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Palynological Study of the Genus *Sonchus* from Pakistan

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Abstract: Pollen morphology of four different species, *Sonchus uliginosus, S. arvensis, S. asper, S. maritimus, S. oleraceous & S. palustris,* belonging to genus *Sonchus* of family Asteraceae was studied from Pakistan. Morphology of pollen grains of each of the species is based on specimens selected at random. Proposed characters i.e. grain class, shape of pollen grain, equatorial view, polar view, equatorial diameter (E), polar diameter (P), P/E ratio, length of colpus, exine surface, exine thickness, inter poral distance, inter spinal distance, inter spinal outline, length of spines, number of spines between colpi in each species were recorded for comparison. At species level, micromorphological differences and distribution of surface pattern, shape and size of pollen have been found to exist. The pollen grain are tetrazonocolporate. Maximum spine length, exine thickness, colpus length was observed in *Sonchus uliginosus*. *Sonchus oleraceous* has studies in distinguishing some taxonomic groups in the Asteraceae.

Key words: *Sonchus*, palynology, Asteraceae, pollen grain

Introduction

Pollen morphology of the Lactuceae (Cichorieae) is probably the more distinctive tribe in the family Asteraceae. The ligulate corolla, milky sap and echinolophate pollen from a unique combination of characters which it can be readily distinguished from the rest. This tribe consists of about 70 genera and 2300 species (Tomb, 1977). Stebbins (1953) proposed a natural system of classification for this tribe using geographical distribution, pollen morphology and chromosomal data in addition to traditional morphological characters. This method produced eight subtribes.
(I)Scolyminae, (ii)Cichorinae, (iii)Microseridinae, (iv)Stephanomeriinae, (v) Dendroseiidinae, (vi) Scoronerinae, (vii)Leontodontinae and (viii) Crepidinae. Jeffrey (1966) revised Stebbin's classification recognizing the importance of microcharacters like length of collector hairs on the style, shapes of hairs on stigmatic surfaces ad pubescence on the corolla tube. He divided this tribe into five groups, eleven subgroups and 23 series.
Many workers regard pollen grains of Lactuceae as "Liguliflorae-type" (Faegri and Iverson, 1975; Moore and Webb, 1978) and contrast this with a "Tubiliflorae-type" for most of the reminder of the family Asteraceae. Wodehouse (1928, 1935) examined a large number of taxa of this tribe primarily in an effort to formulate phylogenetic trends within the tribe. His studies led to the characterization of several basic echinolophate patterns common in the Lactuceae.
Pausinger (1951) divided the tribe into two main types based on pollen characters. His Leontodon type was charactered by the possession of poral lacunae, and this tragopogon types of the lack of poral lacunae and the poral ones communicating to form long lacunae. While working on the comparative pollen morphology of *Sonchus*, Boulos (1960) found that this genus was closely related to Launaea. Tomb *et al*. (1974) studies the pollen morphology of Stephanomeriinae and showed that pollen grains of most of the tribe were echinolophate or tricolporate with same, or almost the same number and shape or lacunae and demonstrated strikingly different exine stratification in several genera. Feuer (1974) examined the pollen grains of Microseridinae, which in contrast with the stephnomeriinae, were predominantly echinolophatte. Skvarla *et al*. (1977) summarized much that was taken about pollen structure in the Asteraceae, where two major pollen types were categorized, namely, anthemoid and helianthoid, with various subtypes. Taxonomic, evolutionary and functional studies of the Asteraceae pollen grains on the basis of ultrastructure and sculpture were made by Bolick (1978), who noted two basic exine patterns. The caveate helianthoid and non-caveate anthemoid. El Ghazaly (1980) studied the pollen grains of 35 species of the subtribe Hypochoeridinae. Regarding the sub tribe Scorzonerinae with reference to its taxonomic significance, Blackmore (1982) recorded seven pollen types, which could be distinguished by a key constructed on the basis of number and arrangement of the lacunae of the grains. Blackmore (1984) further dealt with pollen morphology of a large number of taxa of the tribe Lactuceae and recognized seven distinct pollen types, which were further subdivided into smaller groups on the basis of distinguishing characters.
According to Clark *et al*. (1980) Pollen grains of the Astereae have been characterized as basically helianthoid, spherical or slightly flattened, tricolporate and uniformly echinate, having internal foramina, with varying proportions of abnormalities in size and colpus number (Wodehouse, 1930, 1935; Skvarla *et al*., 1977) . Pollen of the few genera examined to date has been difficult or impossible to distinguish by light or electron microscopy (Skvarla *et al*., 1977). However in conjunction with systematic studies of Haplopappus and related genera in the subtribe Solidaginae, we have four a few cases of significant variation in pollen size, spine length, and the number of spine rows between colpi. These characters indicate a potential for utilizing pollen characters in at least some systematic studies in the Astereae.
According to Ali (1988), in most of the plant groups in Angiosperms, at maturity, all the pollen grains are free from each other. As the prime function of the pollen grains is to provide the male gametes to the female counter part, in order to facilitate fertilization and ultimately the formation of the seed, such as category seems logical. However, it is also well known that in some plant groups, the pollen aggregate mis used for all types of compound pollen grain and for various types of situations where pollen grains do not separate at maturity and remain associated together. The term pollen aggregate is used for all types of compound pollen grains and for various types of situations were pollen grains are dispersed in-groups. Char *et al*. (1973) reviewed the evolutionary trends in pollen organization and discussed their adaptive significance.
In the present investigation, the pollen morphology of the genus Crepis, all Pakistan, of the tribe Lactuceae, have been studied systematically. The present writers decided to contribute as much as possibe and to start with different species of the genus Asteraceae. The present study was undertaken to evaluate the morphological differences in the pollen grains of family Asteraceae.

Materials and Methods

Pollen morphology: Pollen morphology of six taxa of Tribe Lactuceae of Asteraceae (Compositae) was studied from Pakistan during 1999 to June 2001 in Quaid-i-Azam University Islamabad. The florets from mature capitula were extracted, either from the herbarium specimens of Quad-i-Adam University, Islamabad or fresh polliniferous material collected from the wild was used in this research.

Pollen staining preparation: Pollen grains were stained with 1 % safranine mixed in glycerin jelly. Glycerin jelly was prepared by dissolving 70gm of gelatin in 42 ml distilled warm water in a beaker. The beaker was placed in another metalloid pot, containing boiling water. The gelating was stirred for 1-2 hours. After this operation, 35ml of gelatin was added in it, followed by 1gm of phenol crystals. This warm gelatin jelly was filtered using filter paper, 1 % safranine solution was poured in it in 1:1 ratio. The homogenized mixture was preserved in a vial and was used for staining pollen grains.

Pollen grains study by light microscopy: Florets taken from herbarium specimens were kept in distilled water in petri dishes for about 24 so as to soften them and were then used, while fresh material was used directly. The florets were separated from capitula and were placed in a few drops of distilled water on a clean glass slide. With the help of dissecting needles, the florets were opened, the anther were opened, the extra material was removed and the anthers were crushed to release pollen grains on the slide. Anther wall material was discarded, while excess of water was removed with filter paper. Pollens were stained with 1 % safranine mixed in glycerin jelly. The slide was placed on a hot plate and when the stain had completely melted, any bubble formed was carefully removed. Cover slip was placed on the prepared pollen glycerin jelly mixture. When cooled, the glass slide was labeled and the cover slip edges were sealed with white transparent nail varnish.

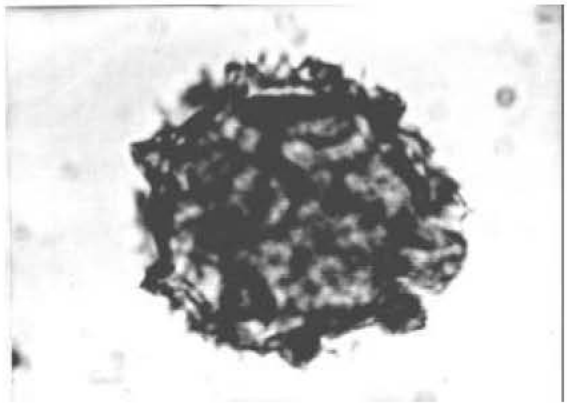
The prepared slides were studied under the light microscope. Eight slides of each taxon were prepared and complete set is kept in the Plant Taxonomy Lab, Department of Biological Sciences. Quad-I-Adam University, Islamabad. Their photographs were taken with Nikon Apaphot Microscope (LM).

For the measurements of pollen grains, following characters were noted as grain, shape of pollen grain, equatorial view, polar view, dimensions, equatorial diameter (E), polar diameter (P), P/E ratio, length of colpus, exine surface, exine thickness, inter oral distance, inter spinal distance, inter spinal outline, length of spines, number of spines between colpi.

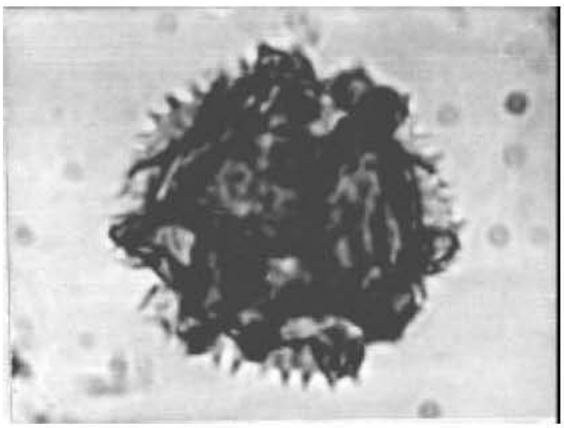
Results

The values of equatorial diameter of *Sonchus* pollen grain are presented in Fig. 1, the average values of pollen diameter in Fig. 2, P/E ratio in Fig. 3, the colpi length in Fig. 4, exine thickness in Fig. 5, interporal distanc in Fig. 6, interspinal distance in Fig. 7 and spine length is presented in Fig. 8. Photographs of polar and equatorial view of verious species of the genus *Sonchus* are shown in Plate1-5.

Sonchus asper (L.) Hill (Plate 1)

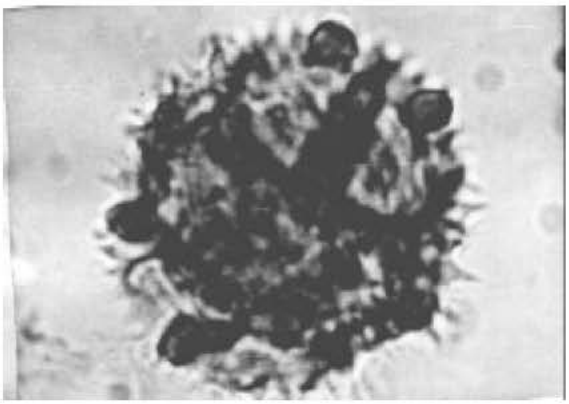


A: Equatorial view

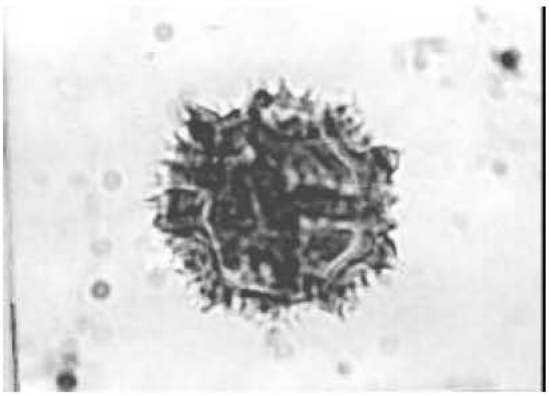


B: Polar view
Grain: Trizonocolporate
Shape of pollen grain : Sub spheroidal
In equatorial view: Sub spheroidal
In polar view: Triangular(obtuse convex)
Dimensions:
Exine surface: Echinate or Spinate
Inter spinal outline: V shaped

Sonchus maritimus (L.) Hill (Plate II)



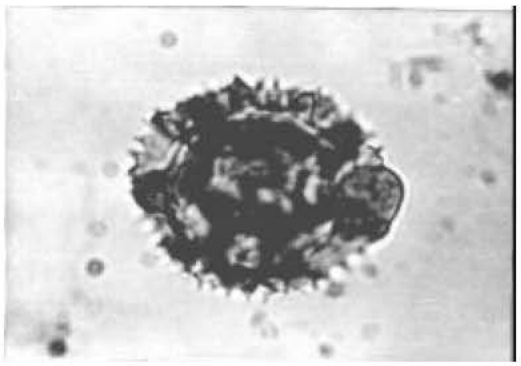
A: Equatorial view



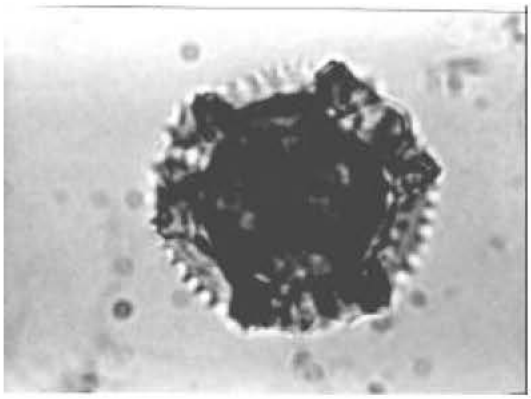
B: Polar view
Grain: Trizonocolporate
Shape of pollen grain : Sub spheroidal
In equatorial view: Sub spheroidal
In polar view: Triangular

Dimensions:
Exine surface: Echinate or Spinate
Inter spinal outline: V shaped

Sonchus oleraceus L. (Plate III)

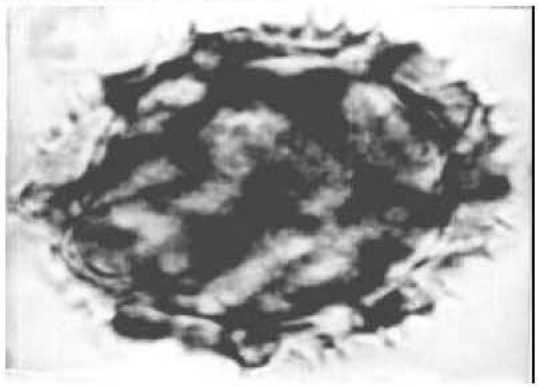


A: Equatorial view

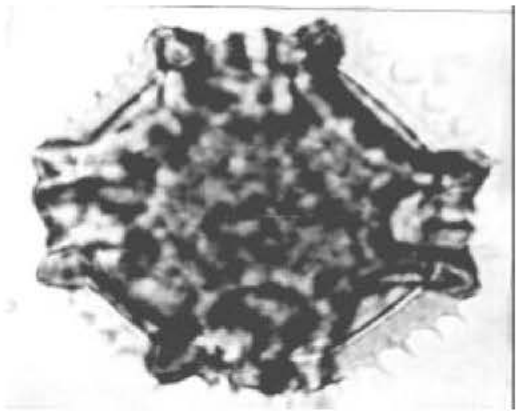


B: Polar view
Grain: Trizonocolporate
Shape of pollen grain : Sub spheroidal
In equatorial view: Sub spheroidal
In polar view: Triangular(obtuse convex)
Dimensions:
Exine surface: Echinate or Spinate
Inter spinal outline: V shaped

Sonchus palustris L. (Plate IV)

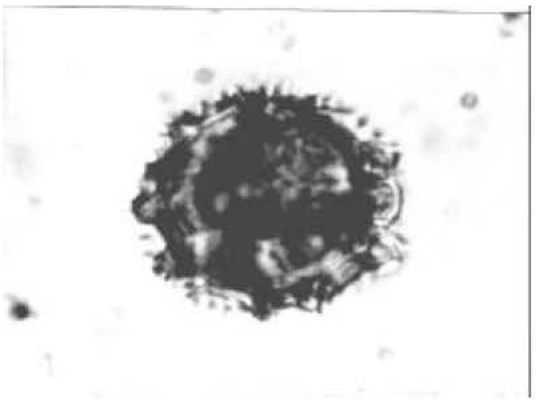


A: Equatorial view

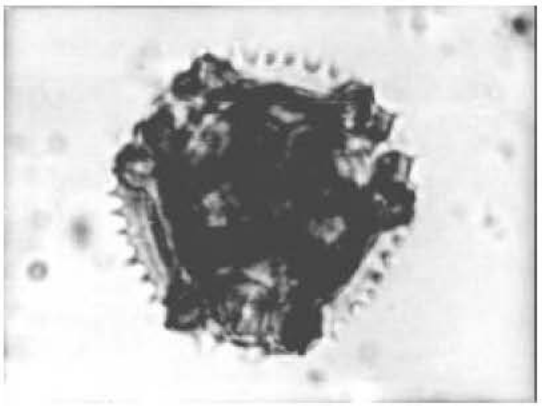


B: Polar view
Grain: Trizonocolporate
Shape of pollen grain : Sub spheroidal
In equatorial view: Sub spheroidal
In polar view: Triangular/ quadrangular
Dimensions:
Exine surface: Echinate or Spinate
Inter spinal outline: V shaped

Sonchus arvensis L. (Plate V)



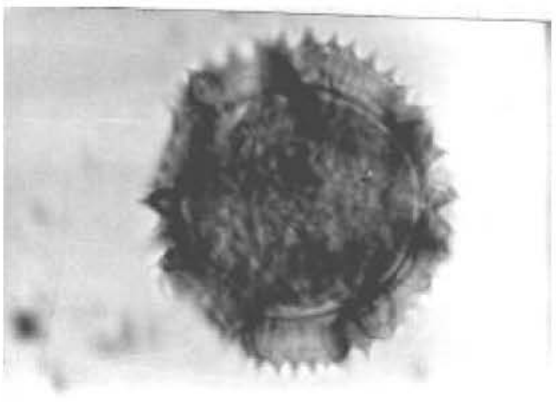
A: Equatorial view



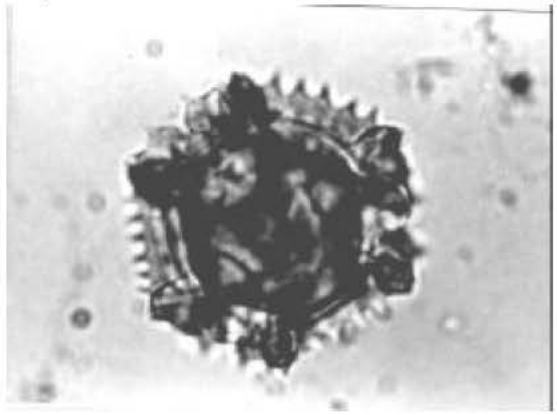
B: Polar view
Grain: Trizonocolporate
Shape of pollen grain : Sub spheroidal
In equatorial view: Sub spheroidal
In polar view: Triangular(obtuse convex)

Dimensions:
Exine surface: Echinate or Spinate
Inter spinal outline: V shaped

Sonchus uliginosus M. B. (Plate VI)



A: Equatorial view



B: Polar view
Grain: Trizonocolporate
Shape of pollen grain : Sub spheroidal
In equatorial view: Sub spheroidal
In polar view: Triangular(obtuse convex)
Dimensions:
Exine surface: Echinate or Spinate
Inter spinal outline: V shaped

Discussion

Although the micro-morphological study of pollen surface pattern, shape and size of Compositae/Asteraceae has demonstrated striking diagnostic features similar to the flora, chromosomal and vegetative characters of the family, these features show some inconsistencies with the tanomic classification of the family into sub families, tribes and sub tribes. However some genera retain individually unique surface pattern and some tribes and sub tribes with small number of genera show homogeneity in their pollen surface pattern, though other characters such as shape and size range of pollen do not distinguish them from other members of the family.

It is concluded that pollen morphology can not be solely used as the base of taxonomic classification of the family. However, if it is accepted that pollen morphology shows evolutionary sequences comparable to those in other organs,

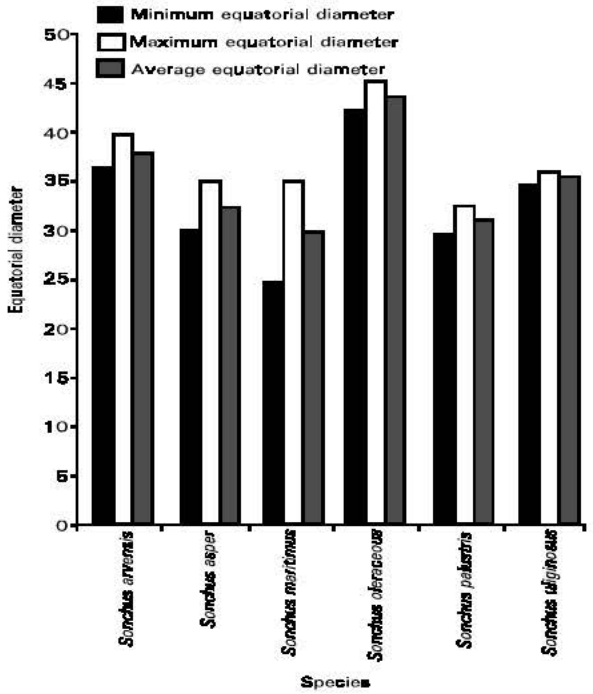


Fig. 1: A graph showing minimum, maximum and average values of equatorial diameter

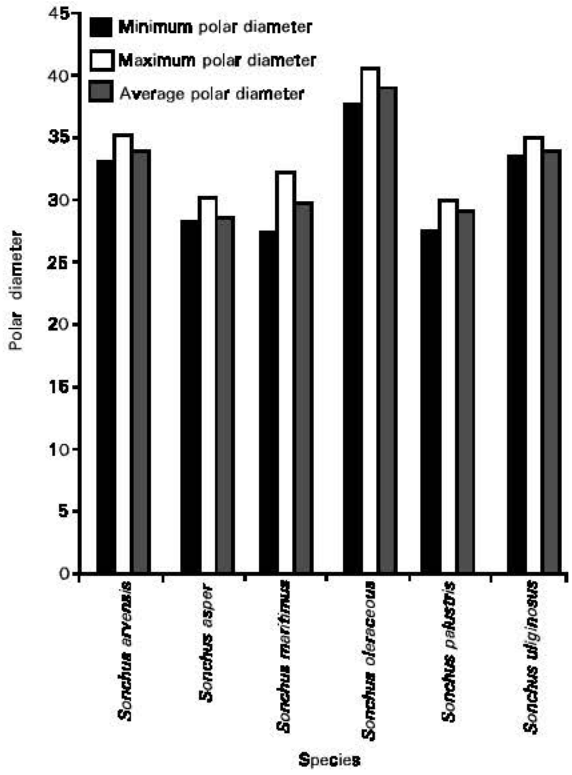


Fig. 2: A graph showing minimum, maximum and average values of polar diameter

Table 1: Minimum, maximum & average values of colpi length, exine thickness & interporal distance

Name of taxa	Length of colpi in microns			Exine thickness in microns			Inter-poral distance in microns		
	Min.	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.
Sonchus arvensis	4.50	5.00	4.75	2.50	3.25	2.87	18.0	20.0	19.00
Sonchus asper	4.75	5.00	4.80	3.57	4.00	3.70	23.5	25.0	24.50
Sonchus maritimus	5.00	6.25	5.60	2.50	3.75	3.12	1.5	2.0	1.75
Sonchus oleraceous	3.50	3.75	3.62	2.50	3.75	3.12	26.0	30.0	28.00
Sonchus palustris	4.75	5.00	4.80	2.00	2.50	2.25	13.0	15.0	14.00
Sonchus uliginosus	6.25	6.79	6.52	3.75	4.00	3.87	21.4	22.5	21.95

Table 2: Minimum, maximum & average values of polar & equatorial diameter

Name of taxa	Polar diameter in microns (P)			Equatorial diameter in microns (E)			P/E diameter in microns		
	Min.	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.
Sonchus arvensis	32.5	35.0	33.75	36.25	39.6	37.92	0.88	0.90	0.89
Sonchus asper	28.0	30.0	29.00	30.00	35.0	32.50	0.85	0.93	0.89
Sonchus maritimus	27.5	32.5	30.00	25.00	35.0	30.00	0.93	1.10	1.00
Sonchus oleraceous	37.5	40.4	38.95	42.50	45.4	43.90	0.88	0.90	0.89
Sonchus palustris	28.0	30.0	29.00	30.00	32.5	31.25	0.92	0.93	0.92
Sonchus uliginosus	34.0	35.0	34.50	35.00	36.0	35.50	0.97	1.05	0.98

Table 3: Minimum, maximum & average values of inter spinal distance, spine length & spine rows b/w colpi

Name of taxa	Inter spinal distance In microns			Length of spines in microns			Number of spine rows b/w colpi in microns
	Min.	Max.	Ave.	Min.	Max.	Ave.	
Sonchus arvensis	2.50	3.0	2.75	2.00	2.50	2.25	6-9
Sonchus asper	2.25	2.5	2.37	1.75	2.50	2.12	6-8
Sonchus maritimus	1.28	2.0	1.64	2.00	2.50	2.25	6-8
Sonchus oleraceous	2.00	2.5	2.25	1.50	1.75	1.62	8-10
Sonchus palustris	0.75	1.4	1.00	1.25	1.80	1.52	5-8
Sonchus uliginosus	2.00	2.5	2.25	2.30	2.50	2.40	7-9

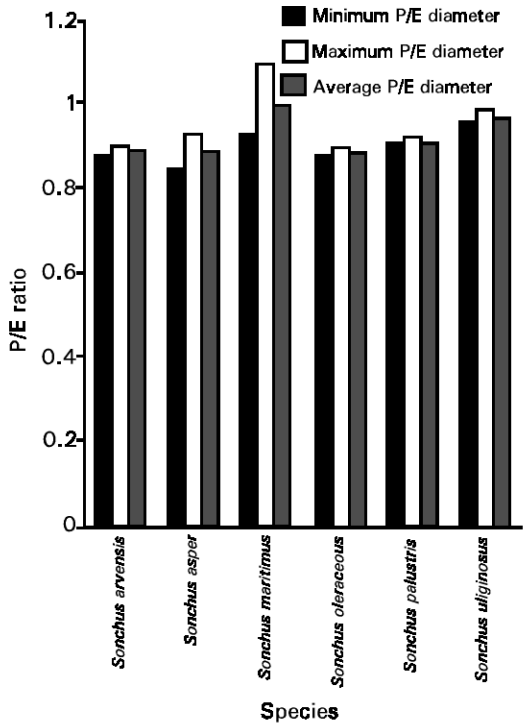


Fig. 3: A graph showing minimum, maximum and average values of P/E ratio

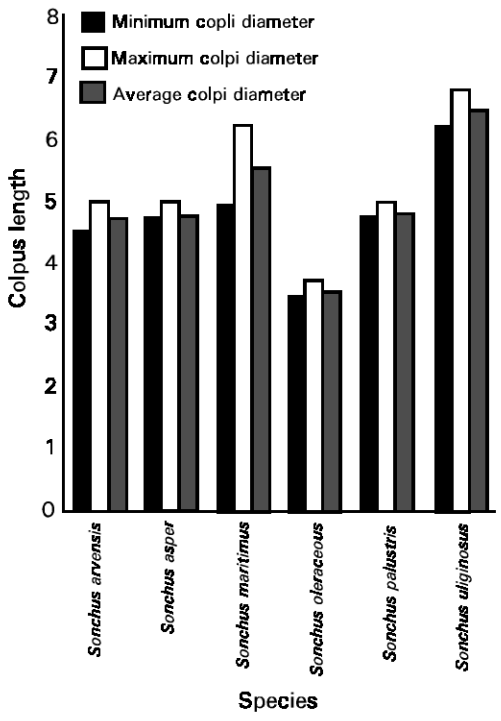


Fig. 4: A graph showing minimum, maximum and average values of coplus length

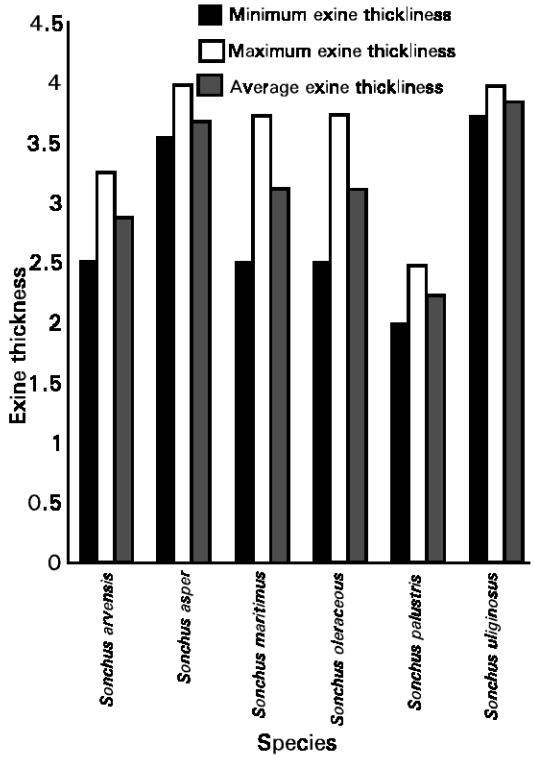


Fig. 5: A graph showing minimum, maximum and average values of exine thickness

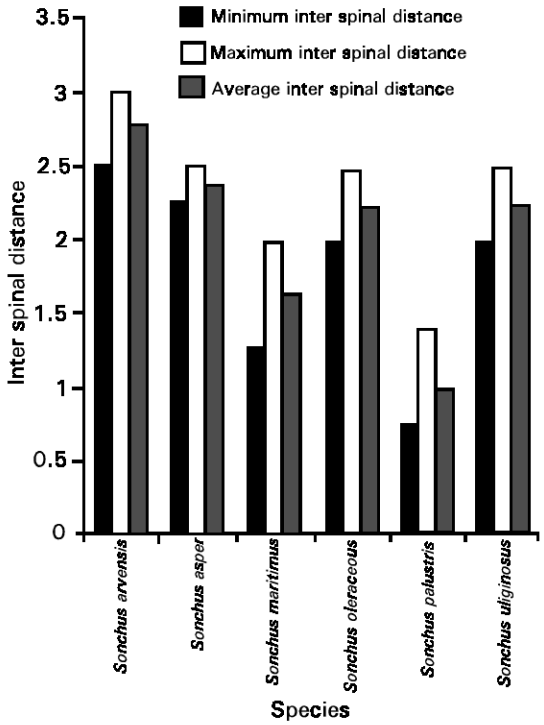


Fig. 7: A graph showing minimum, maximum and average values of inter spinal distance

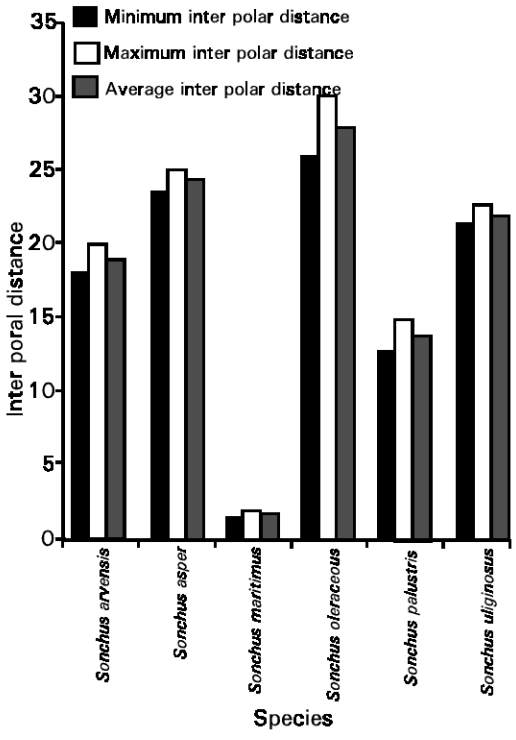


Fig. 6: A graph showing minimum, maximum and average values of inter poral distance

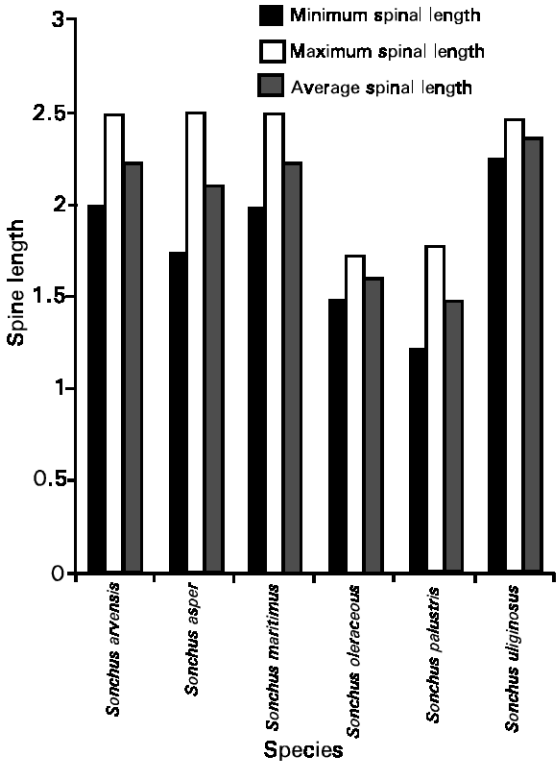


Fig. 8: A graph showing minimum, maximum and average values of spine length

then it may need to be given as much weight as any other morphological character. On this assumption, in the pollen morphology of the species examined, some inconsistencies and alternative relationship have been suggested in correlation with the recent taxonomic classification proposed by Johnson & Briggs (1975). The general features of the Asteraceae, taken together are not repeated in other families. This gives Asteraceae (Composite) a unique taxonomic status. To quote "Combined efforts of systematic, evolutionary and ecological studies are still necessary to help us understand the evolution of this fascinating family. Lactuceae is a tribe of Asteraceae, some plant in this tribe are of medicinal importance palynological studies of Lactuceae from Pakistan are carried out for the first time. The study of pollen morphology has assumed great significance in plant taxonomy and the advancement in microscopy have led to the effective use of new pollen morphological parameters for taxonomic purposes. This research project was conducted to examine the value of pollen morphology in taxonomy of Lactuceae and if the palynonogical characters are correlated to the morphological features, then they have great significance in taxonomy and may be considered as base for taxonomic decisions. For structure and pattern describing different characters, terminology followed is that of Erdtman (1969) and Nair and Lawrance (1985). For clear understanding to the taxonomic status of the species following palynonogical characters like grain class, shape in equatorial and polar view equatorial & polar diameter, P/E ratio, colpus length, exine surface, exine thickness, interporal distance, interspinal distance, interspinal outline, length of spine, number of spine b/w colpi were also considered. It is hoped that added information of pollen grains will help in taxonomic studies of Crepis.

The palynological characters not only provide the additional information but are also helpful to improve the systematic position of 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 Wodehous (1935). Pollen grains of Asteraceae may be resolved into two major groups i.e., lophate and non-lophate grains. The former ones characterize in *Sonchus*, Lactuca of Lactuceae. Among the numbers of Lactuceae the general pollen morphology is similar i.e the exine surface is echinate (spinate) or echinate (spinulate), the pollen grain is trizonocolporate but in some species tetrazonocolporate.

In the genus Sonchus, all the pollen grains were trizonocolporate but in *Sonchus palustris* the pollen grains are tetrazonocolporate. The pollen grain shape in equatorial view were subspheroidal & triangula in polar view. The pollen grain in *Sonchus palustris* are quadrangular in polar view. The equatorial diameter varies from 25 to 42.5 μm (minimum) and 32.5-45.4 μm (maximum) . In *Sonchus oleraceous* equatorial diameter is minimum 45.4 μm but in *Sonchus maritimus* is minimum 25 μm. The size of polar diameter varies between 27.5-37.5μm (minimum) and 30-40.4 μm (maximum). *Sonchus maritimus* has minimum polar diameter 27.5 μm and *Sonchus oleraceous* has maximum polar diameter 40.4μm. Colpi length is maximum in *Sonchus uliginosus* 6.79 μm while minimum in *Sonchus oleraceous* 3.5 μm. In Sonchus the pollen grains are echinate or spinate. *Sonchus palustris* has least exine thickness 2 μm but *Sonchus asper* and *Sonchus uliginosus* has maximum exine thickness 4 μm. In this research project, some more morphological parameters were considered for their application in plant taxonomy, which were found to be useful. Minimum value of interporal distance were found in *Sonchus maritimus* 1.5 μm but maximum value of interporal distance were found in Sonchus oleraceus 30 μm. The maximum interspinal distance were found in Sonchus arvensis 3 μm while least in *Sonchus palustris* 0.75 μm. In Sonchus interspinal outline is V shaped. Spine length were found to be maximum in *Sonchus uliginosus*, *Sonchus anensis*, *Sonchus asper*, *Sonchus maritimus* 2.5 μm but minimum in *Sonchus oleraceus* 1.5 μm. Number of spine rows b/w colpi varies from 5-10 in different species of Sonchus.

In the present investigation the genus Sonchus characteristic pollen grain were observed. Light microscopic observations could not clearly indicated the exine sculpturing. Bolick (1978) suggested that Scanning Electron Microscope (SEM) studies should be carried out for obtaining many characters of great taxnonmic importance. Not only the general morphology but also pollen morphology is of significance in species delimitation and pollen characters are correlated with morphological features. Palynonogy can play a very important role in solving the taxonomic problems if the pollen characters are co-related with morphological characters to become a qualitative character.

It is recommended that pollen grain of the plants should be studied pharmacognistically to determine their importance in medicine, in this way medicinal plants can be explored from Pakistan. According to Clark *et al.* (1980) that the pollen morphology of some genera in the Astereae can be useful in supporting taxonomic suggestions. The divergent pollen types probably represent early or major divergences from the main "Haplopappoid" line. Further pollen studies in the Astereae may lend support to taxonomic suggestions concerning other genera of the family Asteraceae. Pollen morphology bears a number of details of taxonomic interest in sections.

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