Phenological Patterns and Palyno-Morphological Significance of Medicinal Plants by Using Light and Scanning Electron Microscopy

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ABSTRACT

The study was conducted to identify 15 valuable medicinal plants from Khyber Pakhtunkhwa, Pakistan. In the present study, seasonal phenological behavior in leaf droplets, flower and fruit activity was discussed. The morpho-palynological characters studied were size, shape, polar and equatorial diameters LM and SEM were used for the importance of their classification. The present investigations examined the pollen apertures were tricolporate, tricolpate, tetracolporate, hexacolportae and polycolportae. Pollen shape classes according to P/E ratio were identified as oblate spheroidal, spheroidal and prolate. We found the following 11 types of exine sculpture pattern occurred in the investigated species. Similarly, variation in pollen length was prominent and the largest pollen on polar and equatorial view was recorded for *Trianthema portulacastrum* while the smallest one was observed for *Cleome brachycarpa* respectively. Exine thickness of maximum value was examined in *Trianthema portulacastrum* while minimum in *Heliotropium europaeum*. Pollen fertility was estimated highest in *Indigofera linifolia* while highest percentages of sterility were observed in *Croton bonplandianus*. The findings analysis of pollen morphological data demonstrated that pollen characters of studied medicinal plants shows species level variations and of taxonomic importance and shown phenological pattern of species to understand their response to climatic factors.

Key words: Medicinal plants, Taxonomy, Pollen morphology, Phenology, LM, SEM

1. INTRODUCTION

Herbal remedies are the richest source of compounds that can be used in drug development. Plants play an important role in the development of human culture around the world (Jain et al., 2020; Boukhatem and Setzer, 2020). In developing countries, the difficulties encountered in the production of herbal medicines are misidentification and falsification and this situation is further complicated by the lack of quality control standards in the herbal industry. Locally,

more than one plant known by the same name, this poses the major problem in establishing the botanical names or identity of plants used in the traditional Unani system. Researchers are working on modern techniques for the exact botanical identification of plants used in the Unani drug system (Hameed *et al.*, 2020). Valuable and recent trends for the identification of different plant species Pollen morphology are the most important tools for the interpretation of closely related species. The different morphological characteristics of pollen, such as symmetry, shape, diaphragmatic pattern and exine configuration are very important characteristics in the taxonomic evaluation of plants. Nowadays, for the taxonomic identification of flowering plants, pollen grains are widely studied. Relation between time and place Studies in different parts of the world show that climate factors are primarily responsible for plant and reproductive phenology at the community and species level (Mishra *et al.*, 2006). Taxonomists and botanists have reported pollen morphology to classify flowering plants by species and variety (Bahadur *et al.*, 2019 and Ahmad *et al.*, 2013). In phenological observation the flowering having implications for the timing and intensity of the pollen season; this is showing an advancing trend as many species start to flower earlier (Pokorná *et al.*, 2018).

Medicinal plant pollen morphology was first performed in Pakistan; they studied various aspects of plants including anatomy, morphology, palynology and their medicinal uses by (Perveen and Qaiser, 1999; Sardar *et al.*, 2013). However, there is currently no separate documentation on the pollen morphology of some species of medicinal plants collected from the southern regions of Khyber Pakhtunkhwa, Pakistan. Most taxonomists identify plant species based on phenotypic traits of plants such as root, stem and leaf structures, but scientists now believe that Palynological studies could provide a more precise basis for identifying plant species (Amina *et al.*, 2020; Devendere *et al.*, 2016; Kailas *et al.*, 2016).

Microscopy plays an important role in the taxonomic identification of medicinal plants based on vegetative morphology and can also be used for the correct verification and validation of new sources of their adulterants. Researchers identify plants based on comparing the microscopic characters of the specimens examined to determine the differences between them (Park *et al.*, 2020). Light microscopy (LM) and electron scanning (SEM) is the advanced form of microscopy used to characterize the morphological characteristics of plants and their organs (Matthaeus et al. 2020).

Scanning electron microscope (SEM) can be used to differentiate taxonomic traits from closely related genera (Khan *et al.*, 2020). In addition, SEM can also be used to validate raw herbal remedies for their adulterants and also for authentication. It can also play a major role in natural products for quality assurance, as these products are to be used in the herbal market, research

institutes and other industries (Ali *et al.,* 2020; Miralles *et al.,* 2020; Veloz *et al.,* 2012; Zhigila *et al.,* 2014; Albert and Innes, 2020). SEM of pollen collected from medicinal plants is currently used for authentication and identification purposes (Ashfaq *et al.,* 2020; Majeed *et al.,* 2020).

Previous studies have adopted microscopic techniques to add new records of species, systematic studies of plants, anatomical, micro and macro morphological and to find variations between the authenticities of medicinal plants (Ullah *et al.,* 2018; Ashfaq *et al.,* 2018; Naz *et al.,* 2019; Ur Rahman *et al.,* 2019; Hameed *et al.,* 2020; Ahmad *et al.,* 2013; Khan *et al.,* 2020; Raza *et al.,* 2020; Zafar *et al.,* 2020; Gul *et al.,* 2020; Sadia *et al.,* 2020; Nazish *et al.,* 2019; Mir *et al.,* 2019; Singh *et al.,* 2020; Song *et al.,* 2019; Hinojosa *et al.,* 2019; Joujeh *et al.,* 2019). To introduce new species into Pakistani flora, the taxonomist and scientists have contributed. Due to Pakistan's unique geographical position, there are still many chances to find new plant species that were not previously reported in Pakistan.

The main objective of the research was to study the characteristics of constant and diagnostic pollen and to use this data for the subsequent identification, classification and authentication of taxa. The study also aimed to assess the total variations between taxa at different levels of the taxonomic hierarchy and to further contribute to the classification and phylogenetic relationship of the taxa in question and also to analyze the phenological pattern of species to understand their response to climatic factors.

2. MATERIAL AND METHODS

2.1. Collections, identification and preservations

From different areas of southern region of Khyber Pakhtunkhwa, Pakistan including Bannu, Karak, and Lakki Marwat plant collection was done. The members of 15 ethnomedicinally important plants belong to different plant families were collected, that is, *Croton bonplandianus*, *Heliotropium europaeum*, *Cleome viscosa*, *Cleome monophylla*, *Cleome brachycarpa*, *Capparis spinosa*, *Crotalaria burhia*, *Digera muricata*, *Indigofera linifolia*, *Astragalus mongholicus*, *Solanum surattense*, *Physalis minima*, *Trianthema portulacastrum*, *Peganum harmala* and *Tribulus Terrestris* were collected to study pollen morphological diversity.

All plant specimens were collected in fresh form, so that from their ripe anthers, pollen was obtained for further analysis. The identification of the plant specimens was made by comparison with the herbarium samples kept in the Herbarium of Pakistan (ISL) and compared with the flora of Pakistan by cross-checking (http://www.efloras.org). The dried and poisoned plant specimens were then mounted on standard sized herbarium sheets and submitted to

Herbarium of Pakistan (ISL) at Quaid-i-Azam University Islamabad, Pakistan. Details of botanical names, voucher specimen number, family, place of collection and state of cultivation, form of growth and distribution of the plants collected are given in **(Table-1)**.

Table: 1 List of Medicinal plant species with detail information along with their previous Studies

S. No	Voucher specimen	Taxa	Family	Local name	Collection site	Flowering period	Flower Colour	Inflorescence	Collector	Cultivation status / Distribution in Pakistan	Distribution in World	Medicinal importance	Previous studies
1	SA-212	*Croton bonplandianus* Baill.	Euphorbiaceae	Harh Botai	Laka Tizza/District Bannu	April-July	Pale-creamy-Yellow or nearly white	Axillary, Racemose or Spicate	Siraj Khan	Wild/ Narowal, Bannu, Karak, Kohat, Lakki Marwat, Multan, Mardan and Peshawar	Paraguay, Brazil, Argentina, Indonesia, India and Abyssinia	Ring worm, Control scabies, bronchitis, asthma, heal cuts and wounds	(Verma et al. 2020, Reddy & Reddy, 2018).
2	SA-213	*Heliotropium europaeum* Forssk.	Boraginaceae	Herporai	Ameer Zai/ District Bannu	March-April	White	Spike like cymes	Siraj Khan	Wild/ Bannu, Lakki Marwat, Kohat, Karak, Lachi, Mianwali and Chakwal	Afghanistan, India, Russia, N-Africa, Trans Jordan, Syria, Iraq, Iran and Russia	Cardiotonic, anthelmintic, headache and for gout and Snake bites	(Khan et al. 2017, Alasbahi, & Al-Hawshabi, 2020).
3	SA-214	*Cleome viscosa* L.	Capparaceae	Sheen Starga	Nadir bodeen khel/ District Bannu	May-September	Yellow	Receme corymbs	Siraj Khan	Wild/ Swat district, Karak, Bannu, Dera ghazi khan, Chakwal and Hazara	India, Sri Lanka, Philippines, Indonesia, Malaysia, Australia, America and Africa	Hypertension, wound healing, Ear infection, pain and deafness	(Mekap et al. 2020; Anwer et al. 2020).
4	SA-215	*Cleome monophylla* L.	Capparaceae	Sheen Gul	Tujak kala/ District Bannu	May-September	Pink	Racemes	Siraj Khan	Wild/ Chakwal, Hazara, Bannu, Karak, Swat and Karachi	Afghanistan, Oman, Africa, Saudi Arabia, Kenya, India, Egypt, Iran and Afghanistan	Ulcers, and for ear discharges	(Poddar et al. 2020, Kebede et al. 2017).
5	SA-216	*Cleome brachycarpa* Vahl ex-DC	Capparaceae	Sheen Starga	Marghala / District Bannu	May-September	Pale yellow	Racemose	Siraj Khan	Wild/ Punjab, Sindh, Karachi, Chakwal, Hazara, Bannu, Karak, and Swat	Africa, Saudi Arabia, Kenya, India, Egypt, Iran, Afghanistan, Oman	Rheumatism, scabies and leucoderma	(Naeem et al. 2019, Afifi 2014).

6	SA-217	*Prosopis juliflora* (Sw.) DC.	Mimosaceae	Kikrai	BadarkaKalai/ District Bannu	August-May	Greenish yellow	Dense Axillary Pedunculate Spikes	Siraj Khan	Wild/ Bannu, Karak, Karachi, Kashmir, Baluchistan and Sindh	Australia, Brazil, Africa, western Asia, Arabia, India, Arabia, Mexico, America and Iraq	Diabetes mellitus, itch, measles, pinkeye, stomachache and sore throat.	(Haider & Zhong, 2014, Umair et al. 2019).
7	SA-218	*Crotalaria burhia* Buch. - Ham.	Leguminosae	Zergulai Balanza	ZeerakiPeerbaKhel/ District Bannu	January-February	Yellow	Raceme	Siraj Khan	Wild/ Karachi, Punjab, Sind, Baluchistan, Tank Lakki Marwat, Karachi, Bannu, Karak and Kohat	Afghanistan, India, Iran and Brazil	Eczema, swellings, wounds, kidney pain and abdominal problems.	(Ahmad et al. 2014, Yousaf 2018).
8	SA-219	*Indigofera linifolia* subsp. (Wight).	Leguminosae	Sorgul	Sparkai Kala/ District Bannu	July-October	Pink, Red	Sessile or Shortly Peduncled Axillary Raceme	Siraj Khan	Wild/ Karak, Bannu, Peshawar, Kohat, Sindh, Punjab, Azad Kashmir, Kaghan, Narran and Charsada	India, Australia, Angola, Ghana, Tanzania, Botswana, Zambia, Africa, Nepal, Philippines, Saudi Arabia, Sri Lanka	Liver disease, heart palpitation, gout and laxative.	(Suroowa et al. 2019, Alamgir 2017).
9	SA-220	*Dicliptera chinensis* Roe m. & Schult.	Acanthaceae	Ghargul	LakaTizza/District Bannu	Sep-Jan	Pale Purple	Axillary or Apical, Cymose	Siraj Khan	Wild/ Bannu, Karak, Peshawar, Lakki Marwat, Dir, Swat, Swabi, Hazara and Peshawar	Africa, America, Afghanistan, Nepal, Bhutan, Bangladesh, India, China	Wounds healing and digestive problems	(Agize, et al. 2013, Amjad 2015).
10	SA-221	*Solanum surattense* Burm.f.	Solanaceae	Maraghenai	Kasho River / District Bannu	Feb-Dec	Purple	Axillary Cymose	Siraj Khan	Wild/ Bannu, Karak, Kohat, Peshawar, Mardan, Hazara, Swabi, Charsada, Punjab, Azad Kashmir, Baluchistan and Sind	North Africa, South America, Malaysia, Philippines, Australia, Western Assam, Polynesia	Liver tonic, wound healing, rheumatism and for kidney stones	(Wariss et al. 2014, Mahmood et al. 2011).

11	SA-222	*Physalis minima* L.	Solanaceae	Tarhaha botai	Domel /District Bannu	August-October	Off White to Pale Yellow	Solitary	Siraj Khan	Wild/Islamabad, Kashmir, Kohat, Bannu, Karak, Swat, Karachi and Hazara	Afghanistan Nepal, China, India, America, Australia	Diuretic and laxative	(Hassan et al. 2017, Das et al. 2012).
12	SA-223	*Trianthema portulacastrum* L.	Aizoaceae	Aghzai	LakaTizza/District Bannu	May-October	White to Pink	Solitary, Sessile	Siraj Khan	Wild/ Kohat, Bannu, Lakki Marwat, Mardan, Peshawar, Swat and Swabi	Southeast Asia, America, Africa, India and Sri Lanka	Blood purifier, stomachic and liver disorders.	(Nandagopalan et al. 2014, Ajaib et al. 2016).
13	SA-224	*Peganum Harmala* Crantz.	Rutaceae	Sponda	Town Ship/ District Bannu	April-October	White or Yellowish White	Pedicelled, Solitary or Paired in Terminal Cymose	Siraj Khan	Wild/ Gilgit Baltistan, Karak, Bannu, Malakand, Swat and Ghazi khan	USA, Mediterranean region, Africa, India, Tibet, North Africa, Russia, Central Asia, Middle East	Anti-cancerous, antidiabetic, hypothermic and cardiovascular	(Aslam et al. 2014, Khan et al. 2017).
14	SA-225	*Tribulus Terrestris* L.	Zygophyllaceae	Draygotai	Jar Marghalai/ District Bannu	Throught the year	Yellow	Solitary, Axillary	Siraj Khan	Wild/ Islamabad, Karak, Lakki Marwat, Charsada, Malakand and Bannu	New Zealand, Africa, North Australia and America	Kidney problems, skin disorders and for male sexual problems	(Das & Chakraborty, 2020, Sargın et al. 2013).
15	SA-226	*Digera muricata* Mart.	Amaranthaceae	Rhanzaka	Kasho River/ District Bannu	September-December	White Tinged with Pink to Carmine or Red	Long Pedunculate Axillary Racemes	Siraj Khan	Wild/ Karak, Bannu, Lakki Marwat, Kohat, Hazara, Swat, Malakand, Swabi and Dir Lower	Africa, Sudan, Ethiopia, Tanzania, Madagascar, Yemen, Afghanistan, India, Malaysia and Indonesia	Kidney disorders, diabetes, constipation and expectorant	(Fatima et al. 2019; Iqbal et el. 2011).

2.2. Phenology of species

Both short-term and long-term activity was observed by (Mishra *et al.*, 2006) the periodicity of leaf flushing, flowering and fruiting activity by individuals of species populations. Short activity is less than 2 weeks and long activity is longer than 2 weeks. More or less continuous activity of flowering and fruiting throughout the year is called continuous activity. The terms seasonal and long-term activity refer to flowering / fruiting occurs during a given period and extending into more than one period respectively. Marginal activity refers to species that are active during the transitional period of seasonal change. When some individuals of a plant species are in flowering / fruiting simultaneously is referred to as synchronous activity (S). The species showing flower/fruit development during a distinct period is known as asynchronous (A) (Tixier *et al.*, 2020).

2.3. Pollen micro morphological analysis

Pollen grains were obtained from mature buds of heterostyle individuals. For each species, three specimens were used and from each specimen at least three to four anthers were studied, and their pollen grains were prepared for scanning electron microscopy (SEM) and light microscopy (LM) using the prolonged acetolysis procedure of (Harley, 1990; Erdtman, 1960). For LM, the pollen grains were mounted in glycerin jelly and sealed with paraffin. The polar (P) and equatorial (E) shape and length and the P/E ratios were obtained under a light microscope (x 1000). Three replicates were used to measure characters. For the SEM, the pollen grains were transferred directly to double-sided tape affixed stubs; then vacuum coated with gold in the Biorad E5200 car coating (Bio-Rad, Hercules, CA, USA) and examined and photographed by a 10kV CamScan MV2300 scanning electron microscope (Electron Optic Services Inc., Ottawa, Canada). The types and dimensions of the sculpture were studied, as well as their fine structure, as well as the dimensions of the lines and the length of the Mesocolpium. The terminology in this article corresponds to that used by (Talebi *et al.*, 2014).

2.4. Statistical analysis

The results were analyzed quantitatively using SPSS software (Dutra and Gasparino, 2018). Quantitative measurements of pollen, including stroke length / width, exine thickness, polar and equatorial diameter. For the qualitative description of pollen, previously reported terminology was used (Ahmad *et al.*, 2018; Bahadur *et al.*, 2018).

3. RESULT

Out of 15 species, 13 were deciduous, *Dicliptera chinensis* were semi-evergreen and *Prosopis juliflora* were semi- deciduous species. The present research area does not retain its green appearance all year round, as most species are deciduous in the area. However, during the wet months of July to October deciduousness of the southern region is not so conspicuous due to reduced leaf fall in comparison to the drier and cool months. The general phenological stages of all species are presented in **(Table-2)**. Their seasonal phenological behaviours have been discussed under leaf drop, leaf flushing, flowering and fruiting activities.

3.1. Leaf drop

Leaf drop may be total or partial depending upon the species. In some truly deciduous taxa, all or most of the old leaves got abscised before the arrival of new ones and the species was bare for a period of weeks or few months. Examples of this category are *Croton bonplandianus*, *Heliotropium europaeum*, *Cleome viscosa*, *Cleome monophylla*, *Cleome brachycarpa*, *Crotalaria burhia*, *Indigofera linifolia* subsp, *Solanum surattense*, *Physalis minima*, *Trianthema portulacastrum*, *Peganum Harmala*, *Tribulus Terrestris* and *Digera muricata*. In other species such as *Dicliptera chinensis* and *Prosopis juliflora*, leaf fall and leaf flushing processes slightly over lapped in the same specise. In evergreens old leaves were abscised over a period of time throughout the year, thus, retaining a steady population of functional leaves all the time.

3.2. Flowering and fruiting activity

Fruiting activity was observed throughout the year with approximately 1.5 times more activity around May and June than in December. The peak period of fruit maturity in the present study was observed during winter and summer. Next to rapid fruiting activity a larger proportion of species recorded lengthy fruiting behaviour but only very few species had multiple fruiting behaviour **(Table-2)**. Almost all species had phenological patterns that synchronized flowering and fruiting in the dry months i.e. April, May and June. Most of the species flowered at the beginning of April and fruited near the end of May and beginning of June, needing only a short time for the development of fruits. Rest of the species flowered during April and May, fruited during December, with a moderate amount of time required for fruit development. Flowering and fruiting at hottest summer i.e., April and May have selective advantage **(Table 2).**

Table: 2 Phenology of some medicinal plant species from District Bannu, Pakistan

S. No	Name of Plants Species	Altitude (m)/(feet)	Habitat	VT	Leaf Drop	Leaf flushing	Flowering Period	Fruiting Period
1	*Croton bonplandianus* Baill.	100-1800m	Road side, bank of the river, wastelands and yards	D	……	……	04-07 (eA)	06-07. (eR)
2	*Heliotropium europaeum* Forssk.	130m	Roadside	D	4-5 r (A)	3 (e)	04-07 (eS)	06-09 (eR)
3	*Cleome viscosa* L.	1000m	Woodland, grassland, fields, roadside, sandy soils, calcareous and rocky soils	D	4-5 r (A)	3 (e)	04-07 (eS)	06-08 (eR)
4	*Cleome monophylla* L.	2100m	Stony and sandy plains	D	4-5 r (A)	3 (e)	04-07 (eS)	06-08 (eR)
5	*Cleome brachycarpa* Vahl ex-DC	1750m	Stony and sandy plains	D	4-5 r (A)	3 (e)	04-07 (eS)	06-08 (eR)
6	*Prosopis juliflora* (Sw.) DC.	300-1600m	Sandy, rocky, poor and saline soils	SD	10-11 (i) A	9 (e)	04-07 (eA)	06-08 (eR)

7	*Crotalaria burhia* Buch. - Ham.	200-1400m	Sand dunes in arid regions	D	2-3 (S)	3 (e)	03-04 (eS)	04-05 (eR)
8	*Indigofera linifolia* subsp. (Wight).	1500m	Dry grassland and bush land	D	9-10 (i)(A)	5 (e)	04-07 (eA)	07-09 (eR)
9	*Dicliptera chinensis* Roem. & Schult.	1800m	Stream sides and trailside	SE	……	……	…….	……..
10	*Solanum surattense* Burm.f.	3300m	Waste places and road sides	D	5-7 (A)	5 (e)	04-05 (eS)	06-09 (eR)
11	*Physalis minima* L.	1610-981m	Field edges, roadsides and sandy river flat	D	9-11 (i)(S)	3 (e)	04-05 (eS)	07-09 (eR)
12	*Trianthema portulacastrum* L.	900m	Lawns, wastelands, Roadsides, Gardens and cultivated crops fields	D	8-11 (S)	4 (e)	04-05 (eS)	05-08 (eR)
13	*Peganum Harmala* Crantz.	1600-2600m	Dry steppes and Sandy soil	D	9-11 (i) (A)	3 (e)	04-05 (eS)	05-06 (eR)
14	*Tribulus Terrestris* L.	3000-3300m	Roadsides and Waste places	D	4-5 (A)	3 (e)	04-05 (eS)	07-08 (eR)
15	*Digera muricata* Mart.	1000-1500m	Waste ground	D	5-7 (A)	6 (e)	04-05 (eS)	07-09 (eR)

Abbreviation: VT vegetation type, **r** = rapid leaf drop, **l** = lengthy leaf drop > > 2 months, **e** = extended flowering/fruiting extending into more than one period, **S** (synchronous)= flowering/fruiting taking place simultaneously, **A** (a synchronous)= Flower/Fruit development during distinct period, **D**= deciduous, **E**= evergreen, **SE**= semi-evergreen, **b**= brief periods < < 2 weeks per episode, **e**= extended periods > > 2 weeks per episode, **M**= multiple events per year, **R**= rapid fruit maturation < > 4 months

The morpho-palynological characters of deciduous, semi-evergreen and semi- deciduous taxa were studied. The morpho-palynological variation has been described below in terms of size, shape, exine ornamentation, number and size of aperture, length and width of colpi, ornamentation of mesocolpium, and percent fertility and sterility. The representative pollen traits of all the species studied were summarized in **(Tables -3)**.

Table 3: Qualitative palyno-morphological characters of plant species

S. No	Taxa	Exine sculpturing	Aperture condition	Colpi orientation	Mesocolpium	Spines
1	*Croton bonplandianus* Baill.	Papillate-reticulate	------	------	------	Absent
2	*Heliotropium europaeum* Forssk.	Regulate	Tricolporate	Deeply sunken. margins are pointed	Regulate	Absent
3	*Cleome viscosa* L.	Foveolate-verrucate	Tricolporate	Emergent and tapering. Pointed from margins but broader at middle	Foveolate-verrucate	Absent
4	*Cleome monophylla* L.	Spinulose-verrucate	Tricolporate	Sunken and slit like. Margins are pointed.	Verrucate	Present
5	*Cleome brachycarpa* Vahl ex-DC	Foveolate-reticulate	Tri or tetra colporate	------	Foveolate-reticulate	Present
6	*Prosopis juliflora* (Sw.) DC.	Scabrate-reticulate	Tricolporate	Sunken and slit like. Margins are pointed.	Scabrate-reticulate	Absent
7	*Crotalaria burhia* Buch. -Ham.	Reticulate	Tricolporate	Sunken and slit like. Margins are more or less pointed.	Reticulate	Absent
8	*Indigofera linifolia* subsp. (Wight).	Regulate	Tricolpate	Emergent	Regulate	Absent
9	*Dicliptera chinensis* Roem. & Schult.	Echinate-regulate	Tricolpate	Sunken and slit like. Pointed margins	Regulate	Present
10	*Solanum surattense* Burm.f.	Equinate-scabrate	Hexacolpate	Sunken and slit like. Margins are pointed	Equinate-scabrate	Present

11	*Physalis minima* L.	Verrucate	Tricolporate	Sunken and slit like. Margins are rounded	Verrucate	Absent
12	*Trianthema portulacastrum* L.	Reticulate-foveolate	Polycolporate	Sunken and linear. Margins are rounded	Reticulate-foveolate	Absent
13	*Peganum Harmala* Crantz.	Foveolate-verrucate	Tricolpate	Sunken	Foveolate	Absent
14	*Tribulus Terrestris* L.	Bireticulate-verrucate	-------	-------	-------	Absent
15	*Digera muricata* Mart.	Psilate	-----	-----	------	Absent

3.3. Size

The size of the pollen grain ranged from 14.55µm to 71.28µm. At *Cleome brachycarpa* was found with a small size of 14 µm. *Solanum surattense*, *Peganum Harmala* and *Heliotropium europaeum* were also small, while in 10 taxa, namely *Croton bonplandianus*, *Cleome monophylla, Prosopis juliflora, Cleome viscosa, Crotalaria burhia, Indigofera linifolia, Dicliptera chinensis* and *Physalis minima* were medium size. Large sizes were observed in *Trianthema portulacastrum*, that is, 71.28 µm **(Table 4)**.

3.4. Shape

The relationship between the polar axis and the equatorial diameter is as follows: P/E = <0.50 is peroblate, 0.50-0.75 is oblate, 0.76-0.88 is suboblate, 0.89- 0.99 is oblate-spheroidal, 1.00 is spherical, 1.01-1.14 is prolate-spheroidal, 1.15-1.33 is sub-prolate, 1.34-2.00 is prolate and >2.00 is perprolate; While in the present finding four taxa, namely *Cleome viscosa, Cleome brachycarpa, Crotalaria burhia* and *Dicliptera chinensis* in the oblate spheroidal category and four taxa (*Heliotropium europaeum, Indigofera linifolia, Physalis minima* and *Trianthema portulacastrum*) in the spheroidal category. Prolate spheroidal category are found in 3 taxa (*Croton bonplandianus, Cleome monophyla, Solanum surattense*) while Peroblate, Subprolate and Prolate category are found in *Prosopis juliflora, Digera muricata, Peganum harmala* and *Tribulus terrestris*.

3.5. Aperture types

It was observed the size and numbering of pollen grain openings, that 6 taxa exhibited Tricolporate i.e., *Heliotropium europaeum, Cleome viscosa, Cleome monophylla, Prosopis*

juliflora, *Crotalaria burhi*a and *Physalis minima*, 3 taxa represent Tricolpate i.e., *Indigofera linifolia*, *Dicliptera chinensis* and *Peganum Harmala*, one taxa represent Hexacolpate (*Solanum surattense*), Polycolporate found in *Trianthema portulacastrum*, and one taxa shown tri or tetra colporate found in *Cleome brachycarpa* **(Figure 1, 2, 3, 4).**

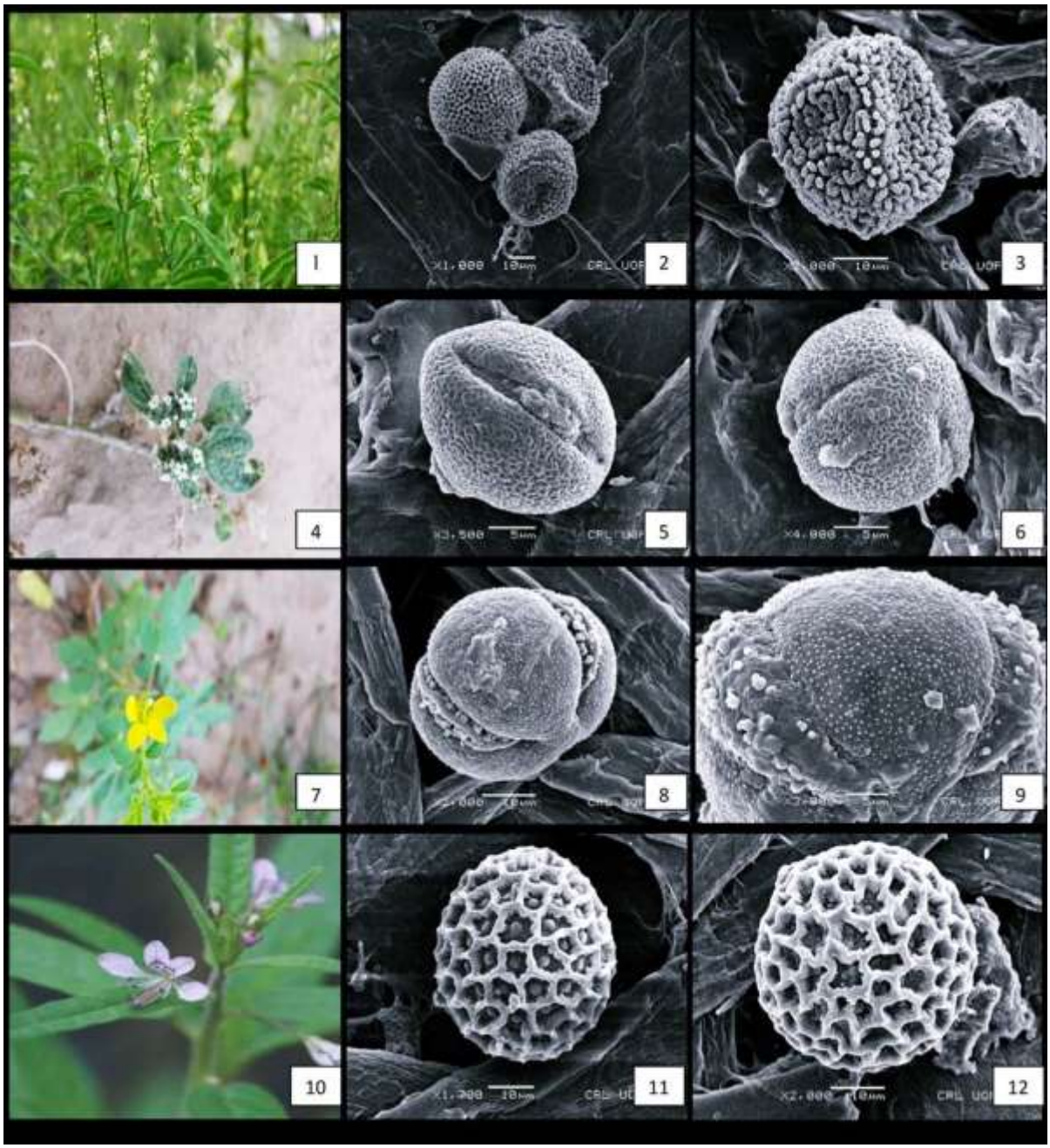

Figure 1: SEM micrographs showing detailed exine ornamentation. PV: polar view; EV: equatorial view; ES: exine sculpture. **(1)** *Croton bonplandianus*, **(2)** *Croton bonplandianus* (EV), **(3)** *Croton bonplandianus* (ES), **(4)** *Heliotropium europaeum*, **(5)** *Heliotropium*

europaeum (EV), **(6)** *Heliotropium europaeum* (ES), **(7)** *Cleome viscosa*, **(8)** *Cleome viscosa* (EV), **(9)** *Cleome viscosa* (ES), **(10)** *Cleome monophylla*, **(11)** *Cleome monophylla* (EV), **(l2)** *Cleome monophylla* (ES).

Figure 2: SEM micrographs showing pollen grains of **(13)** *Cleome brachycarpa*, **(14)** *Cleome brachycarpa* (EV), **(15)** *Cleome brachycarpa* (ES), **(16)** *Prosopis juliflora*, **(17)** *Prosopis juliflora* (EV), **(18)** *Prosopis juliflora* (ES), **(19)** *Crotalaria burhia*, **(20)** *Crotalaria burhia* (EV), **(21)** *Crotalaria burhia* (ES), **(22)** *Indigofera linifolia*, **(23)** *Indigofera linifolia* (EV), **(24)** *Indigofera linifolia* (ES).

Figure 3: SEM micrographs Observed detailed exine ornamentation; PV: polar view; EV: equatorial view; ES: exine sculpture. **(25)** *Dicliptera chinensis*, **(26)** *Dicliptera chinensis* (EV), **(27)** *Dicliptera chinensis* (ES), (**28)** *Solanum surattense*, **(29)** *Solanum surattense* (EV), **(30)** *Solanum surattense*

(ES), **(31)** *Physalis minima*, **(32)** *Physalis minima* (EV), **(33)** *Physalis minima* (ES), **(34)** *Trianthema portulacastrum* (**35)** *Trianthema portulacastrum* (EV), **(36)** *Trianthema portulacastrum* (ES).

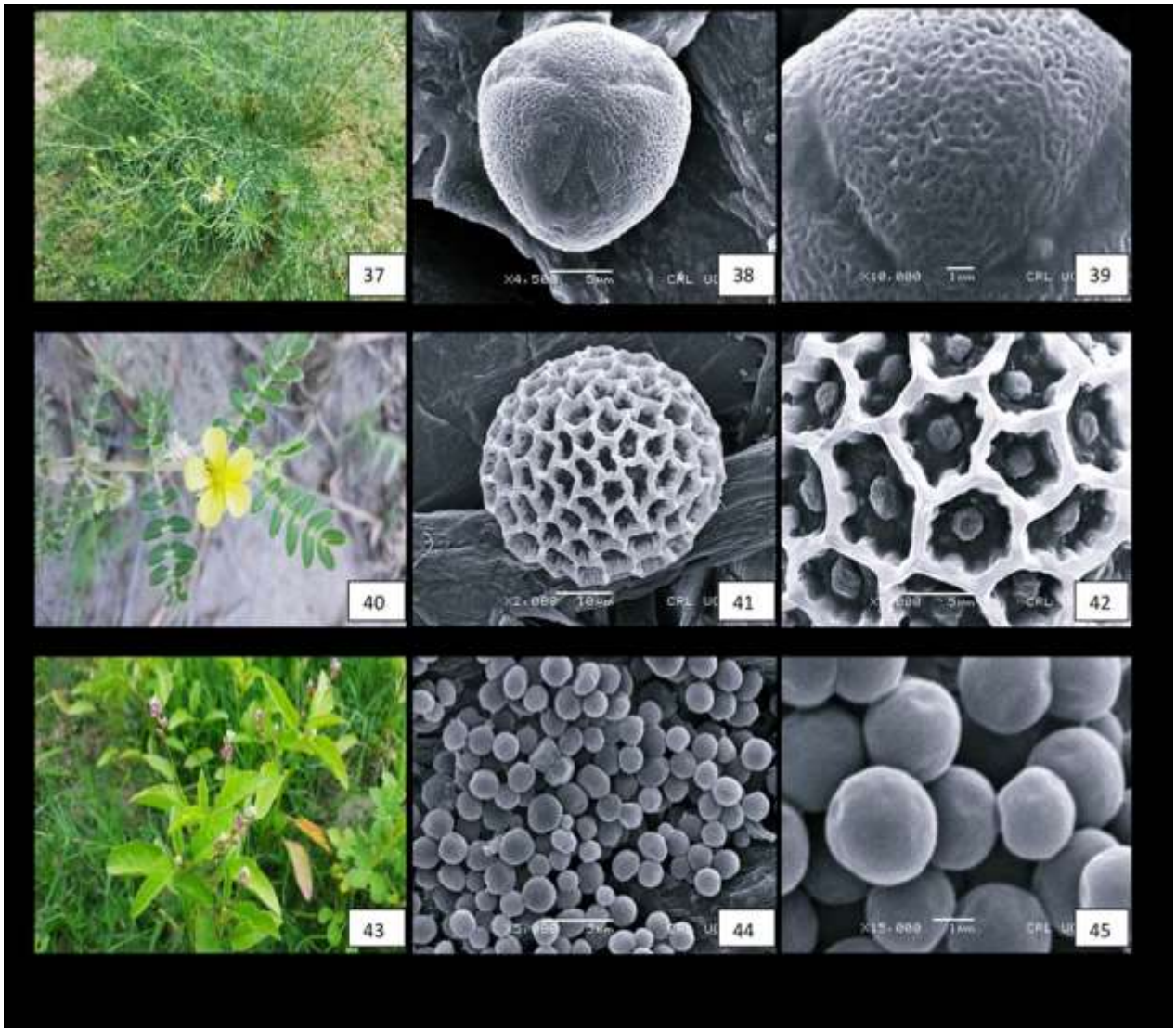

Figure 4: SEM micrographs showing pollen grains of **(37)** *Peganum Harmala*, **(38)** *Peganum Harmala* (EV), *(***39)** *Peganum Harmala* (ES), **(40)** *Tribulus Terrestris*, **(41)** *Tribulus Terrestris* (EV), **(42)** *Tribulus Terrestris* (ES), **(43)** *Digera muricata*, **(44)** *Digera muricata* (EV), **(45)** *Digera muricata* (ES).

3.6. Colpi Size

The LM and SEM pollen study showed a rich diversity in the ornamentation of the colpus surface, as well as in size and width. The largest colpus length and width were found to be variable, the maximum colpus length was observed in *Cleome viscosa* (10.89 μm) and the minimum value is observed in *Paganum harmala* (0.63 μm). The largest width of colpi examined in *Trianthema portulacastrum* (4.14 μm) and the smallest in *Paganum harmala* (0.48 μm) **(Table 4)**.

3.7. Exine sculpturing

Most prominent variations are observed for exine ornamentation patterns. 11 types of sculpturing patterns are found as taxonomic tool among the studied species. *Tribulus terrestris* has Bi-reticulate-verrucate ornamentation. *Dicliptera chinensis* has echinate regulate sculpturing of exine. *Solanum surattense* has exine with equinate-scabrate ornamentation. Foveolate- reticulate ornamentation is observed for *Cleome brachycarpa, Cleome viscosa* and *Peganum harmala* while *Croton bonplandianus* has papillate-reticulate ornamentation. Regulate sculpturing is present in *Heliotropium europaeum* and *Indigofera linifolia.* Reticulate sculpturing is revealed in *Crotalaria burhia* while *Trianthema portulacastrum* has reticulate-foveolate exine. *Prosopis juliflora*, *Cleome monophylla* and *Physalis minima* has scabrate reticulate, spinulose- verrucate and verrucate ornamentation respectively. *Digera muricata* is an exception as no exine sculpturing has been found on its exine, therefore it has psilate exine **(Figure 5, 6).**

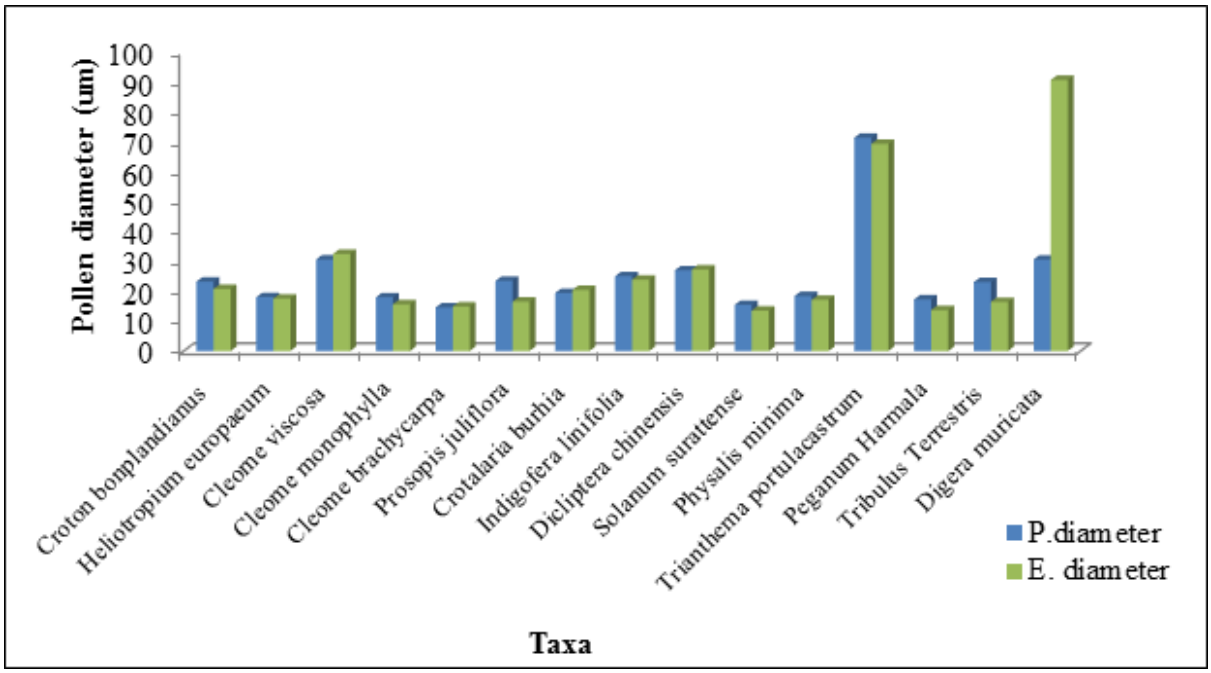

Figure 5: Comparison b/w polar and equatorial diameters of pollen grains of different Plant species

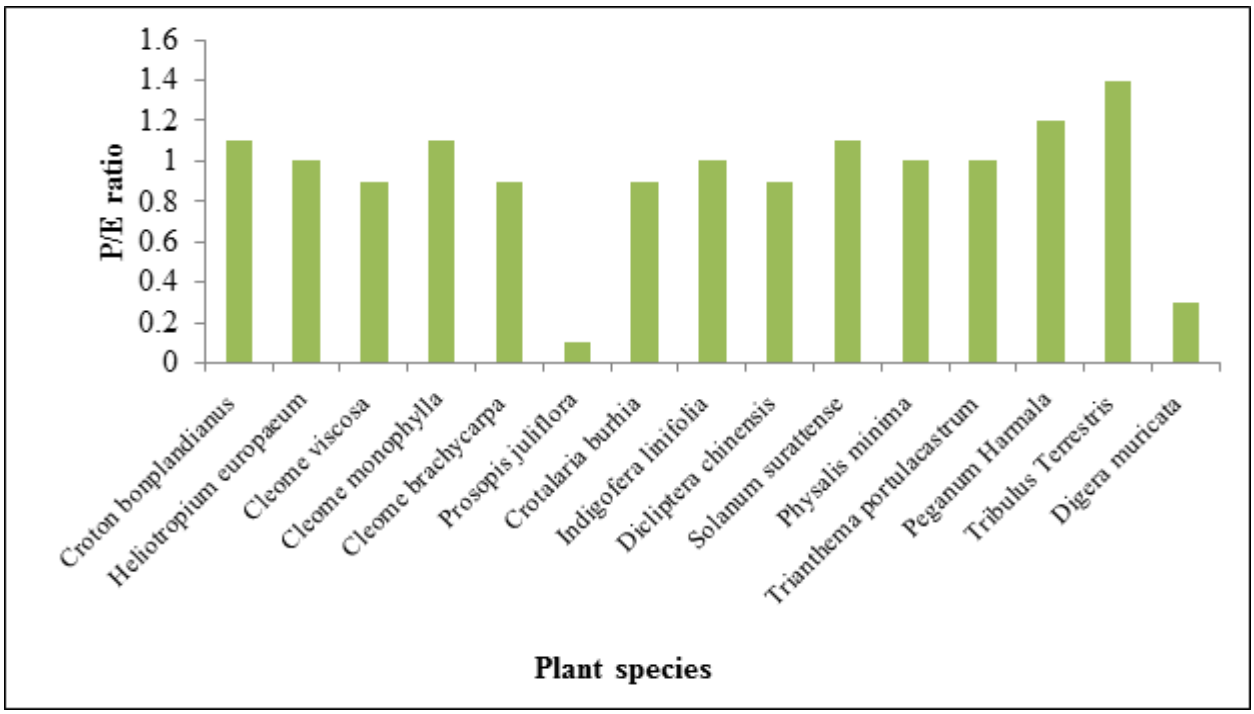

Figure 6: Polar to equatorial (P/E) ratio of different plant species

3.8. Mesocolpium

Mesocolpium ornamentation is significant taxonomically as it can aid in plant identification. Most of pollen has same mesocolpium ornamentation as exine but some pollen has shown variations as they exhibit differences between the sculpturing of exine and mesocolpium. *Peganum harmala* has foveolate mesocolpium while its exine shows foveolate-verrucate sculpturing. Exine of *Dicliptera chinensis* has echinate-regulate pattern while mesocolpium is regulate. Spinulose-verrucate exine is observed for *Cleome monophyla* but its mesocolpium has verrucate patterns. Mesocolpium is not observed in *Croton bonplandianus*, *Digera muricata* and *Tribulus terrestris*. Remaining species has same exine sculpturing as well as mesocolpium sculpturing **(Figure 7, 8).**

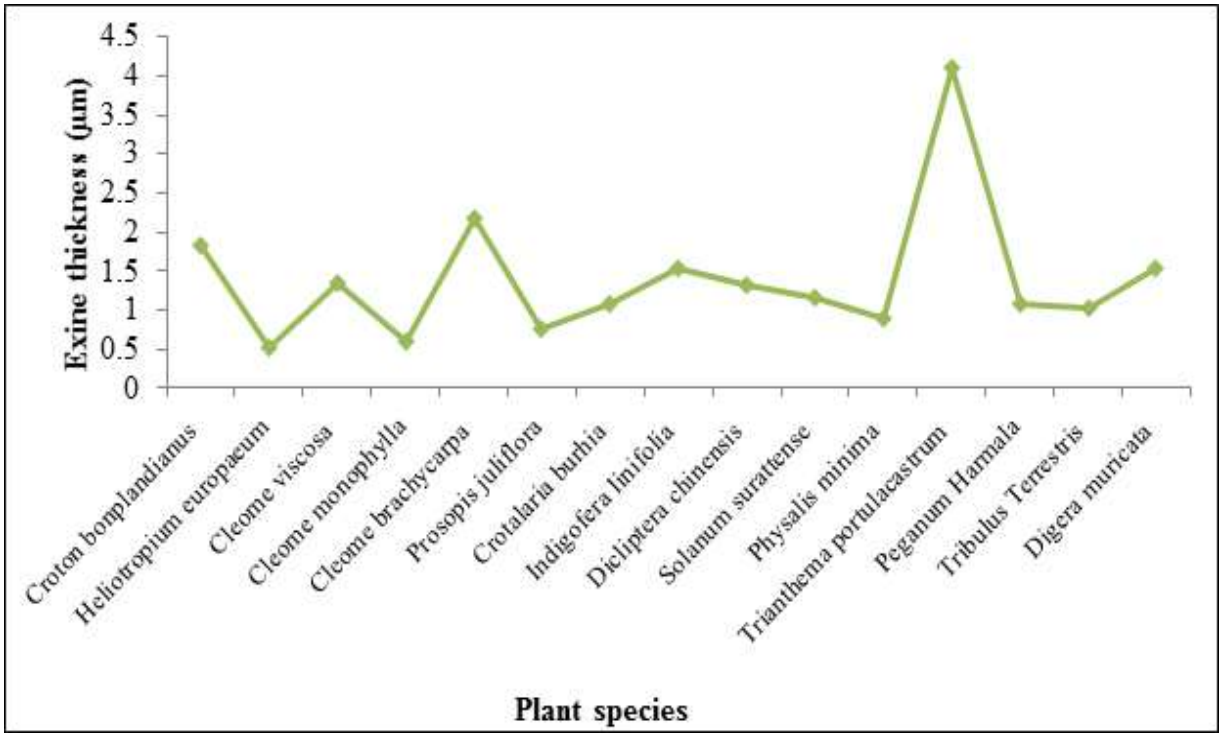

Figure 7: Variations in exine thickness of different species

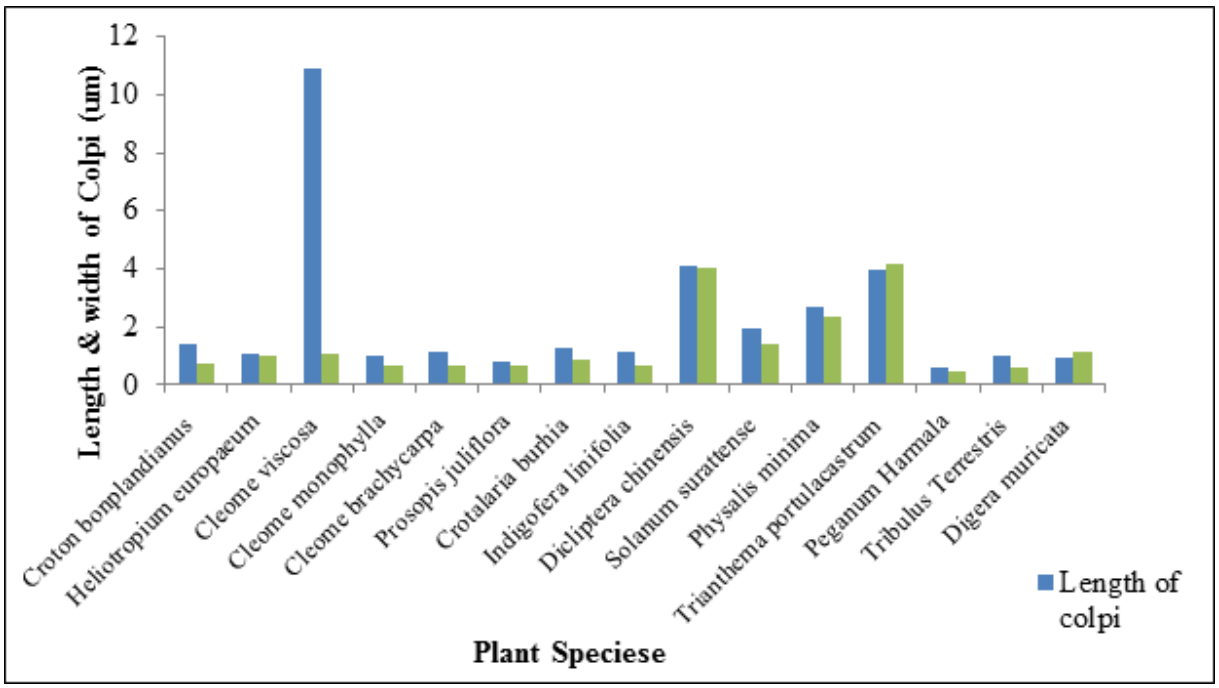

Figure 8: Comparison between length and width of Colpi

3.9. Pollen fertility and sterility

The fertility and sterility percentage has been determined for each species shown in (Table 3). Maximum number of fertile pollen percentage was counted in *Indigofera linifolia* (88.46%), while the highest sterility percentages were observed in *Croton bonplandianus* (60%). Lowest fertility and sterility percentage are observed in *Croton bonplandianus* and *Indigofera linifolia* (40 % and 11.53%) respectively **(Table 4)**.

Table 4: Quantitative palyno-morphological findings of selected medicinal plant species

S.NO	Taxa/family	P/E ratio	ET M (Min-Max) ±SE (µm)	PD M (Min-Max) ±SE (µm)	ED M (Min-Max) ±SE (µm)	Colpi (L) M (Min-Max) ±SE (µm)	Colpi (W) M (Min-Max) ±SE (µm)	Spine (L) M (Min-Max) ±SE (µm)	Spine (W) M (Min-Max) ±SE (µm)	Pollen fertility (%)	Pollen sterility (%)
1	*Croton bonplandianus* Baill. (Euphorbiaceae)	1.1	1.83 (1.05-2.55) ±0.27	23.19 (22.50-24.00) ±0.27	20.76 (19.50-22.50) ±0.49	1.38 (.75-2.25) ±0.25	0.75 (.45-1.05) ±0.10	A	A	40	60
2	*Heliotropium europaeum* Forssk. (Euphorbiaceae)	1.0	0.51 (.15-0.90) ±0.13	18.00 (16.05-20.55) ±0.76	17.55 (14.85-20.40) ±1.07	1.05 (.60-1.50) ±0.17	0.99 (.45-1.80) ±0.24	A	A	42.10	57.89
3	*Cleome viscosa* L. (Capparaceae)	0.9	1.35 (.30-2.25) ±0.33	30.60 (29.25-31.95) ±0.51	32.46 (30.75-35.10) ±0.72	10.89 (8.40-15.45) ±1.20	1.05 (.75-1.35) ±0.10	A	A	43.75	56.25
4	*Cleome monophylla* L. (Capparaceae)	1.1	0.60 (.30-0.90) ±0.10	17.94 (16.80-19.05) ±0.38	15.63 (14.25-17.25) ±0.51	0.99 (.60-1.35) ±0.13	0.66 (.30-1.05) ±0.13	A	A	61.53	38.46
5	*Cleome brachycarpa* Vahl ex-DC (Capparaceae)	0.9	2.19 (1.35-3.15) ±0.37	14.55 (13.65-15.45) ±0.30	14.88 (14.25-15.75) ±0.30	1.14 (.75-1.35) ±0.11	0.69 (.30-1.05) ±0.12	A	A	77.27	22.72
6	*Prosopis juliflora* (Sw.) DC. (Mimosaceae)	0.1	0.75 (.45-1.05) ±0.10	23.49(21.45-25.80) ±0.82	16.53(14.25-18.75) ±0.78	0.78(.30-1.35) ±0.19	0.69(.30-1.05) ±0.13	A	A	58.82	41.17
7	*Crotalaria burhia* Buch. -Ham. (Leguminosae)	0.9	1.08 (.60-1.50) ±0.17	9.41 (17.55-22.80) ±0.89	20.41 (19.05-21.90) ±0.47	1.29 (.75-1.80) ±0.18	0.87 (.45-1.20) ±0.12	A	A	67.85	32.14
8	*Indigofera linifolia* subsp. (Wight). (Leguminosae)	1.0	1.53 (1.05-1.95) ±0.16	25.02 (23.25-27.30) ±0.75	23.85 (19.80-28.35) ±1.44	1.17 (.45-1.80) ±0.24	0.69 (.45-1.05) ±0.12	A	A	88.46	11.53
9	*Dicliptera chinensis* Roem. & Schult. (Acanthaceae)	0.9	1.32 (.75-1.95) ±0.20	26.97 (25.80-28.05) ±0.39	27.24 (26.40-28.05) ±0.32	4.08 (3.45-4.95) ±0.25	4.02 (3.16-4.65) ±0.25	0.28 (.10-0.30) ±0.03	0.18 (.10-0.32) ±0.04	68.18	31.81

10	*Solanum surattense* Burm.f. (Solanaceae)	1.1	1.17 (.75-1.50) ±0.12	15.42 (14.25-16.80) ±0.47	13.50 (12.75-14.55) ±0.32	1.98 (1.50-2.40) ±0.14	1.38 (.45-2.25) ±0.30	A	A	47.82	52.17
11	*Physalis minima* L. (Solanaceae)	1.0	0.90 (.60-1.20) ±0.10	18.39 (16.20-20.25) ±0.70	17.13 (15.75-19.20) ±0.58	2.67 (1.95-3.30) ±0.24	2.37 (1.05-4.50) ±0.67	A	A	50	50
12	*Trianthema portulacastrum* L. (Aizoaceae)	1.0	4.11 (3.00-4.15) ±0.29	71.28 (70.05-72.45) ±0.48	69.24 (68.55-70.35) ±0.30	3.96 (3.15-4.50) ±0.24	4.14 (3.00-4.95) ±0.33	0.33 (.13-0.53) ±0.07	0.18 (.07-0.27) ±0.03	70.83	29.16
13	*Peganum Harmala* Crantz. (Rutaceae)	1.2	1.08 (.75-1.50) ±0.12	17.25 (15.90-19.35) ±0.60	13.68 (12.60-15.30) ±0.53	0.63 (.30-1.05) ±0.12	0.48 (.15-0.90) ±0.12	A	A	50	50
14	*Tribulus Terrestris* L. (Zygophyllaceae)	1.4	1.02 (.45-1.65) ±0.20	23.01 (21.30-24.30) ±0.53	16.41 (14.70-18.45) ±0.73	0.99 (.45-1.50) ±0.18	0.60 (.30-.90) ±0.10	A	A	80.95	19.04
15	*Digera muricata* Mart. (Amaranthaceae)	0.3	1.53(1.05-2.25) ±0.21	30.57 (29.55-31.20) ±0.28	90.63 (87.00-93.75) ±1.23	0.96 (.60-1.50) ±0.15	1.17 (.45-2.25) ±0.30	A	A	56.25	43.75

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4. DISCUSSION

The current study illustrated the comprehensive analysis of Phenological pattern and micromorphological characteristics of 15 medicinal plants in the southern region of Khyber Pakhtunkhwa, Pakistan. The vegetative and reproductive phenology of 15 plant species including 13 herbs, shrub and one tree were recorded through field visits in different seasons for one year. The phenological events were divided into vegetative and reproductive phases (flowering and fruiting). During summer season 5 plant species were reported in flowering while in winter 4 species have been recorded in flowering and 5 species were accounted in flowering during monsoon season. Similarly, 6 species in fruiting were noted in summer while 5 plant species documented to be in fruiting during winter and 4 plants were reported in fruiting throughout monsoon. One species were reported to be in flowering and fruiting throughout the year. Similar phenology pattern was studied and conducted by (Mishra et al. 2006; Maul 2008; Waman 2017; Ali et al. 2012). For the first time comprehensive study for the unique Phenological pattern of *Cleome viscosa, Cleome brachycarpa, Cleome monophylla, Crotalaria burhia, Dicliptera chinensis, Heliotropium europaeum, Indigofera linifolia, Physalis minima, Trianthema portulacastrum, Prosopis juliflora, Peganum Harmala* and *Tribulus terrestris* were conducted.

In the present observation, leaf fall can be total or partial depending on the species. In some truly deciduous taxa, all or most of the old leaves disappeared before the new ones arrived and the species was left empty for weeks or months. Examples of this category are *Croton bonplandianus*, *Heliotropium europaeum*, *Cleome viscosa*, *Cleome monophylla*, *Cleome brachycarpa*, *Crotalaria burhia*, *Indigofera linifolia* subsp, *Solanum surattense*, *Physalis minima*, *Trianthema portulacastrum*, *Peganum Harmala*, *Tribulus Terrestris* and *Digera muricata*. In other species such as *Dicliptera chinensis* and *Prosopis juliflora*, leaf fall and leaf flushing processes slightly over lapped in the same species. In evergreens old leaves were abscised over a period of time throughout the year, thus, retaining a steady population of functional leaves all the time. Majority of the species start leaf shedding in dry months i.e., from January and extending up to May and being low in other months. Mishra et al. (2006) stated that leaf drop is delayed due to rain and high temperature and advanced due to drought and low temperature. In the present investigation it was observed that the leaf drops of species had positive significant correlation with rain fall because of arid region. Wang et al. (2013), stated that seasonal changes in leaf particulate matter accumulation, surface wettability and micromorphology in urban tree species, including *Sophora japonica* and *Platanus acerifolia*. The synchronization of flowering with leaf flushing seems to be related to moisture,

temperature and photoperiod (Yadav and Yadav, 2008; Singh, 2013). Cool and dry winter period is responsible for maximum leaf drop whereas increase in temperature during warm and dry periods induces the leaf flushing and flowering in most of the species.

Flowering and fruiting activity was observed throughout the year with approximately 1.5 times more activity around May and June than in December. The peak period of fruit maturity in the present study was observed during winter and summer. Next to rapid fruiting activity a larger proportion of species recorded lengthy fruiting behaviour but only very few species had multiple fruiting behaviour **(Table-2)**. Almost all species had phenological patterns that synchronized flowering and fruiting in the dry months i.e., April, May and June. Frankie et al. (1974) stated the same investigation to comparative phenological studies of trees in tropical wet and dry forests. Most of the species flowered at the beginning of April and fruited near the end of May and beginning of June, needing only a short time for the development of fruits. Rest of the species flowered during April and May, fruited during December, with a moderate amount of time required for fruit development. Mishra et al. (2006) observes similar parameters working on phenology of species of moist deciduous forest sites of Similipal biosphere reserve. Flowering and fruiting at hottest summer i.e. April and May have selective advantage. It is more efficient to transfer assimilates directly into growing organs rather than having to store them and mobilize and translocate them latter (Antoniadis et al., 2017). We observed strong positive correlations between the temperature of hottest months and the number of species fruited in the same period. This perhaps establishes that increased temperature favors formation of fruits in most of the species.

For the identification of deciduous, semi-evergreen and semi- deciduous taxa at different taxonomic levels Palyno-morphological characterizations play an essential role. There are large variations observed in the micromorphological characteristics of the pollen between the thickness of the exine, polar diameter, equatorial diameter, length and width of colpi, length and width of the spine, pollen shape and colpi/pore.

SEM is a modern technique that has been primarily used to solve taxonomic problems by studying morphological details which, in turn, help identify and authenticate medicinal plants (Ahmed et al., 2019). Medicinal plants play an important role in the development of effective therapeutic agents. By some estimates, two-thirds of the world's plant species have potential medicinal values (Hosseini et al., 2018). Therefore, the present study is an attempt to introduce new herbs with their micromorphological details, so that researchers can use the correct herbs identified for future development of medicinal plants.

Heliotropium europaeum is deciduous belongs to the Boraginaceae family, its common name is Herporai, widespread in Afghanistan, India, Russia, North Africa, Transjordan, Syria, Iraq, Iran and Russia and Pakistan. During Phenological study the flowering periods of *Heliotropium europaeum* is 04-07 (eA) while fruiting period is 06-07 (eR) respectively. The exine sculpture of pollen was adjusted; aperture condition was Tricolporate which are also documented in the previous study by (Khatamsaz, 2001) while it was different from the study of (Pérez-Gutiérrez et al., 2015). The orientation of the colpi is deeply submerged, the edges are straightened while the mesocolpium is adjusted and the spine is absent. The present investigation is different from the previous work of (Khatamsaz, 2001; Saad-Limam et al., 2005).

Croton bonplandianus belongs to the Euphorbiaceae family, native to Paraguay, Brazil, Argentina, Indonesia, India and Abyssinia. The exine sculpture is Papillate-reticulate of *Croton bonplandianus*. In the present finding, *Croton bonplandianus* does not show such similarities with the previous work by (Layek et al., 2020; Nandi and Karmakar, 2018).

Cleome viscosa, Cleome monophylla and *Cleome brachycarpa* of the Capparaceae family are distributed in India, Sri Lanka, Philippines, Indonesia, Malaysia, Australia, America, Africa, Afghanistan, Oman, Saudi Arabia, in Kenya, Egypt, Iran and Pakistan. The pollen of *Cleome viscose* and *Cleome monophylla* have Foveolate- verrucate, Spinulose-verrucate and Foveolate- reticulate exine sculpturing and the aperture conditions are Tricolporate and Tri or tetra colporate were recorded which were not similar to (Patchell et al., 2014). Colpi orientation of *Cleome viscosa* and *Cleome monophylla* have emergent and conical sharp at the edges, but wider in the middle and sunken and slit-like, the edges are sharp, while the mesocolpium is Foveolate-verrucate, Verrucate and Foveolate-reticulate. Spines are absent in *Cleome viscosa* and present in *Cleome monophylla* and *Cleome brachycarpa*, our current result from *Cleome viscosa Cleome monophylla* and *Cleome brachycarpa* has similarities with previous work from (Linn, 2020; Mir et al., 2019) while the paper conducted by (Ragho, 2020; Shilla et al., 2019) is different from current surveys. The current result does not correspond to the previous work of (Riaz et al., 2019; Lippi et al., 2007 and Ahmad et al., 2010). *Prosopis juliflora* from the Mimosaceae family, the local name is Kikrai distributed in Australia, Brazil, Africa, West Asia, Arabia, India, Arabia, Mexico, America and Iraq. The exine sculpture is reticulated with the scabrate; the condition of the diaphragm is Tricolporate, which is similar to the results of previous research conducted by (Khan et al., 2019). The orientation of colpi is sunken and slit like, Margins are pointed, while mesocolpium are Scabrate- reticulate, while the mesocolpium are Scabrate-reticulated. In previous surveys, the same results were observed by (Quamar et al., 2017).

In this study, *Crotalaria burhia* and *Indigofera linifolia* were noted with Reticulate and Regulate exine sculpturing, which is consistent with the results (Perveen and Qaiser, 1998) while reticulate exine Sculpturing was reported for same plant by (Al-Watban et al. 2013).

Dicliptera chinensis of family Acanthaceae naturally distributed in Pakistan Africa, America, Afghanistan, Nepal, Bhutan, Bangladesh, India and China. Exine sculpturing are Echinate-regulate, aperture condition is Tricolpate and colpi orientations are Sunken and slit like, Pointed margins. Mesocolpium are regulated and spines are present. The same observations were also noticed by (Kiel et al., 2017; Shamso and Fouad, 2019).

Solanum surattense commonly known as Aghzai of family Solanaceae, widely distributed in North Africa, South America, Malaysia, Philippines, Australia, Western Assam, Polynesia. Pollen were Hexacolpate and exine sculpturing are Equinate- scabrate while colpi orientations are Sunken and slit like and Margins are pointed, are accordance with the past study of (Ashfaq et al., 2020; Khan et al., 2020). Mesocolpium were Equinate- scabrate and spines are also present, while same results were noted by (Perveen and Qaiser, 2007).

Physalis minima commonly present in Field edges, roadsides and Sandy River flat of family Solanaceae distributed in Afghanistan Nepal, China, India, America and Australia. Pollen was Tricolporate, exine sculpturing were Verrucate while colpi orientation are Sunken and slit like and Margins are rounded. The result deviates from the previous result (Prabhakar and Ramakrishna, 2014; Song et al., 2019). Mesocolpium were Verrucate and spines were absent, shows dissimilar to our observations (Da Silva et al., 2017; Azeez et al., 2019).

Trianthema portulacastrum commonly known as Aghzikai of family Aizoaceae widely distributed in Southeast Asia, America, Africa, India and Sri Lanka. Pollen was Polycolporate and exine sculpturing is Reticulate-foveolate were recorded that were dissimilar with previous finding (Branch and Sage, 2018; Mazari et al., 2017). Colpi orientations were Sunken and linear, Margins are rounded and mesocolpium are Reticulate-foveolate while spines are absent. Nnamani and Nwosu, (2012) noticed different colpi orientations and mesocolpium that shows dissimilarities to our findings.

Peganum Harmala of family Solanaceae, widely distributed in USA, Mediterranean region, Africa, India, Tibet, North Africa, Russia, Central Asia, and Middle East. Pollen was Tricolpate and exine sculpturing is Foveolate-verrucate while colpi orientations are Sunken and mesocolpium are Foveolate which is in accordance with the findings of (El-Atroush et al., 2015; Semerdjieva and Yankova-Tsvetkova, 2017).

Tribulus Terrestris is commonly known as Maklindai of family Zygophyllaceae distributed in New Zealand, Africa, North Australia and America. The exine sculpturing of pollen of *Tribulus*

Terrestris is Bireticulate-verrucate related to the previous finding (Naghiloo & Siahkolaee 2019; Semerdjieva et al., 2011) while *Digera muricata* have Psilate exine sculpturing linked with previous observation by (Hussain et al., 2018; Kanwal and Abid, 2017).

A combined LM and SEM technique for palynological study has assumed indispensable part in the accurate identification of plants up to generic and species level. This examination uncovers that palyno-morphological characters can be utilized as taxonomic guide for accurately distinguishing proof of medicinal plants as it can diminish the danger of adulteration. Also, as a large portion of these plants are essential for traditional medicinal system, this examination can be useful in portrayal of these significant plants for their characterization in various aspects.

5. CONCLUSION

The contemporary study elucidates the pollen micromorphological attributes and Phenological pattern of 15 medicinal plants using light and scanning microscopy provided information previously unknown plant species from southern region of Khyber Pakhtunkhwa, Pakistan. Both SEM and LM study of pollen grains has played a vital role in identifying the plants up to genus and species level. Furthermore, strengthen the systematic position of the flora of the particular region. Based on present findings, it is concluded that LM and SEM can be used as a tool to identify the species at micro level. This present study along with previous study comparison reveals palynological characters under light and scanning electron microscopy are of immense value to correctly identify the species. The results of the study show clear difference in pollen diameter, exine thickness, sculpturing and the shape of pollens and pollen fertility. *Cleome brachycarpa* was found with a small size of 14 µm, while in 10 taxa, namely *Croton bonplandianus, Cleome monophylla, Prosopis juliflora, Cleome viscosa, Crotalaria burhia, Indigofera linifolia, Dicliptera chinensis* and *Physalis minima* were medium size. Large sizes were observed in *Trianthema portulacastrum*, that is, 71.28 µm. The highest pollen fertility percentage was counted in *Indigofera linifolia* (88.46%), while the highest sterility percentages were observed in *Croton bonplandianus* (60%). Lowest fertility and sterility percentage are observed in *Croton bonplandianus* and *Indigofera linifolia* (40 % and 11.53%) respectively. It is therefore recommended to study pollen micro morphological features to identify taxonomically problematic taxa. The aim of the research is to expand the morphological knowledge of the species, thus contributing to taxonomic knowledge and subsequently conservation of species in the arid zone.

6. ACKNOWLEDGMENT

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7. CONFLICT OF INTREST

All the authors have no conflict of interest.

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