae have been examined with light and electron microscopy (Jensen et al., 1974; Niezgodna and Tomb, 1975; Argue, 1981, 1982; Inceoglu, 1982; Hong, 1984). Although a lot of work had already been done on pollen morphology of various members of the family but about 70% species do not overlap with previous researches, for example, Dutta and Chanda (1979),

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Table 1. Quantitative analysis of the pollen morphology.

S/N	Name of species	Polar diameter (µm)	Equatorial diameter (µm)	P/E ratio	Length of colpi (µm)	Width of colpi (µm)	Exine thickness (µm)
1	<i>Kickxia incana</i>	16.25 (15-17.5)	25 (30-35)	0.65	6.25 (5-7.5)	2.75	1.87
2	<i>Kickxia ramosissima</i>	28.75 (25-32.5)	32.5 (30-35)	0.88	2.5	0.625	3 (2.5-3.75)
3	<i>Pedicularis oederi</i>	35 (27.5-40)	34.16 (27.5-40)	1.02	1.25	0.625	1.87
4	<i>Scrophularia robusta</i>	20 (17.5-22.5)	23.75 (22.5-25)	0.84	3.75	3.75	2.12
5	<i>Scrophularia scabiosaefolia</i>	24.37 (20-30)	23.12 (22.5-23.75)	1.05	3.12	0.625	1.56 (1.25-1.87)
6	<i>Verbascum thapsus</i>	23.75 (22.5-25)	25	0.95	5.62 (5-6.25)	3.37 (2.12-4.62)	1.75 (1.25-2.12)
7	<i>Veronica anagalis-aquatica</i>	25 (15-32.5)	21 (19-23)	1.2	3	1	2.12
8	<i>Veronica melissifolia</i>	34.5 (27.5-42.5)	25	1.38	9.37 (8.75-10)	2.43 (2.75-2.12)	2.31 (2.12-2.5)
9	<i>Veronica persica</i>	25.83 (20-35)	27.5	0.93	2.5	1.25	2.56 (2.5-3.12)

Saeidi and Zarrei (2006) etc.

The main goal of the research was to investigate constant and diagnostic pollen characteristics and to utilize such data for identification, classification and further authentication of the taxa. The study also aimed to evaluate the total variations among the taxa at different levels of taxonomic hierarchy and to further aid in the classification and phylogenetic relationship of the concerned taxa.

METHODOLOGY

The studies were conducted during 2010 to 2011 in Experimental Taxonomy Laboratory of Quaid-i-Azam University of Islamabad. Pollen studies were confined to pollen morphology and fertility estimation of 9 species of family Scrophulariaceae. Pollen material of dried herbarium specimens collected from Quaid-i-Azam University Herbarium (ISL), Islamabad were used.

The pollen grains were prepared for acetolysis by the modified procedure of Ronald (2000). Glycerin jelly was prepared according to modified method of Ahmad et al. (2008). Pollen characters such as type, shape in polar and equatorial view, polar and equatorial diameter, P/E ratio, length and width of colpi and exine thickness of selected species were studied (Table 1). Microphotographs of pollen

were taken by using Nikon Labophot light microscope fitted with camera by using oil immersion (Figures 1 and 2).

Pollen fertility estimation

Percentage of pollen fertility was calculated on the basis of staining by glycerin jelly. Pollen fertility estimation was confined to viability test of 9 species of Scrophulariaceae. Data on botanical name, sterile pollen count, fertile pollen count and percentage fertility estimation is presented in Table 3.

RESULTS AND DISCUSSION

The results of the present work reveal the utility of the palynological characters for distinguishing the examined genera in Scrophulariaceae. The results clearly demonstrate that palynological characters are useful in recognition of taxa at specific level and agreed with all the previous researches which offer similar results concerning shape, apertures and sculpturing of pollen grains. The present study showed the usefulness of both qualitative and quantitative characters in taxonomic studies.

In this study, an account was made, which was impounded to the detailed palynological studies of both pollen morphology and fertility estimation of 5 genera and 9 species of family Scrophulariaceae. Scrophulariaceae is a eurypalynous family (Erdtman, 1952) and most of its pollen are radially symmetrical, isopolar, oblate-spheroidal or sub-spheroidal or sub-prolate, tricolporate rarely tetracolporate (Perveen, 1993). The present study showed that there is a great diversity in pollen morphology of Scrophulariaceae. The variation is mostly found in size, shape and exine thickness. Maximum pollen size that is, 35 µm was found in *Pedicularis oederi* in polar view and minimum pollen size that is, 16.25 µm in polar view was observed in *Kickxia incana*. Whereas maximum pollen size in equatorial view was 34.16 µm in *P. oederi* and minimum was 21 µm in *Veronica anagalis-aquatica* (Saeidi and Zarrei, 2006). The highest colpi length was 9.37 µm in *Veronica melissifolia*, where as smallest in colpi length was observed in *P. oederi* that was 1.25 µm (Table 1).

A clearly defined set of correlated pollen characters was shared among these genera. Pollen shape in polar view was circular in almost

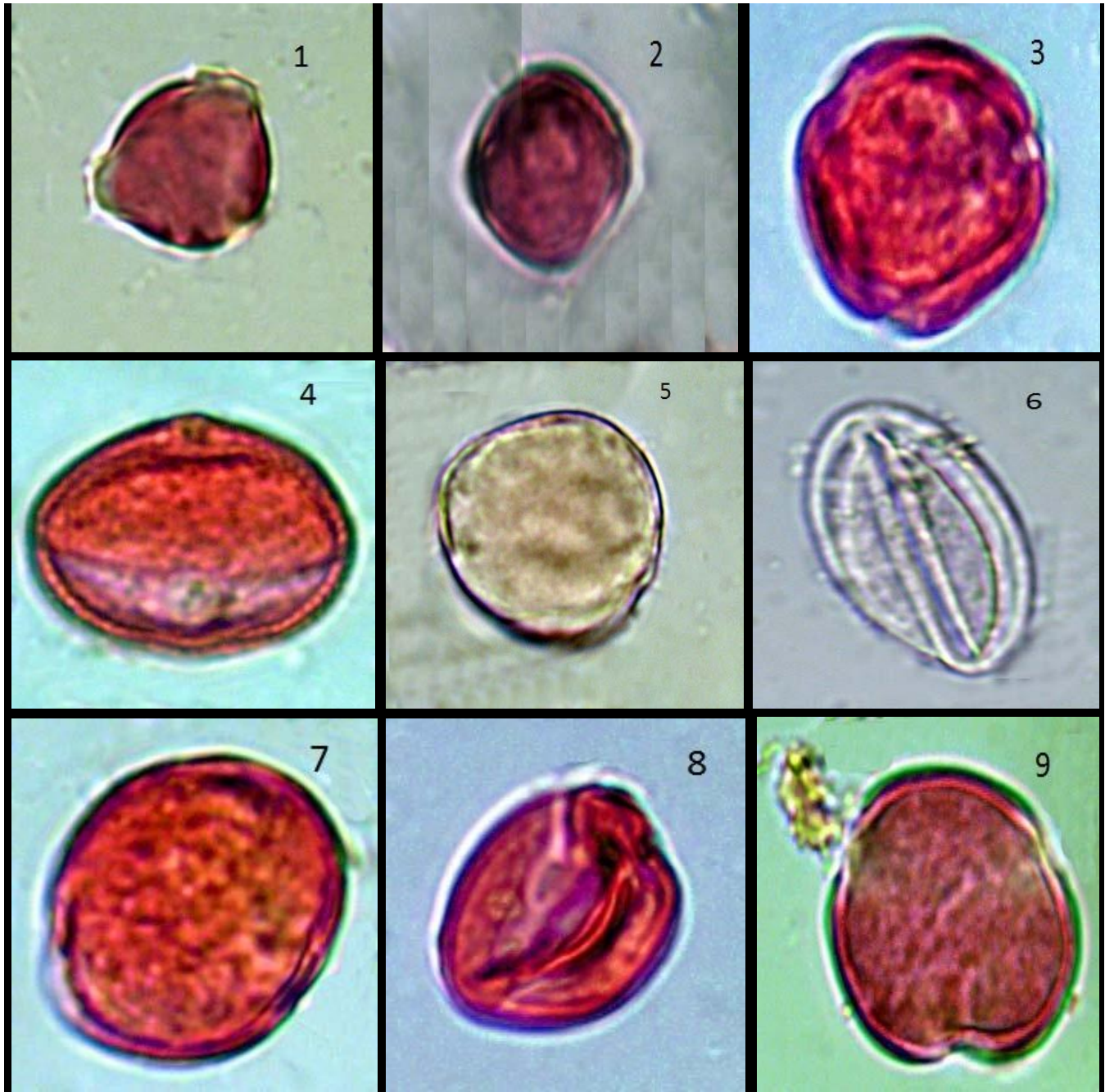


Figure 1. Micro photographs of pollen (40X). 1: Polar view of *Kickxia incana*. 2: Equitorial view of *Kickxia incana*. 3: Polar view of *Kickxia ramossisima*. 4: Equitorial view of *Kickxia ramossisima*. 5: Polar view of *Pedicularis oederi*. 6: Equitorial view of *Pedicularis oederi*. 7: Polar view of *Scrophularia robusta*. 8: Equitorial view of *Scrophularia robusta*. 9: Polar view of *Scrophularia scabiosaefolia*.

all species except *Kickxia ramossisima* and *V. mellisifolia* being circular to semiangular while the shape of pollen in equatorial view varied between different species even among the same genera. In *K. incana* it was oblate, while in *P. oederi* and *Scrophularia scabiosaefolia* the shape was prolate-spheroidal. The pollen of *Verbascum thapsus* and *Veronica persica* were oblate-spheroidal. On the other hand the pollen of *V. anagalis-aquatica* and *V.*

mellisifolia were sub-prolate and prolate respectively, while rest of the species were sub-oblate. The shared pollen type had tricolporate apertures with psilate colpus membranes except *P. oederi* which was bisyncolporate (Table 2).

Parveen (1993) gave a rough idea that Scrophulariaceae do not show much pollen diversity. Pollen are mostly tricolporate. However, sufficient

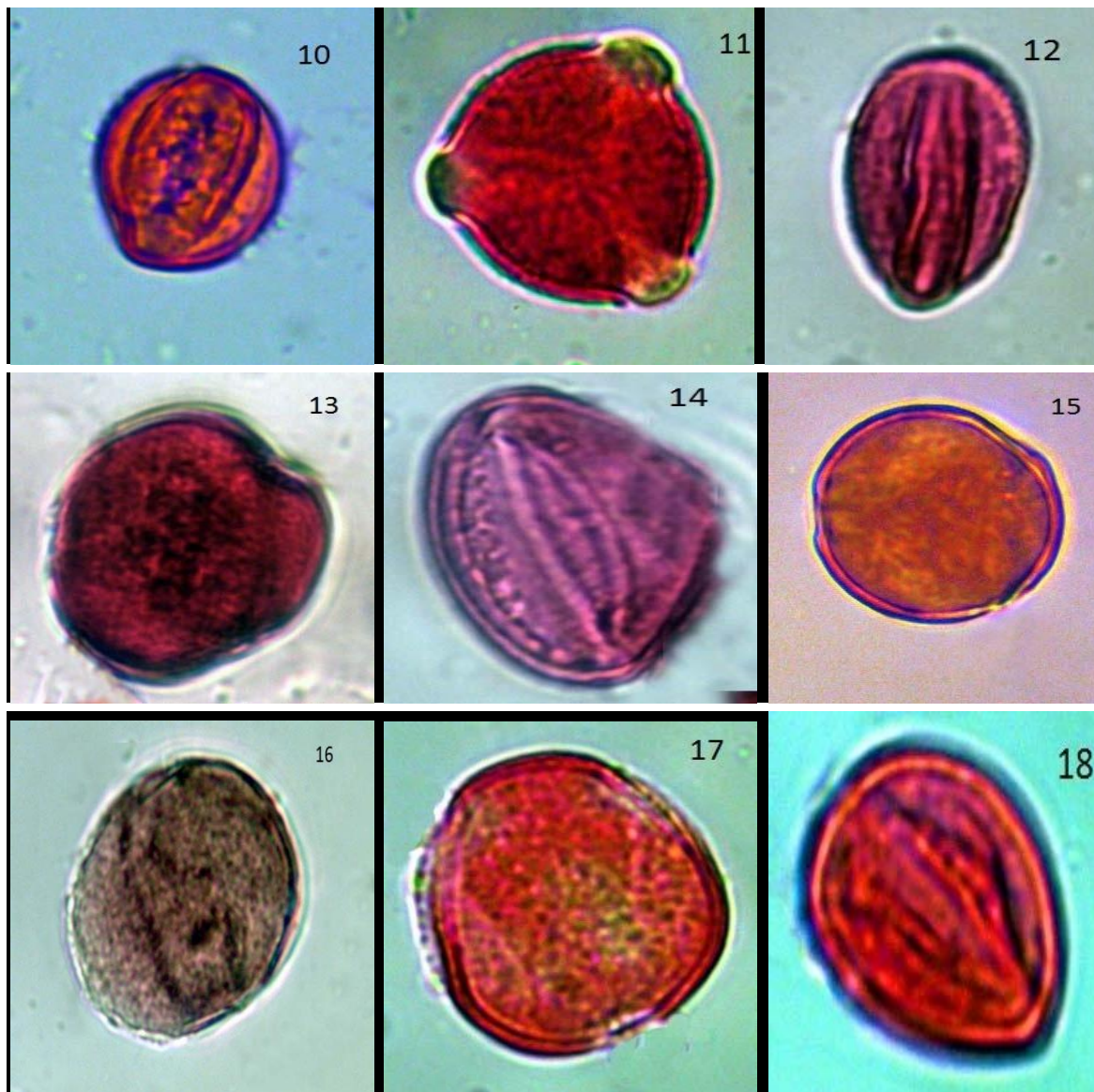


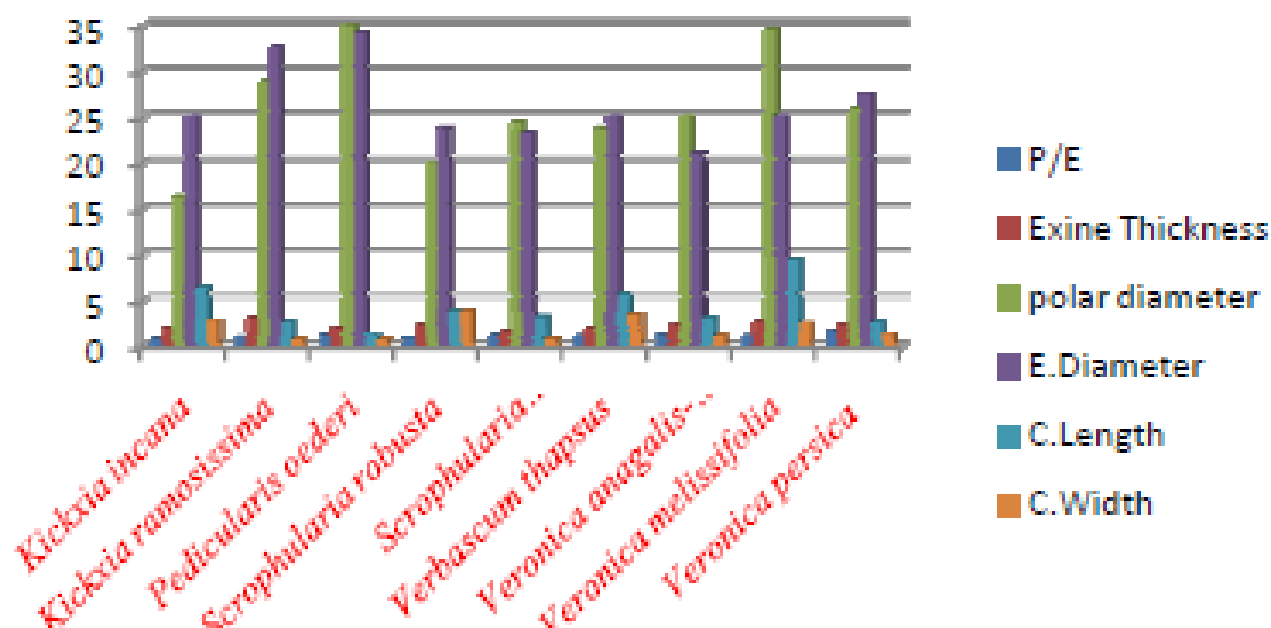
Figure 2. Micro photographs of pollen (40X). 10: Equatorial view of *Scrophularia scabiosaefolia*. 11: Polar view of *Verbascum thapsus*. 12: Equatorial view of *Verbascum thapsus*. 13: Polar view of *Veronica anagallis-aquatica*. 14: Equatorial view of *Veronica anagallis-aquatica*. 15: Polar view of *Veronica mellisifolia*. 16: Equatorial view of *Veronica mellisifolia*. 17: Polar view of *Veronica persica*. 18: Equatorial view of *Veronica persica*.

Table 2. Qualitative analysis of pollen morphology.

S/N	Name of species	Shape in Polar view	Shape in equitorial view	Type	Sculpturing
1	<i>Kickxia incana</i>	Circular	Oblate	Tricolporate	Psilate
2	<i>Kickxia ramosissima</i>	Semi angular	Sub-oblate	Tricolporate	Psilate
3	<i>Pedicularis oederi</i>	Circular	Prolate-spheroidal	Bisyncolpate	Psilate
4	<i>Scrophularia robusta</i>	Circular	Sub-oblate	Tricolporate	Psilate
5	<i>Scrophularia scabiosaefolia</i>	Circular	Prolate-spheroidal	Tricolporate	Psilate
6	<i>Verbascum Thapsus</i>	Circular	Oblate-spheroidal	Tricolporate	Psilate
7	<i>Veronica anagallis-aquatica</i>	Circular	Sub-prolate	Tricolporate	Psilate
8	<i>Veronica persica</i>	Circular	Oblate-spheroidal	Tricolporate	Psilate
9	<i>Veronica mellisifolia</i>	Semi angular	Prolate	Tricolporate	Psilate

Table 3. Percentage pollen fertility estimation.

S/N	Name of species	Sterile pollen	Fertile pollen	Fertility (%)
1	<i>Kickxia incana</i>	1	5	83.4
2	<i>Kickxia ramosissima</i>	6	15	71.5
3	<i>Pedicularis oederi</i>	2	26	92.8
4	<i>Scrophularia robusta</i>	30	150	83.3
5	<i>Scrophularia scabiosaefolia</i>	20	150	88.2
6	<i>Verbascum thapsus</i>	1	10	90.9
7	<i>Veronica anagalis-aquatica</i>	1	50	98
8	<i>Veronica persica</i>	8	22	73.3
9	<i>Veronica melissifolia</i>	5	25	83.3

**Figure 3.** Graphical representation of quantitative results.

variations are observed within the shape, polar, equatorial diameter and exine thickness by which these genera can be separated (Figure 3). Our results regarding palynology of veronica are in accordance with the findings of Saeidi and Zarrei (2006) and Bigazzi and Tardelli (1990) that used colpus shape, colpus membrane together with three subtypes based on grain shape as distinguishing taxonomic characters in palynology of family Scrophulariaceae.

Pollen fertility is helpful for the taxonomists in order to distinguish the presumed hybrids from the parent plants and is also useful to determine the degree of fecundity in those plants which are grown under unfavorable conditions (Lawrence, 1951). Pollen fertility of the selected species ranged from 70 to 98%. It indicates that the flora of Dir is well established and the species are somewhat stable in the area.

Conclusion

This data could provide sufficient information that supports the relation character between the species. The data derived from palynological characters of the examined species could also contribute to the taxonomy of the family Scrophulariaceae. These studies will be supportive for assembling such work and identifying the flora according to their pollen morphology and fertility for broader circulation.

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