# Tendril Anatomy and Palynological Studies of Cucurbitaceous Taxa from District Bhakkar, Punjab, Pakistan



**By**

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# Tendril Anatomy and Palynological Studies of Cucurbitaceous Taxa from District Bhakkar, Punjab, Pakistan

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# CERTIFICATE

This is to certify that this dissertation submitted by **Mr. Naveed Abbas** entitled **"Tendril Anatomy and Palynological Studies of Cucurbitaceous Taxa from District Bhakkar, Punjab, Pakistan"** is accepted in its present form, by the Department of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan as satisfying the thesis requirements for the degree of Master of Philosophy (M.Phil.) in Plant Sciences.

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**DEDICATED**

**To**

**My Beloved Late**
**Mother & My Father**

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Up and above anything else, all praises to The Allah Almighty alone, the Omnipotent, the Merciful and Compassionate. Knowledge is limited and time is short to express the dignity, the Propitious, the Benevolent and Sovereignty of ALLAH, Whose blessings and glories have flourished my thoughts and thrived my ambitions. Peace and blessings of Allah be upon last Prophet Hazrat Muhammad (PBUH). Trembling lips and wet eyes pray for the Holy Prophet Hazrat Muhammad (PBUH) for enlightening our conscience with an essence of faith in Allah, converging all His kindness and mercy upon him.

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## ABSTRACT

This research is confined to presenting taxonomic information on the family Cucurbitaceae from the District Bhakkar Punjab-Pakistan. This study elucidates the micromorphological characterization of Cucurbitaceous species adapted to arid environment were analyzed using light (LM) and scanning electron microscope (SEM). A total of 17 Cucurbitaceous taxa were categorized into 07 genera collected from March 2022 to August 2022 including *Cucumis* (five species), *Cucurbita* and *Luffa* (three species each), *Citrullus* and *Momordica* (two species each) while *Lagenaria* and Praecitrullus (one species each). Tendril transverse sections were cut with a Shandon Microtome to prepare slides. Tendril shapes observed are irregular, slightly oval-shaped, slightly C shaped, angular, and star shaped. *Cucurbita pepo* had a maximum tendril diameter length of 656.1 µm and a minimum in *Momordica balsamina* of 123.05 µm. The highest number of vascular bundles (12) were noticed in *Luffa acutangula* var. *amara.* Morphological variations in Cucurbitaceous pollen grains may aid in the delimitation of Cucurbitaceous species using visualized scanning sculptured elements patterns. Pollen grains were processed through the acetolysis technique, measured, and photographed. The pollen grains are monads, medium to large in size, shape prolate-spheroidal (nine species), oblate-spheroidal (six species and spheroidal in two species with a circular to triangular amb. Tricolporate types of the apertures analyzed in the genera are to be separated into different varieties. The pollen diameter measured maximum for the polar axis was 93.8 µm and the equatorial distance was 97.4 µm in *Cucurbita maxima*. Exine thickness was calculated highest at 5.9 µm for *Praecitrullus fistulous.* Mesocolpium ornamentation shows scabrate-reticulate, reticulate, scabrate-perforate, gemmate-verrucate, baculate-echinate, reticulate-gemmate and scabrate-echinate sculptural features. Statistical UPGMA dendrogram and PCA clustering of micromorphological traits can be used to identify species and will play a vital role in future taxonomic and phylogenic linkages. A dichotomous key based on palyno-anatomical characters to the Cucurbitaceous species was also constructed. The taxonomic micromorphology of Cucurbitaceous species provides a better understanding of their systematics and plays a significant role in their accurate identification. The purpose of this study is to provide an overview of the tendril microanatomical and pollen morphological features as a foundation for future research at the molecular systematic level.

# CHAPTER: 1

# Introduction

## 1.1 Family Cucurbitaceae

The Cucurbitaceae family contains approximately 825 species divided into 118 genera, with a mainly tropical distribution (90% of the species) across Africa, Central and South America, and Southeast Asia (Lima et al., 2010). According to Schaefer and Renner (2011), Cucurbitaceous species, which are primarily found in tropical and subtropical regions of the planet, are rare in temperate hotspots. In Pakistan, this family is categorized into 17 genera and 32 specific and intraspecific taxa (Akhtar et al., 2019). Prostrate plants, monoacious or dioecious; with tendrils; alternate leaves, palmately veined; actinomorphic, dichlamydeous flowers, with hypanthium; sepals 3, and petals 5; stems 3-5, ovary inferior and generally berry fruit.

The Cucurbitaceae family, or cucurbits, are most widely distributed in subtropical and tropical climates, with hotspots in West Africa, Southeast Asia, Mexico, and Madagascar (Schaefer and Renner, 2011). Cucurbitaceous members (watermelons, cucumbers, luffas, pumpkin, courgettes, zucchini, and summer squash) are all edible and can be found growing across all continents. Around 800 species and 130 genera can be distributed worldwide (Jeffrey, 2005). In West Africa, this family is represented by 24 genera and 54 species (Hutchinson and Dalziel, 1954). In Pakistan, it has 33 species across 17 genera, including both domesticated (22 species) and wild species (11 species) (Okoli, 2013). The wild genera are *Coccinia*, *Lageneria*, *Luffa*, *Momordica*, and *Zehneria*, whereas cultivated include *Citrullus*, *Cucumis*, *Cucurbita*, *Cucumeropsis*, *Lagenaria*, *Telfairia*, and *Trichosanthes* (Okoli, 2013). However, Pakistan's cucurbit species with high nutritious potential remain unexplored (Jeffrey, 1964).



Cucurbitaceous family members have lianous plant bodies, peculiar fleshy fruits (known as pepo), and a similar system of sex determination. The Cucurbitaceous species are primarily herbaceous plants with diverse pubescence and tuberous roots (Jeffrey, 2005; Loy, 2013). They are also physically distinguished by frequently angled stems and bicollateral vascular bundles that are frequently grouped in two concentric rings. The leaves are petiolate, exstipulate, alternating, often palmately veined, simple, or sedately complex, with extra-floral nectaries. The tendrils are lateral to the base of the petiole, usually one to four at each node, branching, simple-lobed, with a non-spiraling base (Okoli, 2013).

## 1.2 Tendrils: Synapomorphy for Cucurbitaceae

Tendrils are filamentous plant organs that coil on contact with an object, thereby providing mechanical support for climbing to reach more sunlight. Plant tendrils are considered to be modified structure of leaves, stems, or inflorescence, but the origin of cucurbit tendrils is still argued because of the complexity in the axillary organ patterning. Most cucurbitaceous plants commonly form tendrils, which have variously been interpreted as modified flowers modified stems or modified stem–leaf complexes in structural aspect (Mizuno et al., 2015). Cucurbits are distinguished from their closer relatives by the presence of tendrils, a specialized organ that allows them to adopt a climbing habit. This family exhibits a complex evolutionary history and contains many species of agronomical value. Tendrils are an excellent example of convergent evolution because they have evolved numerous times among angiosperms. They can be found in lineages that are not closely related, such as Magnoliales and Asterales (Hernandes-Lopes et al., 2019). Tendrils are a perfect demonstration of their wide variation in morphology and ontogeny over the course of evolution. Tendrils can develop from modified twigs, pedicel, stipules, entire leaves, leaflets, leaf bracts, leaf apex, and inflorescences (Sperotto et al., 2020). Guo et al. (2020) were interested in the evolution of tendrils, as the emergence of climbing habits likely played an important role in the diversification and evolutionary success of Cucurbitaceae.

## 1.3 Diagnostic Characters

Plants are annual or perennial herb or shrubs. Roots are fibrous or tuberous. Stems are usually angular. Leaves are alternate, tendrils solitary, and exstipulate. Plants are monoecious or dioecious. Flowers are unisexual, paniculate, racemose, or subumbellate. Calyx is usually 5-lobed and imbricate. Corolla is valvate and involute. Stamens are usually five. Ovary is usually inferior. Fruit is often a pepo. Seeds are often numerous, exalbuminous. The Cucurbitaceae family is related to the Begoniaceae, Datiscaceae, and Tetramelaceae, with which it shares inferior ovaries and parietal placentation (Zhang et al. 2006).

## 1.4 Taxonomy and Systematics

The word taxonomy is combination of two Greek words “taxis” means arrangement and “nomos” means law. According to (Lincoln and Clarke, 1998) and (Wagele, 2005) taxonomy is related to classification and description of organisms. The word systematics is derived from Greek word “Systema” related to the classification of organisms as well as their phylogenetic relationships. According to (Guerra Garcia et al., 2008) systematics is the study of arrangement of organisms to know about their evolutionary relationships. Taxonomy and systematics seems to be same however besides classifications, identification and nomenclature, systematics also deals with the phylogeny. According to (Wagele, 2005) systematics tells us about the phylogenetic relationships among intra-specific taxa but not related to the identification of new species. Systematics is the study and classification of organisms for the determination of the evolutionary relationships of organisms. That’s why the systematics covers the both taxonomy and evolution (Coccia, 2019). Their tendrils readily distinguish Cucurbitaceae from their closest relatives, and the family’s monophyly is well supported by molecular analyses that have included a dense sampling of Cucurbitaceae (Renner and Schaefer, 2016).



## 1.5 Anatomical Significance

According to biosystematics point of view, the foliar epidermal study is one of the most conspicuous taxonomic features and based on leaf epidermis, the various taxonomic investigations are contrived by studying different families. Some of the taxonomist admitting that taxonomic thesis is now contemplated incomplete without epidermal studies as they are important microscopic structures (Essiett et al., 2012). Anatomical investigation of vascular plants brings evolutionary changes within the last few decades and its implementation in taxonomy have been considered noteworthy (Ahmad et al., 2010) .The ecological condition may be influenced by the epidermal features of plants with huge distinction have been investigated (Talebi et al. 2017).

Foliar epidermis is one of the most substantial taxonomic character in context with biosystematics and taxonomic studies of various families are completed on the basis of leaf epidermis (Bostanci Ordu et al., 2021). Anatomical studies have been used effectively to explain taxonomic position and support in the identification of different species (Belmonte et al., 2021). The leaf epidermal characters have been evidenced to be very important in the taxonomic study. In ancient times both anatomical and morphological studies were used for solving taxonomic difficulties of monocots (Nitu et al., 2021).

Environmental changes do not affect a plant's anatomical characteristics (Stace, 1980). Anatomical information was utilized to identify plant species, genera, and families. It is frequently employed in systematic identification, giving anomalous groupings a better classification position and illuminating relationship patterns that morphological traits may not have entirely conveyed. Nevertheless, it has been documented that cucurbit species in Pakistan can be distinguished by their plant morphology and anatomy (Hutchinson and Dalziel, 1954). Several (Agbagwa and Ndukwu, 2004; Ajuru and Okoli, 2013; Josephine et al., 2015) researchers describe anatomical characters of Cucurbitaceous species however, tendril micromorphology is not visualized (Amonwu and Okoli, 2013). Plant cell and tissue distributions such as sclerenchyma, vascular bundles, and other anatomic traits have been described at various systematic levels for species delimitation (Luchian and Teodosiu, 2019). The presence of trichomes on the lower surface of the leaves, which reflects significant taxonomic relevance, is well recognized in the Cucurbitaceae (Ielciu et al., 2015). Comparative and systematic studies on the anatomy of the various vegetative organs (root, stem, and leaf) of the species of the Cucurbitaceae family were carried out (Agbagwa and Ndukwu, 2004; Ajuru and Okoli, 2013; Mohammed and Guma, 2015; Săvulescu and Hoza, 2011).



### 1.6 Palyno-morphological Studies

Palynological features provide ancillary knowledge as well as indulge the systematic stand of species with its individual family. Pollen morphology can help and support the taxonomic approach (Munsif et al., 2007). The word palynology was first confined by Hyde and William (1944). Since then in Botanical sciences has become an advanced sub-division with distinct applications. Microscopic investigation of spores, pollen, and some other imperceptible structures are known as palynology. Fungal and algal spores, dinoflagellates, acritarchs and their cyst (dinocysts) are included in other structures. All these microscopic bodies are also known as palynomorphs and its diameter ranges from 5 µm-500µm.It was first considered only the morphological study of spores and pollen thus palynology can also describe as the study of organic-walled microfossils (acritarchs).Earlier concept about pollen was that it causes can only a high level of hay-fever allergies in human beings but the perception becomes change later on. Pollen is the micro-gametophyte or male gametophyte of phanerogams (seed-producing plants) produced male gametes. Some of the flowering plants of angiosperm-like conifers pollen grains exist with some unique microscopic size features like bilateral air bladder helping anemophilous transport of grains and some pollen-produced hook-like structures in exine wall for entomophilous pollination (Ajipe and Adebayo, 2018). Previous studies revealed that pollen morphology show advancement in taxonomic characteristics like pollen type, exine and sexine appearance, position, measurement, no of aperture, type, ornamentation. Pollen grains are contemplated as advantageous way in morphological and phylogenetic studies. Pollen grains are used to solve taxonomic difficulties, study the effects of climate change on plants, and keep conservation efforts up to date (Ullah et al., 2022). Traditionally, the potential of morpho-palynological attributes has been a subject in plant systematics that has recently been expanded significantly. It is necessary to explain pollen data for the unstudied plant families, especially for those with important phylogenetic positions (Majeed et al., 2022b). Pollen morphology has provided phylogenetic useful information in microteaching at the community level by utilizing diverse Angiosperm families. Pollen grains are distinguished by conspicuous morphological characteristics represented in their exine, serving as crucial criterion for morphological comparisons in blooming plants (Raza et al., 2020).

Pollen characteristics are important in classifying taxonomic groups such as subfamilies, tribes, genera, and species. Several investigations on the pollen morphology of the group, such as those by Lima et al., (2011), Lekhak et al., (2018), and Cruz-Barros et al. (2011), contributed to the family identification (Zienkiewicz et al., 2012). According to Lima et al., (2010), the pollen of Cucurbitaceae is eurypalynous, with significant changes in grain form, ornamentation pattern, and aperture position amongst species. However, among tribes, subtribes, and genera, pollen is often stenopalynous, with only minor variation between pollen species. Palynological data are thus extremely valuable as a taxonomical tool at the tribal or sub-tribal level (Hassan et al., 2019). In Pakistan, Perveen and Qaiser (2008) morpho-palynological observed nine Cucurbitaceae species belonging to seven taxa.

Pollen morphology supports the split of the Cucurbitaceae into two subfamilies, according to accounts on the relevance of pollen features in the Cucurbitaceae (van der Ham et al., 2010). These findings indicate that the pollen of the Fevilleoideae family is quite uniform: 3-colporate, medium-sized and striate, and perforated to reticulate. Cucurbitoideae pollen was characterized as significantly more diverse: often bigger than 40 m in length with varied aperture and exine ornamentation types, although (sub) prolate forms and striate ornamentation were reported as extremely unusual. Kocyan et al. (2007) linked pollen diversity to molecular phylogeny in the family. The tectum is usually reticulate, but it can also be verrucate or reticulate-rugulate (Perveen & Qaiser, 2008). Cucurbitaceae pollen grains are tectate to semi-tectate and shed in monads or tetrads. Pollen grains in the Cucurbitaceae family exhibit radial symmetry and are isopolar, triporate, or tricolporate, with tectum that is finely to coarsely reticulate (Barth et al., 2005).



### 1.7 Economic Importance

Cucurbitaceae is botanically highly specialized family of mostly climbing and trailing plants that is extremely important to man as a food source. The family is economically significant as a source of food ornamentals (Tin et al., 2020). The Cucurbitaceae comprises many important agronomic crops such as melons, zucchini, cucumber, pumpkins, and watermelons. Cucurbits are robust, prostrate vines that provide fruit in a variety of forms, sizes, and flavors (Zienkiewicz et al., 2012). Many Cucurbitaceae members, including cultivated species, are economically significant, owing mostly to the utilization of their edible fruits and seeds (Lutz et al., 2021). The fruits and seeds can also be consumed and have rich medicinal value (Zheng et al., 2021; Mukherjee et al., 2022). These plants have many pharmacological effects, such as anti-cancer, anti-oxidation, antithrombotic, and antibacterial effects (Karrar et al., 2019).

*Citrullus lanatus* is a succulent species belonging to the *Citrullus* genus and are desert vines and the only genus in the family with pinnatifid leaves. Watermelon (three varieties) and brown-seeded melon, both with bitter pulp, are members with solitary staminate flowers, tiny sepals, a basal corolla sectioned into five parts, and fleshy fruits. These are members of the subspecies *C. lanatus*, which is commonly cultivated in Pakistan. *Cucumis*, true melons, honey melons, and West Indian gherkins are all members of the twinning, tendril-bearing plants that belong to the *Cucumis* genus (Ghebretinsae et al., 2007). The leaves rarely split beyond the center. The fruits are smooth, green-lined, or hairy, with the appearance of a ground trailer. *Cucurbita* is a genus with approximately 20 species. The primary cultivated squash and pumpkin are four different *Cucurbita* species*: C. pepo, C. sativus, C. maxima,* and *C. pepo* var *cylindrica.* Ripe and immature fruit is the most important edible plant parts, although some species also consume seeds, flowers, roots, and even leaves. Cucurbitaceous species are not the only source of food, but are also used as a nutraceutical and pharmacotherapeutic potential. The pepo's sweet, delicately flavored, juicy flesh is eaten raw, frequently as a dessert. *Cucumis melo,* often known as sweet melon, is a fruit, not a vegetable (Omokhua-Uyi et al., 2020).

### 1.8 Justification of the Study

In this project, we link taxonomic assemblages to specific arid vegetation types with a new spectrum with specific morphological-anatomical and palynological, specific Cucurbitaceous species and related habitats, providing a solid foundation for further tracing the evolutionary linkages. Although in recent year's field explorations, regional floras and taxonomic reviews regarding the Cucurbitaceae have been increasingly published, for many taxa the taxonomic data are lacking and/or show significant gaps of knowledge.



Systematic and detailed investigations are of great importance to record the occurrence of plant species. Their taxonomic investigation will enable one to assess the botanical potentialities for useful exploration and researches on economic utilization. To undertake such investigation it is important that the dicot flora of the region should be well known. It would be possible to assess the vegetable wealth of the region only when all the plants are properly collected and preserved, accurately identified and described for a system of classification. In Pakistan, Systematics is now well recognized field and explored various eminent angiosperm plant species to identify their taxonomic characters to accurately identify the species but still many regions such as District Bhakkar as a deserted region are still unexplored taxonomically.

With respect to spectrum of systematics exploration Cucurbitaceous medicinal plant species in the Bhakkar zone has been ignored for many reasons. Major reasons are unavailability of research and academic institutes in this region. The study of taxonomic features representing family Cucurbitaceae has proved very helpful in taxonomy at different hierarchical levels. Therefore, this study is in the continuity of previous work conducted on the district Bhakkar (Ahmed et al., 2014; Chaudhari et al., 2013; Khan, 2009; Malik et al., 2015; Shaheen et al., 2014), however, the majority of above-reported studies from district Bhakkar presents only checklist of plant species along with ethnomedicinal documentation without any focus on pollen, and anatomical micromorphological traits of angiosperm species. Vegetation of Thal in general is not constant; it varies from year to year, depending upon the moisture level. The establishment, growth, regeneration and distribution of the plant communities in the deserts are controlled by many factors, such as geographical position, physiographic features and human impacts (Fakhireh et al., 2012).

The surveys and identification of the Cucurbitaceous plants growing in the district Bhakkar, the vegetation types, life forms and floristic categories of the collected species were taken into consideration. For future reference, voucher herbarium specimens of different Cucrbitaceous specimens were pressed and deposited in the Herbarium of Pakistan (ISL). Therefore, the present study was evaluate the taxonomic value of medicinally important Cucurbitaceous species, based upon multiple parameters including morpho-anatomy and palynology using scanning electron microscopy aid in correct identification of species.



### 1.9 Aims of the Study

- Enlist floristic checklist of Cucurbitaceous along distribution localities and their geographical coordinates.
- Identification and preservation of Cucurbitaceous using flora of Pakistan and herbarium techniques.
- Micromorphological studies based on taxonomic tools using tendril anatomical and palynological (LM and SEM), characterization for classification of Cucurbitaceous species.
- Elucidation of differences based on micromorphology of palyno-anatomical structure of Cucurbitaceous species at generic, species and varieties level.

# CHAPTER: 2

# Materials and Methods

The present study shows the tendril anatomical and palynological investigations of Cucurbitaceous species

## 2.1 General background

Extensive surveys of various localities desert areas of Bhakkar district were conducted for the collection of Cucurbitaceous plants from March to July 2022. The laboratory research work was carried out during August to October 2022 in the Plant systematic and biodiversity laboratory of Quaid-e-Azam University Islamabad. The main goal of this research is to expand the palyno-anatomical study of family Cucurbitaceae for their accurate identification.

## 2.2 Study area: District Bhakkar

The area of study of the present thesis work is Bhakkar district, located in Punjab province of Pakistan. It shares its border with Mianwali in the North, Layyah in the South, Khushab in the North East, Jhang in the East and Dera Ismail khan in the West as shown in figure 1. It covers an area of 8,153 km2 with the city of Bhakkar, Darya Khan being the principal town. t is sandy humid desert located in Southern Punjab. It lies in humid subtropical climatic category with mild and warm temperatures. According to census of 2017 the total population of the district Bhakkar was 1.651 million (Hussnain et al., 2019). Thal sand dunes and xeric vegetation are the main features of the desert landscape. Bhakkar is divided into four tehsils: Kalurkot, Darya Khan, Bhakkar and Mankera. Average annual temperature is 24.6 o C while mean annual precipitation is 213 mm (Khan et al., 2021).



## 2.3 Plant sampling and Field Data Collection

For systematic studies 17 Cucurbitaceous species were collected including their root, stem, leaves and floral parts of plants also collected for pollen study. During the collection of plants, details of collected species such as local name, medicinal use and voucher number was given as shown in (Table 1). Habit, habitat, flower color, voucher number, date of collection, collectors name and flowering periods of plant and other diagnostic characters were noted. Cucurbitaceous species sampling sites were georeferenced using a GPS device (German eTrex Venture).


**Plate 1.** Panoramic view of District Bhakkar areas; (a) Patti Bulanda (b) Mian form



**Plate 2.** Panoramic view of Thal desert areas; (a) Gohar Wala (b) Rakhhonda lala



**Plate 3.** Field plant collection from Bhakkar localities; (a) *Citrullus colocynthis* (L) Schrad. (b) *Cucurbita pepo* var. *cylindrica*

### 2.4 Identification

The identification of Cucurbitaceous plants was done by matching the plants with specimens mounted on herbarium sheets in herbarium of Pakistan (ISL) Quaid-i-Azam University Islamabad, Pakistan and also with the help of flora of Pakistan. The online source was also used in the conformation of synonyms and perfect spelling of scientific names of plants (https://wfoplantlist.org/plant-list).

### 2.5 Plant Preservation

Cucurbitaceous specimens collected from field were pressed in presser along with flowers place in newspaper. Dried plants were then poisoned with chemicals. For the purpose of poisoning five gram of Mercuric chloride were mixed with one liter of Ethyl alcohol. In this solution plant sample dipped for some time and then dried with the help of blotting paper, which were then mounted on sheets of Herbarium and then submitted to the of Herbarium of Pakistan (ISI) Quaid-i-Azam University Islamabad.



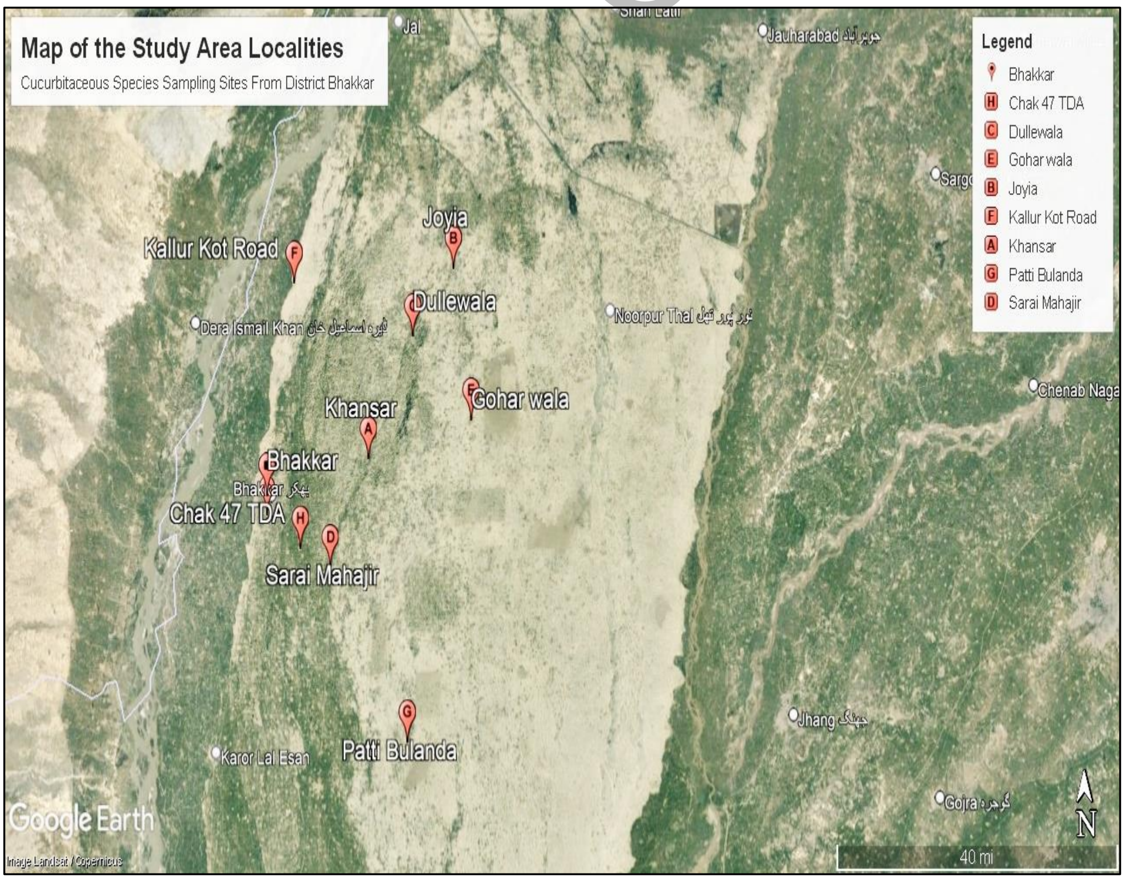


**Figure 1.** Map of the study area localities: District Bhakkar (Google Earth)

(a)

(b)

**Plate 4.** Cucurbitaceous specimens (a) Preservation (b) Mounting of on herbarium sheets.


**Plate 6.** Mounted Herbarium Specimens: (a) *Citrullus colocynthis* (L) Schrad. (b) *Cucumis melo* var.*cantalupensis* Naudin (c) *Luffa acutangula* var.*amara* C.B.Clarke (d) *Luffa cylindrica*(L) M.Roem

## 2.6 Taxonomic studies of Cucurbitaceous taxa

Taxonomic study of Cucurbitaceous species was carried out and two aspects of species were studied under light microscope and scanning electron microscope.

- Tendril anatomy
- Palynology

### 2.6.1 Tendril Anatomy

#### a) Tendril Fixation

A solution of FAA (one part of 40% formaldehyde, 18 parts of 70% ethanol, and one part of glacial acetic acid) was used to fix mature tendrils for anatomical study for 12 h. They were moved for 2 h in 50% ethanol and then dipped into 70% ethanol for 2 h. Afterward, put in absolute ethanol at room temperature (Ekeke et al., 2015).



#### b) Histological Sectioning

Successive fixation of tendrils were sectioned using the standard technique outlined by (Okwuchukwu and Uwabukeonye, 2017) with several changes. Tendril sections were cleaned for about a minute, and any extra water was then removed by treating the sections with a series of alcohols ranging from 70% to 100%. The dehydrated pieces were then submerged in xylol for one hour to permeate the wax. At 60 °C, molten wax was used to preserve the tissues. Sections were transferred to cast using forceps and needles, and a cast was used for this. This wax-filled cast and its sections were chilled with cold water. The cast was afterward lifted out and processed for microtomes. A piece of around 15–20 µm was cut from half of the length from the base with the aid of a Shandon Microtome (Finesse 325). On a glass slide, these sections were moved, and egg albumen was scattered all over the slide. The slide was moved onto a hot plate and placed in an oven at 60 °C, where the wax expanded. The tissues were extracted from the wax using xylol for five minutes. Sections were washed thoroughly before being successively dehydrated with alcohol at concentrations of 100%, 90%, 80%, and 70%. For staining, fast green stain and Safranin O were applied. Slides were stained for 15 to 20 min before being cleaned with distilled water. Rehydration using a sequence of alcohol concentrations of 70%, 80%, 90%, and 100%, respectively was then performed. To make the slide better visible, xylol was employed.

DPX mountant was placed on the slide for mounting, and the area received a cover slip. Each slide had a proper label and was dried (Akhtar et al., 2022).

**c) Tendril Micromorphology**

Slides were examined using a 40x-objective LM (OPTIKA Microscope, Italy). Digital cameras were used to capture photos of each sample under the 4, 10, and 40 objective lenses.

**d) Light Microscopy**

A reading sheet was used to record quantitative data. 10 to 15 readings of each species were taken under the Meiji (MT 4300H) LM at a magnification of 40×. For each species, minimum, maximum, mean and standard deviation values of various microanatomical parameters, including the length and width of the tendrils, the vascular bundles, collenchymatous cells, sclerenchyma cells, chlorenchyma cells and parenchyma cells, abaxial and adaxial epidermal cells, and the vessel elements, were calculated (Boris and Andrey, 2019).

**e) Statistical Analysis**

The statistical SPSS 16.0 tool was used to analyze the corresponding average data for the measured values of tendril anatomical traits (Jehanzeb et al., 2020). The effectiveness of the quantitative and qualitative features were evaluated using the UPGMA clustering analysis based on the Euclidean distance coefficient using PAST 4.03 software (Hammer et al., 2001).



**2.6.2 Palynology**

**a) Pollen slides Preparation using Acetolysis Technique**

The acetolysis procedure was followed to prepare pollen slides. Pollen were extracted from the dried flowers, by scratching the anthers using pointed needle. After that pollen were crushed on glass slide for 30 seconds using few drops of acetic acid. After crushing the anthers to pour pollen dust on slide removed the anther debris with help of brush. Then 1 to 2 drops of glycerin jelly was added on the slide for staining the pollen (Zafar et al., 2022). 5 slides of each Cucurbitaceous species were prepared was observed under optical microscope at 40x magnification. The pollen photographs were taken using MEIJI Microscope Model: MT 4300H. Qualitative and quantitative morpho-palynological features were examined such as pollen type, size, shape, polar and equatorial axis diameter, aperture diameter, mesocolpium distance, exine thickness, spine features and sculpturing elements.

**b) Scanning Electron Microscopy (SEM)**

Pollen was observed under SEM following the protocol of Majeed et al., (2020a), anthers from flowers were separated and crushed in the 45% acetic acid. Afterward, pollen was sustain in 90% ethanol and finally mounted on metallic stubs. Then sputtering with gold palladium coating was done before visualization of the pollen micromorphology of ultrasculpture wall under SEM JEOL JSM5910 Model installed at the Centralized Resource Laboratory University of Peshawar.

**c) P/E Index**

The pollen shape was determined based on P/E ratio measurement using the formula of Raza et al., (2022).

$$P/E = PA/EDx100$$

Where PA indicated polar axis while ED indicates equatorial diameter of pollen

**d) Statistical Analysis**

SPSS software (16.0) tools was used to find out the arithmetic mean, minimum, maximum values, and standard error of the pollen quantitative measurement for each observed parameter. The values were presented as (Min-Max)M±SE (Zafar et al., 2022).

The software PAST3 was used to evaluate the statistics data based on UPGMA based on Euclidean distance linkages. Principal Component Analysis was also quantified to extra plot the variance factor along with Eigenvalues functions among pollen morphometrics (Majeed et al., 2022b).


**Plate 6.** Taxonomic studies (a) Microscopic slide preparations (b) Morphological characters data measurement

# CHAPTER: 3

# Results & Discussion

## 3. Summary

The District Bhakkar Punjab, Pakistan has been bestowed with rich floristic diversity. Some endemic and medicinal plants of the country are restricted to this arid desert area. The current study was conducted from different localities of District Bhakkar. This region was explored first time from taxonomic point of view. Previous work was fragmented but the present research topic is comprehensive and includes the taxonomic study of selected 17 Cucurbitaceous species classified into 07 genera. The results are compiled and presented separately in three sections.

**Section 1:** Floristic checklist of Cucurbitaceous species along with their GPS coardinates.

**Section 2:** Qualitative and quantitative tendril microanatomical characteristic include include tendril and vascular bundle shape, variation in the number of vascular bundles, tendril length, trichomes, layers of collenchyma and shape of parenchyma, scelerenchyma and epidermal cells.

**Section 3:** Qualitative and quantitative pollen micromorphological characters. Qualitative characters include pollen type, pollen shape, exine peculiarities while quantitative characters include polar diameter, equatorial distance, P/E ratio, pore size, pore number, exine thickness, colpi size, mesocolpium distance, spine size.

# Floristic checklist of Cucurbitaceous species

**Table 1.** Cucurbitaceous plant sampling along with localities, GPS coordinates, and herbarium vouchers.

| Sr. No. | Cucurbitaceous species | Locality | GPS Coordinates | Collection Date | Voucher Number | Accession Number |
|---|---|---|---|---|---|---|
| 1. | *Citrullus colocynthis* (L) Schrad. | Patti Bulanda | 31°15′24.67” N<br>71°25′28.66” E | 7-052022 | QAU-NA-3 | 132045 |
| 2. | *Citrullus lanatus* (Thunb.) Matsum.& Nakai | Mian farm | 31°17′46.31” N<br>71°24′41.17” E | 19-03-2022 | QAU-NA-16 | 132053 |
| 3. | *Cucumis melo* L. | Kallur Kot Road | 31°17′46.31” N<br>71°24′41.17” E | 19-03-2022 | QAU-NA-15 | 132059 |
| 4. | *Cucumis melo subsp. agrestis* (Naudin) Pangalo | Rakhhonda lala | 31°19′01.56” N<br>71°25′50.80” E | 07-05-2022 | QAU-NA-7 | 132049 |
| 5. | *Cucumis melo* var. *flexuosus* (L.) Naudin | Patti Bulanda | 31°15′24.67” N<br>71°25′28.66” E | 08-05-2022 | QAU-NA-6 | 132047 |
| 6. | *Cucumis melo* var.*cantalupensis* Naudin | Khansar | 31°19′01.56” N<br>71°25′50.80” E | 07-05-2022 | QAU-NA-9 | 132046 |
| 7. | *Cucumis sativus* L. | Mian farm | 31°17′46.31” N<br>71°24′41.17” E | 19-03-2022 | QAU-NA-14 | 132060 |
| 8. | *Cucurbita maxima* Duchesne | Joyia | 31°31′46.92”N<br>71°09′56.33”E | 22-03-2022 | QAU-NA-8 | 132052 |
| 9. | *Cucurbita pepo* L. | Rakhhonda | 31°31′46.92” N<br>71°09′56.33” E | 20-03-2022 | QAU-NA-13 | 132056 |
| 10. | *Cucurbita pepo* var. *cylindrica* | Dullewala | 31°33′09.40” N<br>71°10′17.71” E | 20-03-2022 | QAU-NA-11 | 132051 |
| 11. | *Lagenaria siceraria* (Molina) Standl. | Joyia | 31°33′09.40” N<br>71°10′17.71” E | 20-03-2022 | QAU-NA-17 | 132050 |
| 12. | *Luffa acutangula* (L.)Roxb | Basti Thind | 31°15′31.94” N<br>71°27′20.64” E | 22-03-2022 | QAU-NA-2 | 132055 |
| 13. | *Luffa acutangula* var.*amara* C.B.Clarke | Khansar | 31°16′13.57” N<br>71°25′03.28” E | 11-05-2022 | QAU-NA-5 | 132044 |
| 14. | *Luffa cylindrica*(L) M.Roem | Basti Thind | 31°15′31.94” N<br>71°27′20.64” E | 10-05-2022 | QAU-NA-10 | 132054 |

| | | | | | |
|---|---|---|---|---|---|
| 15. *Momordica charantia* L. | Sarai Mahajar | 31°33′09.40” N<br>71°10′17.71” E | 20-03-2022 | QAU-NA-1 | 132057 |
| 16. *Momordica balsamina* L. | Patti Bulanda | 31°09′43.53” N<br>71°12′38.90” E | 14-07-2022 | QAU-NA-18 | 132143 |
| 17. *Praecitrullus fistulosus* (Stocks) Pangalo | Gohar Wala | 31°10′28.68” N<br>71°13′32.71” E | 10-05-2022 | QAU-NA-4 | 132048 |

# Tendril Microanatomy of Cucurbitaceous Species

## 3.2 Tendril Microanatomy of Cucurbitaceous Species

Tendril anatomical traits observed in the current investigation were outline, vascularization forms, vessel elements, collenchyma, chlorenchyma, sclerenchyma, as well as parenchyma tissues as illustrated (Plate 7–15). The summarized qualitative and quantitative results are given in (Tables 1–4). Observing their recorded taxonomic evaluation, cucurbitaceous taxa showed significant size and shape variation (Plates 7–15).

Various studies described morpho-anatomical features to classify Cucurbitaceous species (Agbagwa and Ndukwu, 2004; Ajuru and Okoli, 2013). Different researchers have carried out the tendril anatomical investigation of a few species of Cucurbitaceae (Ekeke et al., 2017) and studied the anatomical characteristics of some species of the genus cucurbita, like flower stalk, petiole, and stem, and examined some tendril characteristics. Leaf anatomy of Cucurbitaceous species has been briefly mentioned by (Abdulrahaman et al., 2011), but no information is available on the tendril anatomy of Cucurbitaceous taxa, so this study elaborates tendril histology of 12 Cucurbitaceous taxa to find out taxonomic markers for their correct identification. The tendril's shape was four and five angled furrows in *Momordica charantia* and *Cucumis sativa* (Ekeke et al., 2015), which shows similarities with current results. While current studies show tendrils outlined in transverse view in different cucurbitaceous taxa are mostly irregular, slightly oval-shaped, slightly C shaped, angular (four-angled, six-angled, or polygonal), and star-shaped (Table 2). The maximum tendril length was observed in *Cucurbita pepo* (656.1 µm) and minimum was observed in *Momordica balsamina* (123.05 µm) as shown in Figure 2. Whereas the maximum width of tendril was noted in *Cucurbita maxima* (489.6 µm) and minimum in *Momordica balsamina* (112.95 µm).



There was a single-layered epidermis present in the tendril histological section and it was predominantly irregular in shape while oval-shaped cells were recorded (Ekeke et al., 2015). However, (Hasson et al., 2019) elaborates on rectangular cells whereas our findings show square, oval, isodiametric, irregular, pentagonal, hexagonal, and polygonal types. Significant variation in the tendril micromorphology of Cucurbitaceous taxa was observed on both the adaxial and abaxial epidermal sides. There was variation in the epidermal cell length and width of the studied species (Table 4). The largest cell lengthwise was noted on the adaxial side in *Praecitrullus fistulosus*

(28 µm) and the smallest in *Luffa cylindrica* (13 µm). The maximum epidermal cell width was noted in *Cucumis melo* var.*cantalupensis* (18.2 µm) and minimum in *Luffa acutangula* (8.05 µm) as shown in Figure 3.

Correspondingly, the largest cell length was calculated along the abaxial in *Praecitrullus fistulosus* (27.65 µm) and the shortest in *Cucumis melo* subsp. *agrestis* (12.85 µm). The cell width was observed to be maximum on the abaxial surface in *Cucurbita pepo* var. *cylindrica* (18 µm) and minimum in *Luffa acutangula* var. *amara* (4.1 µm). Layers of sclerenchyma and chlorenchyma (both one to eight layers) and collenchyma cells (two to six layers). However, (Ekeke et al., 2015) mentioned some variations in sclerenchyma two to nine, chlorenchyma one to three and collenchyma two to six as mentioned in Figure 4.

Angular collenchyma cells were observed from Iraq by (Hasson et al., 2019) among Cucurbitaceae species from Iraq, while angular lamellar types were examined in this study (Table 2). In previous studies, two to four layers of collenchyma were present (Ekeke et al., 2015), while present measurements revealed a distinct continuous layering of cells below the epidermis with two to six layers of collenchyma (Table 3). The maximum layers were present in *Cucurbita maxima* and *Luffa acutangula* var.*amara* both having six layers, while the minimum number of layers was present in *Citrullus lanatus*, with two or three layers. Collenchymatous cell size showing maximum length in in *Cucumis sativus* (27.85 µm) while minimum length was in *Cucurbita pepo* (12.85 µm). Whereas the largest width was calculated for *Cucumis sativus* (17.7 µm) the lowest width was for *Cucumis melo* var. *flexuosus* (8.55 µm) as illustrated in Figure 5.

There were mostly two or three layers of chlorenchyma in tendrils of Cucurbitaceae species (Ekeke et al., 2015), while in recent studies, a single layer of chlorenchyma cells lies beneath collenchyma but in some species, more than single layers noticed two or two to three layers (Table 3). Maximum chlorenchymateous layers were present in *Lagenaria siceraria* in two to three layers. Different shapes of chlorenchyma cells were inspected, such as rectangular, irregular, pentagonal, hexagonal, and polygonal (Table 2). The chlorenchymateous cell size range from largest lengthwise was measured in *Cucumis sativus* (44.25 µm) while the shortest was in *Cucumis melo* subsp. *agrestis*

(20.6 µm). Likewise, the largest cell widthwise was seen in *Cucumis melo* var. *cantalupensis* (27.45 µm), and the smallest cell widthwise was observed in *Cucumis melo* subsp. *agrestis* (8.9 µm) Figure 6.

Species like *Citrullus colocynthis* and *Citrullus lanatus* have continuous sclerenchyma cells, similar to our findings. All the studied species illustrated the continuous sclerenchymatous cells except *Cucumis melo* var *catanlupensus*, *Cucurbita pepo* var *cylindrica,* and *Momordica charantia*, discontinuous sclerenchymatous cells were recorded in these species. The continuous layer of sclerenchymatous cells that makes up the tendril acts as an anchor and requires it to be robust enough to hold the weight of the plant and its fruits, especially when it is rising (Ekeke et al., 2015). The most prominent and darkly stained layers of sclernchyma cells were just below the chlorenchyma cell layers. Sclerenchyma cell layers range from a minimum of two to three layers in three species of *Cucumis melo* subsp. *agrestis*, *Cucumis melo* var. *flexuosus*, and *Cucumis melo* var. *cantalupensis*, while the maximum number of layers was noticed in *Lagenaria siceraria* seven to nine layers, Table 2. Shapes of sclerenchyma cells are dissimilar in tendrils of studied plant species. Mainly, sclerenchyma was perceived as irregular, trigonal, tetragonal, hexagonal, and polygonal shaped, Table 1. There were variations in sclerenchyma cell size ranges, maximum cell sizes lengthwise were seen in *Luffa acutangula* var. *amara* (38.05 µm) compared to minimum cell size lengthwise in *Cucumis melo* subsp. *agrestis* (13.55 µm). The maximum width of sclerenchyma cells was noted in *Luffa acutangula* var. *amara* (17.95 µm), while minimum was observed in *Cucumis melo* subsp. *agrestis* (9 µm) Figure 7.



In earlier studies, different parenchyma cells were irregular, pentagonal, and polygonal (Table 1), butt angular parenchyma cells were also recorded (Hasson et al., 2019). In the current studies, parenchymatous cells were present in all species, mostly occupying the pith region in tendrils. The number of parenchyma cell layers differed in all studied species in Table 3. The maximum parenchyma layers were present in *Cucumis sativus* six-layers, whereas the minimum was observed in Luffa acutangula var. amara of two-layers. Parenchyma cells were the largest cells in size present in tendrils. The largest cell lengthwise is Praecitrullus fistulosus (91.55 µm), while mini cells were present in *Cucumis melo* (29 µm). The sizeable cells, widthwise, are present in *Citrullus colocynthis* (63.35 µm), and compact cells widthwise were existingin *Luffa acutangula* (13.5 µm) Figure 8.

Vascular bundles were mainly arranged in a subsidiary manner, and few were centrally arranged in the case of *Cucumis melo* var. *cantalupensis* and *Momordica charantia*, Table 2. Bicollateral-types of vascular bundles were present in tendrils. Various writers have reported this feature in the Cucurbitaceae, which is constant across the analyzed taxa (Ajuru and Okoli, 2013; Hutchinson and Dalziel, 1954; Metcalfe and Chalk, 1950; Okoli, 2013). Their shapes vary from oval, elliptical, rounded, irregular, and dumbbell, Table 2. Each studied species has a different number of vascular bundles (Table 2). The maximum number of vascular bundles was detected in *Luffa acutangula var. amara*, having 12 vascular bundles, while the minimum number of vascular bundles was in *Momordica balsamina*, having three vascular bundles. Vascular bundles also vary in size. The largest vascular bundle lengthwise was present in *Cucumis sativus* (224.25 µm), while the smallest was observed in *Cucurbita pepo* var. *cylindrica* (26.1 µm). The largest vascular bundle widthwise was observed in *Cucumis sativus* (125.3 µm), and the smallest vascular bundle was present in *Cucurbita pepo* var. *cylindrica* (19.35 µm) Figure 9.

There was no record found about the vessel elements of the vascular bundle previously, while the current study showed a distinct number of vessel elements in the xylem of the studied species' tendrils. Their numbers and size vary from species to species. The highest number of vessel elements were present in *Cucumis sativus*, of about 15, and the lowest number of vessel elements existed in *Cucumis melo* var. cantalupensis, at around four elements (Table 3). The biggest vessel element lengthwise was found in *Cucumis sativus* (42.55 µm), while the smallest vessel element lengthwise was present in *Momordica balsamina* (9.5 µm). The largest vessel element widthwise was observed in *Cucumis melo* var. *cantalupensis* (30.45 µm); meanwhile, the shortest was analyzed in *Citrullus colocynthis* (7.82 µm) Figure 10.

No tendril anatomical data were found about *Luffa aegyptiaca*. However, the species was used against hydrocarbon-contaminated soil through rhizoremediation, and chemical analysis of this species was also carried out in research papers (Ani et al., 2021). In Western Africa, three genera of the Cucurbitaceae family, e.g., *Momordica*, *Luffa,* and *Trichosanthes,* were studied for their foliar epidermis and tendril morphology. The significant differences in their leaf and tendril morphology provided additional data for classifying three genera in separate tribes (Kadiri and Olowokudejo, 2016). Furthermore, many authors studied *praecitrullus fistulosu*s for its medicinal, anthelmintic, and anticancer activities (Ishnava and Patel, 2020; Madhu et al., 2022; Shivhare and Jain, 2020). Among the tendril anatomical characters presented in the study, only a few discussed characters have been studied earlier for selected species (Ekeke et al., 2017; Ekeke et al., 2015; Hasson et al., 2019), while other species investigated in the current project have not been investigated. A detailed review of the literature revealed that there is no comprehensive study regarding the tendril anatomical features of these plants.

### 3.2.1 UPGMA Dendrogram Clustering

The UPGMA clustering dendrogram for Cucurbitaceous taxa is presented in Figure 11. Seventeen taxa of Cucurbitaceae fall into two major clusters based on the difference in qualitative features. Similarity relationships among different Cucurbitaceous species were explored using UPGMA clustering using tendril anatomical characters. The UPGMA phenogram shows two main clusters, C1 and C2. The first principle cluster, C1, represents sections *C. maxima* and *C. pepo*. The second cluster C2 further divided into two sub-clusters comprising C2a1 of 5 species in which *C. melo* and *C. balsamina* were closely related based on Euclidean distance mapping. The second sub-cluster, C2a2, represents ten species, among which, based on Euclidean distance *L. cylindrica* and *M. charantia* was placed at the minimum distance in this sub-cluster, showing the similarity in tendril qualitative features.

### 3.2.2 Identification Keys Based on Cucurbitaceous Tendril Features

1 + Lamellar Collenchyma …………………………………………………...............**2**

− Angular Collenchyma……..…………………………………..………..............**5**

2 + Vascular bundle with round shape, irregular tendril outline…...…........***L. siceraria***

- Lamellar and angular collenchyma……………….……….……………..…...……**3**

3 + Irregular vascular bundle, 6-angled tendril outline……………….……….***C. pepo***

- Oval and irregular vascular bundle………………..……………………….…..……..**4**

4 + C shaped tendril shape…………………………...……….***C. pepo* var. *cylindrica***

- Angular collenchyma cell layers……..……………………....………………….**5**

5 + Tendril outline oval, vascular bundle slightly oval……..…..….……***C. colocynthis***

- Subsidiary type vascular bundles…………………………………………………**6**

6 + V shaped tendril, elliptical shape vascular bundle…………….………...***C. lanatus***

- Irregular tendril outline…………...………………………………………………**7**

7 + Subsidiary type vascular bundle, rectangular epidermal cells……………...***C. melo***

- Polygonal sclerenchyma cells……………………………………………………..**8**

8 + 4-angled tendril outline, irregular vascular bundle…………………………………………...……..***C. melo* subsp. *agrestis***

- Irregular tendril shape…………..…………………………………………….......**9**

9 + Tetragonal sclerenchyma cells………………………………***C. melo* var. *flexuosus***

- Triangular to polygonal sclerenchyma cells………..……………….……………**10**

10 + Central vascular bundle type..……….........................***C. melo* var. *cantalupensis***

- Elliptical and irregular vascular bundle shape…………………………….…...**11**

11 + Star shape tendril outline……………………..……………………***C. sativus***

- Rectangular epidermal cells, irregular tendril………………………………………**12**

12 + Dumbbell type vascular bundle……………………………………..***C. maxima***

- Rounded vascular bundle shape……………………..……………….…………**13**

13 + Irregular with hollow pith tendril sape…………………………..…..***L. acutangula***

- Rounded to oval vascular bundle shape…………..………………………………**14**

14 + Polygonal tendril outline…………………………...… ***L. acutangula* var.*amara***

- Hexagonal tendril outline.…………………………………………………**15**

15 + Hexagonal to polygonal chlorenchyma cells……………………..……***L. cylindrica***

- 4-angled tendril, rectangular to polygonal chlorenchyma cells……………………**16**

16 + Central type vascular bundle, tetragonal sclerenchyma cells……………………………………………………………………***M. balsamina***

- Pentagonal chlorenchyma cells………………………………………..……………**17**

17 + Rounded and elliptical vascular bundle type……………..…………***M. charantia***

**-** Rectangular epidermal cells, subsidiary vascular bundle type…………………..……**18**

18 + Polygonal slightly U shaped tendril outline………………………….***P. fistulosus***

**Table 2.** Qualitative tendril anatomical characters of Cucurbitaceous species.

| Sr No. | Cucurbitaceous Taxa | Epidermal Cell Shape | Collenchyma Cell Type | Chlorenchyma Cell Shape | Sclerenchyma Cell Shape | Parenchyma Cell Shape | Vascular Bundle Shape | Vascular Bundles | Tendril Outline in Transverse View |
|---|---|---|---|---|---|---|---|---|---|
| 1. | *Citrullus colocynthis* (L) Schrad. | Rectangular and isodiametric | Angular | Rectangular to irregular | Tetragonal to polygonal | Polygonal | Oval | Subsidiary | Slightly oval |
| 2. | *Citrullus lanatus* (Thunb.) Matsum.& Nakai | Rectangular | Angular | Irregular | Polygonal to irregular | Irregular | Elliptical | Subsidiary | Irregular, slightly v shaped |
| 3. | *Cucumis melo* L. | Rectangular | Angular | Irregular | Irregular | Irregular | Irregular | Subsidiary | Irregular shaped |
| 4. | *Cucumis melo subsp. agrestis* (Naudin) Pangalo | Oval to irregular | Angular | Irregular | Polygonal | Polygonal | Irregular | Subsidiary | 4 angled |
| 5. | *Cucumis melo var. flexuosus* (L.) Naudin | Rectangular, square, and isodiametric | Angular | Irregular | Tetragonal to polygonal | Polygonal | Irregular | Subsidiary | Irregular shaped |
| 6. | *Cucumis melo* var.*cantalupensis* Naudin | Rectangular to square | Angular | Polygonal | Triangular to polygonal | Irregular | Irregular | Central | Irregular shaped |
| 7. | *Cucumis sativus* L. | Rectangular | Angular | Rectangular | Tetragonal to polygonal | Irregular | Elliptical and irregular | Subsidiary | Star-shaped |
| 8. | *Cucurbita maxima* Duchesne | Rectangular | Angular | Rectangular to polygonal | Irregular | Irregular | Dumbbell and irregular | Subsidiary | Irregular shaped |
| 9. | *Cucurbita pepo* L. | Rectangular to square | Lamellar and angular | Polygonal | Irregular | Irregular | Irregular | Subsidiary | Six angled |
| 10. | *Cucurbita pepo* var. *cylindrica* | Rectangular, square to polygonal | Lamellar and angular | Polygonal | Irregular | Irregular | Oval and irregular | Subsidiary | Slightly c shaped |
| 11. | *Lagenaria siceraria* (Molina) Standl. | Rectangular | Lamellar | Rectangular to irregular | Irregular | Irregular | Rounded | Subsidiary | Irregular with hollow pith |
| 12. | *Luffa acutangula* (L.) Roxb. | Irregular | Angular | Irregular | Irregular | Irregular | Rounded | Subsidiary | Irregular with hollow pith |
| 13. | *Luffa acutangula* var. *amara* C.B.Clarke | Rectangular, square to polygonal | Angular | Polygonal | Polygonal and irregular | Irregular | Round and oval | Subsidiary | Irregular polygonal |
| 14. | *Luffa cylindrica* (L) M.Roem | Rectangular to square | Angular | Hexagonal to polygonal | Tetragonal to polygonal | Irregular | Round and elliptical | Subsidiary | Six angled |
| 15. | *Momordica charantia* L. | Rectangular to irregular | Angular | Pentagonal to polygonal | Irregular | Irregular | Rounded and elliptical | Subsidiary | 4 angled |
| 16 | *Momordica balsamina* L. | Pentagonal to hexagonal | Angular | Rectangular to polygonal | Tetragonal to hexagonal | Pentagonal to irregular | Oval and irregular | Central | 4 angled |
| 17 | *Praecitrullus fistulosus* (Stocks) Pangalo | Rectangular | Angular | Pentagonal to polygonal | Irregular | Irregular | Irregular | Subsidiary | Polygonal, slightly U shaped |

**Table 3.** Quantitative analysis of tendril anatomy of Cucurbitaceous taxa.

| Sr No. | Cucurbitaceous Taxa | No. Of Vascular Bundles | Epidermal Cell Layer | Collenchyma Cell Layer | Chlorenchyma Cell Layer | Sclerenchyma Cell Layer | Parenchyma Cell Layer | Vessel Elements |
|---|---|---|---|---|---|---|---|---|
| 1. | *Citrullus colocynthis* (L) Schrad. | 4 | 1 | 3–4 | 1–2 | 3–5 | 4 | 6 |
| 2. | *Citrullus lanatus* (Thunb.) Matsum.& Nakai | 6 | 1 | 2–3 | 1 | 3–4 | 4 | 5 |
| 3. | *Cucumis melo* L. | 5 | 1 | 3 | 1 | 3 | 4 | 8 |
| 4. | *Cucumis melo subsp. agrestis* (Naudin) Pangalo | 5 | 1 | 3 | 1–2 | 2–3 | 4 | 8 |
| 5. | *Cucumis melo* var. *flexuosus* (L.) Naudin | 7 | 1 | 3 | 2 | 2–3 | 3 | 5 |
| 6. | *Cucumis melo* var.*cantalupensis* Naudin | 6 | 1 | 3 | 2 | 2–3 | 4 | 4 |
| 7. | *Cucumis sativus* L. | 5 | 1 | 4 | 1 | 2–4 | 6 | 15 |
| 8. | *Cucurbita maxima* Duchesne | 7 | 1 | 3 | 2 | 5 | 4 | 9 |
| 9. | *Cucurbita pepo* L. | 6 | 1 | 6 | 2 | 3–4 | 4 | 7 |
| 10. | *Cucurbita pepo* var. *cylindrica* | 7 | 1 | 4 | 2 | 3–4 | 3 | 9 |
| 11. | *Lagenaria siceraria* (Molina) Standl. | 7 | 1 | 3–4 | 2–3 | 7–9 | 5 | 8 |
| 12. | *Luffa acutangula* (L.) Roxb. | 8 | 1 | 5 | 1 | 6–8 | 3 | 8 |
| 13. | *Luffa acutangula* var.*amara* C.B.Clarke | 12 | 1 | 6 | 2 | 6 | 2 | 9 |
| 14. | *Luffa cylindrica* (L) M.Roem | 8 | 1 | 5 | 1 | 6 | 3 | 9 |
| 15. | *Momordica charantia* L. | 7 | 1 | 5 | 1 | 4 | 3 | 9 |
| 16 | *Momordica balsamina* L. | 3 | 1 | 3–5 | 1 | 1 | 3–4 | 10 |
| 17 | *Praecitrullus fistulosus* (Stocks) Pangalo | 10 | 1 | 2–5 | 2 | 4 | 5 | 5 |

**Table 4.** Quantitative tendril anatomical data of Cucurbitaceous species.

| Sr No. | Cucurbitaceous taxa | L x W | Tendril Diameter (µm) | Upper Epidermal Cell (µm) | Lower Epidermis Cell (µm) | Collenchyma Cell (µm) |
|---|---|---|---|---|---|---|
| 1. | *Citrullus colocynthis* (L) Schrad. | L | 314 − 326.25 = 321.65 ± 5.02 | 16.75 − 23.25 = 19.85 ± 2.57 | 16.75 − 22.50 = 19.65 ± 2.25 | 10.50 − 21.25 = 16.80 ± 4.74 |
| | | W | 297 − 304.75 = 301.10 ± 2.77 | 12.25 − 17.75 = 14.95 ± 2.13 | 12.75 − 18 = 15.10 ± 2.19 | 4.25 − 16.75 = 11 ± 4.72 |
| 2. | *Citrullus lanatus* (Thunb.) Matsum.& Nakai | L | 512.50 − 517.75 = 514.95 ± 2.18 | 15.75 − 25.25 = 20.90 ± 3.75 | 14.75 − 25.75 = 20.55 ± 4.93 | 16.25 − 23.25 = 19.80 ± 2.56 |
| | | W | 250 − 258.75 = 253.50 ± 3.24 | 10 − 20 = 12.9 ± 4.14 | 10.25 − 20.50 = 13.55 ± 4.12 | 7.50 − 12.50 = 10.05 ± 1.97 |
| 3. | *Cucumis melo* L. | L | 126.75 − 145.25 = 134.10 ± 7.44 | 10.50 − 21.75 = 16.20 ± 4.50833 | 10 − 20.75 = 15.55 ± 4.28442 | 12.00 − 21.25 = 16.20 ± 3.77 |
| | | W | 122.75 − 138 = 130 ± 6.38 | 8.75 − 13.75 = 11.05 ± 2.07 | 8.75 − 13 = 10.6 ± 1.85 | 7.50 − 13.75 = 10.65 ± 2.75 |
| 4. | *Cucumis melo subsp. agrestis* (Naudin) Pangalo | L | 200.50 − 212.50 = 205.35 ± 5 | 3.75 − 25.50 = 16.70 ± 9.13 | 4.75 − 25.50 = 12.85 ± 9.49 | 13.00 − 35.50 = 25.50 ± 9.78 |
| | | W | 183.50 − 188.50 = 186.30 ± 2.13 | 3.75 − 18 = 11.55 ± 6.18 | 2.50 − 15.25 = 7.25 ± 4.97 | 8.25 − 25.50 = 15.45 ± 6.54 |
| 5. | *Cucumis melo var. flexuosus* (L.) Naudin | L | 325.25 − 333 = 328.85 ± 3.18 | 13 − 50.25 = 23.15 ± 15.28 | 13.50 − 45.25 = 22.50 ± 12.90 | 11 − 28 = 18.50 ± 6.98 |
| | | W | 250.25 − 267.75 = 257.30 ± 7.73 | 7.75 − 16.75 = 11.15 ± 3.81 | 8 − 14.25 = 10.75 ± 2.97 | 2.75 − 13.25 = 8.5 ± 3.83813 |
| 6. | *Cucumis melo* var.*cantalupensis* Naudin | L | 250.50 − 256.50 = 253.75 ± 2.384 | 18.75 − 26.25 = 22.7500 ± ± 2.87 | 19.50 − 26.50 = 22.6500 ± 2.831 | 13.00 − 24.75 = 18.7000 ± 4.396 |
| | | W | 225 − 231.25 = 227.60 ± 2.36 | 13.75 − 23.25 = 18.20 ± 3.40221 | 13.25 − 19.50 = 16.70 ± 2.558 | 12 − 14.25 = 13.1500 ± .91 |
| 7. | *Cucumis sativus* L. | L | 524.75 − 531.25 = 527.40 ± 2.71 | 20.75 − 30.00 = 26.0500 ± 4.052 | 17.75 − 30.50 = 25.9500 ± 5.7047 | 23.25 − 33.50 = 27.8500 ± 3.7358 |
| | | W | 225.00 − 238.25 = 229.40 ± 5.375 | 10.25 − 13.00 = 11.5500 ± 1.242 | 10.25 − 13.00 = 11.5500 ± 1.267 | 13.75 − 21.25 = 17.7000 ± 2.808 |
| 8. | *Cucurbita maxima* Duchesne | L | 589.75 − 600.25 = 595.60 ± 3.9115 | 19.25 − 42.25 = 27.3000 ± 10.24 | 19.50 − 41.00 = 27.2000 ± 9.8256 | 12.25 − 20.00 = 16.8500 ± 3.5820 |
| | | W | 486.25 − 494.75 = 489.60 ± 3.3241 | 9.25 − 20.00 = 14.2500 ± 4.10 | 9.75 − 19.75 = 14.4000 ± 3.91152 | 8.75 − 13.25 = 10.7000 ± 1.68077 |
| 9. | *Cucurbita pepo* L. | L | 650.00 − 662.75 = 656.10 ± 4.735 | 8.75 − 19.75 = 13.9000 ± 4.211 | 8.00 − 20.00 = 13.5000 ± 4.53114 | 9.50 − 15.25 = 12.8500 ± 2.29538 |
| | | W | 315.50 − 321.25 = 317.60 ± 2.1837 | 7.50 − 17.50 = 11.4500 ± 3.8786 | 7.25 − 18.00 = 11.3000 ± 4.298 | 7.25 − 14.75 = 10.4500 ± 3.0893 |
| 10. | *Cucurbita pepo* var. *cylindrica* | L | 361.25 − 368.00 = 364.45 ± 2.6774 | 22.50 − 31.25 = 26.5000 ± 3.579 | 22.75 − 31.00 = 26.5500 ± 3.188 | 16.75 − 25.00 = 21.7500 ± 3.292 |
| | | W | 162.75 − 170.25 = 1.67.00 ± 2.93 | 13.00 − 22.50 = 18.0500 ± 3.692 | 13.25 − 22.00 = 18.0000 ± 3.592 | 10.50 − 19.25 = 14.8500 ± 3.223 |
| 11. | *Lagenaria siceraria* (Molina) Standl. | L | 471.25 − 487.75 = 477.85 ± 6.125 | 14.50 − 27.50 = 20.4500 ± 5.874 | 14.25 − 27.75 = 20.7000 ± 6.2759 | 22.50 − 30.50 = 27.7500 ± 3.172 |
| | | W | 210.00 − 223.75 = 217.65 ± 5.641 | 9.75 − 13.00 = 11.1000 ± 1.526 | 9.50 − 13.00 = 11.1500 ± 1.61632 | 8.75 − 12.75 = 10.7000 ± 1.71756 |
| 12. | *Luffa acutangula* (L.) Roxb. | L | 250 − 676.25 = 582.85 ± 186.37 | 8.50 − 20.50 = 15.05 ± 4.56002 | 7.25 − 20.50 = 14.7 ± 5.03550 | 13.00 − 28.50 = 21.20 ± 6.13 |
| | | W | 251.75 − 270.25 = 258.85 ± 7.86 | 5.25 − 10.50 = 8.05 ± 1.89 | 5.00 − 10.25 = 7.75 ± 1.87 | 7.50 − 17.50 = 12.20 ± 3.7 |

| | | | | | |
|---|---|---|---|---|---|
| 13. *Luffa acutangula var.amara* C.B.Clarke | L | 451.25 − 480.50 = 472.45 ± 11.99 | 17.50 − 26 = 21.05 ± 4.19 | 17.75 − 27.50 = 21.3 ± 4.8 | 15.50 − 25 = 20.30 ± 3.83 |
| | W | 219.75 − 252.75 = 229.20 ± 13.64 | 12.50 − 17 = 14.4 ± 1.82 | 12.75 − 16.25 = 4.1 ± 1.47479 | 12.75 − 22.75 = 15.9500 ± 3.98 |
| 14. *Luffa cylindrica* (L) M.Roem | L | 401.25 − 413.75 = 406.2 ± 4.84897 | 11.25 − 15.75 = 13.0000 ± 1.677 | 11.50 − 15.75 = 13.0000 ± 1.6488 | 15.75 − 23.25 = 19.7000 ± 3.2948 |
| | W | 172.25 − 180.25 = 175.75 ± 2.915 | 8.00 − 15.25 = 11.6500 ± 2.637 | 8.25 − 15.25 = 11.7000 ± 2.51496 | 13.00 − 18.00 = 15.0500 ± 2.041 |
| 15. *Momordica charantia* L. | L | 350.00 − 357.75 = 353.25 ± 3.177 | 11.75 − 17.00 = 14.7500 ± 2.186 | 12.00 − 17.00 = 14.6000 ± 2.2262 | 16.25 − 20.00 = 18.2500 ± 1.3578 |
| | W | 160.75 − 167.25 = 164.55 ± 2.7064 | 8.75 − 11.00 = 9.9000 ± .96177 | 9.00 − 10.75 = 9.9500 ± .71589 | 9.25 − 11.75 = 10.5500 ± 1.10962 |
| 16 *Momordica balsamina* L. | L | 122.25 − 124.0 = 123.05 ± .81777 | 8.75 − 16.25 = 13.3000 ± 2.9811 | 8.50 − 16.00 = 13.3000 ± 3.15436 | 14.00 − 25.50 = 19.4500 ± 5.5884 |
| | W | 112.25 − 114.00 = 112.95 ± .64711 | 7.75 − 12.25 = 10.3000 ± 1.6240 | 8.00 − 12.00 = 10.3500 ± 1.49583 | 12.75 − 14.75 = 13.8500 ± .89443 |
| 17 *Praecitrullus fistulosus* (Stocks) Pangalo | L | 575.25 − 584.00 = 578.95 ± 3.49 | 22.50 − 32.75 = 28.0000 ± 4.172 | 23.50 − 33.00 = 27.6500 ± 4.207 | 17.25 − 26.25 = 22.2000 ± 3.858 |
| | W | 238.00 − 244.75 = 241.90 ± 2.7477 | 13.00 − 19.25 = 16.2500 ± 2.417 | 13.25 − 18.75 = 16.1000 ± 2.043 | 10.50 − 20.00 = 14.6000 ± 3.529 |

**Table 5.** Quantitative tendril anatomical features among Cucurbitaceous species.

| Sr No. | Cucurbitaceous taxa | L x W | Chlorenchyma cell (µm) | Sclerenchyma Cell (µm) | Parenchyma Cell (µm) | Vessel Elements(µm) | Vascular Bundle (µm) |
|---|---|---|---|---|---|---|---|
| 1. | *Citrullus colocynthis* (L) Schrad. | L | 31.75 − 50.25 = 40.50±7.65 | 11.25 − 25 = 25±5.76 | 50.50 − 84.75 = 66.25±15.04 | 18.00 − 26.25 = 21.70±3.52 | 135.50 − 188 = 161.55±21.20 |
| | | W | 12.00 − 19.25 = 16.05±3.04 | 10.50 − 22.25 = 16.10±4.73 | 47.00 − 77.25 = 63.35±13.40 | 5.70 − 9.90 = 7.82±1.69 | 74.25 − 90.75 = 81.40±6.79 |
| 2. | *Citrullus lanatus* (Thunb.) Matsum.& Nakai | L | 16.25 − 27.00 = 21±4.25 | 10.75 − 27.75 = 22.40±6.76 | 38.75 − 101 = 74.65±28.14 | 25.50 − 37.50 = 29.05±4.85 | 89.50 − 200.25 = 146.15±48.33 |
| | | W | 8.25 − 14.75 = 10.65±2.56 | 8.50 − 21 = 16.10±4.84 | 26.25 − 28.75 = 27.35±.96 | 15 − 25 = 18.30±4.44 | 27 − 89.75 = 64.25±29.42 |
| 3. | *Cucumis melo* L. | L | 18.75 − 26.25 = 22.15±2.98 | 16.75 − 28 = 22.10±4.83 | 18 − 43.75 = 29±11.15 | 11.25 − 16.25 = 13.75±2.26 | 89 − 113.75 = 99.90±10.29 |
| | | W | 8.75 − 11.75 = 10.35±1.23 | 9.75 − 22 = 14.90±5.23 | 9.75 − 22 = 14.90±5.23 | 8.50 − 11.25 = 9.55±1.10 | 30.50 − 55.25 = 38.20±9.97 |
| 4. | *Cucumis melo subsp. agrestis* (Naudin) Pangalo | L | 15.50 − 25.25 = 20.60±4.05 | 8.25 − 20.25 = 13.55±4.54 | 20.50 − 77.75 = 53.55±26.50 | 9.75 − 13.25 = 11.50±1.47 | 47.25 − 97 = 74±19.78 |
| | | W | 7.75 − 10.25 = 8.9000±1.24 | 2.75 − 18.50 = 9±6.02 | 27.75 − 62.75 = 46.25±13.21 | 5.25 − 10.25 = 7.90±1.78 | 22.50 − 33.75 = 28.20±4.25 |
| 5. | *Cucumis melo var. flexuosus* (L.) Naudin | L | 13 − 41.25 = 25.65±10.97 | 5.25 − 20.50 = 16.15±6.39 | 23.25 − 112.50 = 61.50±37.46 | 7.75 − 13.75 = 11.75±2.60 | 75.25 − 163 = 117.10±39.43 |
| | | W | 10.50 − 20 = 14.35±4.27 | 3.75 − 15.25 = 10.15±4.71 | 15.50 − 55.25 = 33.50±14.27 | 5.50 − 13.25 = 9.60±3.02 | 26.75 − 51.50 = 42.55±9.86 |
| 6. | *Cucumis melo* var.*cantalupensis* Naudin | L | 42.00 − 46.25 = 43.80±1.67 | 19.25 − 25.75 = 21.35±2.61 | 49.25 − 86.25 = 68.10±13.73 | 29.75 − 40.75 = 34.95±4.48 | 77.25 − 106.25 = 89.10±11.14 |
| | | W | 24.50 − 29.50 = 27.45±2.06 | 10.50 − 14.25 = 12.20±1.47 | 36.25 − 52.75 = 44.15±6.78 | 21.25 − 37.75 = 30.45±6.66 | 26.25 − 37.25 = 30.95±4.10 |
| 7. | *Cucumis sativus* L. | L | 25.50 − 62.75 = 44.25±13.87 | 10.25 − 30 = 19.05±8.56 | 13.75 − 88 = 56.95±32.34 | 38.25 − 47.50 = 42.55±4.09 | 151.75 − 301.25 = 224.15±56.12 |
| | | W | 13.75 − 20.25 = 17±2.54 | 7.75 − 20.25 = 14.25±5.60 | 11.00 − 39.25 = 24.45±10.77 | 20.50 − 38 = 29.25±7.35 | 101.50 − 202.25 = 125.30±43.31 |
| 8. | *Cucurbita maxima* Duchesne | L | 20.50 − 51.25 = 33.60±11.74 | 13.75 − 27.75 = 20.70±5.67 | 24.75 − 64.50 = 45.15±16.02 | 24.75 − 43 = 35.10±6.83 | 30.50 − 38.75 = 34±3.15 |
| | | W | 11.75 − 18 = 13.55±2.60 | 9.50 − 12.50 = 11.45±1.19 | 14.50 − 28.25 = 20.80±6.11 | 18.75 − 27.75 = 22.90±3.92 | 24.75 − 30.75 = 27.35±2.24 |
| 9. | *Cucurbita pepo* L. | L | 21.75 − 29.25 = 26.60±3.12 | 20.25 − 30 = 24.05±3.68 | 30.50 − 66.25 = 53.35±14.31 | 25.75 − 35.25 = 30.80±3.69 | 37.25 − 44.50 = 41.55±3.12 |
| | | W | 7.25 − 19.75 = 10.95±5.05 | 9.75 − 14.25 = 11.25±1.76 | 17.75 − 50.75 = 30.95±12.30 | 15.25 − 33.75 = 24.75±8.65 | 24.50 − 29.50 = 27.55±1.93 |
| 10. | *Cucurbita pepo var. cylindrica* | L | 26.75 − 46.25 = 33.55±7.57 | 19.50 − 25.25 = 23.05±2.34 | 47.25 − 98.75 = 63.45±20.67 | 16.25 − 22.25 = 19±2.34 | 22.50 − 28.75 = 26.10±2.87 |
| | | W | 12.50 − 28 = 19.10±7.40 | 12.25 − 18 = 16.35±2.32 | 12.25 − 25 = 18.45±5.13 | 11.25 − 15 = 13.30±1.52 | 17.25 − 21.25 = 19.35±1.51 |
| 11. | *Lagenaria siceraria* (Molina) Standl. | L | 20 − 46.25 = 32.50±10.08 | 22.50 − 31.25 = 27.60±3.43 | 43.75 − 105.25 = 74.15±22.10 | 20 − 37.50 = 28.30±7.28 | 63.25 − 103.75 = 86.25±17.20 |
| | | W | 11.75 − 17.50 = 13.40±2.38 | 11.75 − 20.25 = 15.05±3.65 | 26.75 − 36.50 = 31.40±4.32 | 18 − 30.75 = 24.80±5.14 | 25.50 − 63.50 = 38.95±14.65 |
| 12. | *Luffa acutangula* (L.)Roxb. | L | 17.75 − 45.50 = 30.50±10.42 | 15.75 − 35.75 = 26.70±8.98 | 24.25 − 38.75 = 31±5.76086 | 25.50 − 32.75 = 27.60±3.04 | 76.25 − 125.25 = 109.85±19.32 |
| | | W | 11.25 − 15.25 = 13±1.60 | 11.00 − 15.50 = 13.50±1.81 | 10 − 15.25 = 13.50±2.05 | 13.25 − 30 = 23.95±6.43 | 46.50 − 101.75 = 87.60±23.57 |
| 13. | *Luffa acutangula* var. *amara* C.B.Clarke | L | 15.25 − 45 = 29.85±10.89 | 30.75 − 46 = 38.05±6.45 | 23.25 − 82.81 = 41.61±24.37 | 12.75 − 25.25 = 17±5.54 | 76.25 − 125.25 = 109.90±19.33 |
| | | W | 10.25 − 15.75 = 13.45±2.25 | 13.00 − 22.75 = 17.95 ±3.97 | 12.75 − 20.75 = 16.40±3.83± | 10.25 − 22.75 = 15.80±5.58 | 46.50 − 101.75 = 87.60±23.57 |
| 14. | *Luffa cylindrica* (L) M.Roem | L | 17.50 − 37.75 = 28.80±7.97 | 21.25 − 31.25 = 26.50±3.99 | 53 − 75.75 = 65.34±9.45473 | 10.25 − 31.25 = 21.70±9.30 | 101 − 139.25 = 119.90±17.32 |
| | | W | 11.00 − 20.50 = 15.60±3.76 | 11.25 − 17.50 = 13.80±2.68 | 19.50 − 45.25 = 38.50±10.88 | 7.50 − 27.75 = 17.55±8 | 76 − 101.75 = 84.75±10.84 |
| 15. | | L | 31.25 − 53.50 = 45.20±9.05 | 21.25 − 26.25 = 24.45±2.01 | 30.25 − 41.75 = 37.20±4.36 | 22.25 − 31 = 26.85±3.83 | 95.50 − 112.75 = 102.90±7.33 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Momordica charantia* L. | W | 11.25 − 28.50 = 19.35±6.64 | 11.50 − 18 = 14.95±2.63 | 11.25 − 19.50 = 15.05±3.83 | 17.50 − 23.75 = 20.75±2.89 | 71 − 77.25 = 73.70±2.61 |
| 16 | *Momordica balsamina* L. | L | 16.25 − 28.75 = 22.45±4.88 | 16.25 − 21.25 = 19.60±1.94 | 38.75 − 64 = 52.05±9.07 | 7.25 − 11 = 9.50±1.57 | 51.25 − 68.75 = 59.15±7.87 |
| | | W | 13.75 − 20.50 = 16.70±3.10 | 11.25 − 17.75 = 13.95±2.58 | 31.25 − 48.75 = 39.95±7.16 | 7.75 − 9.75 = 8.60±.741 | 41.25 − 50.50 = 45.90±3.90 |
| 17 | *Praecitrullus fistulosus* (Stocks) Pangalo | L | 20.00 − 47.50 = 32.70±11.01 | 23.25 − 31.25 = 27±3.08 | 62.50 − 126.75 = 91.55±26 | 13 − 22.75 = 18±4.09 | 138.25 − 153.75 = 147±6.25 |
| | | W | 13.75 − 25.50 = 19.85±4.97 | 12.25 − 17.75 = 14.±2.22 | 20 − 75 = 41.65±27.69 | 10.25 − 18 = 14.35±3 | 104.50 − 127.75 = 118±11.78 |

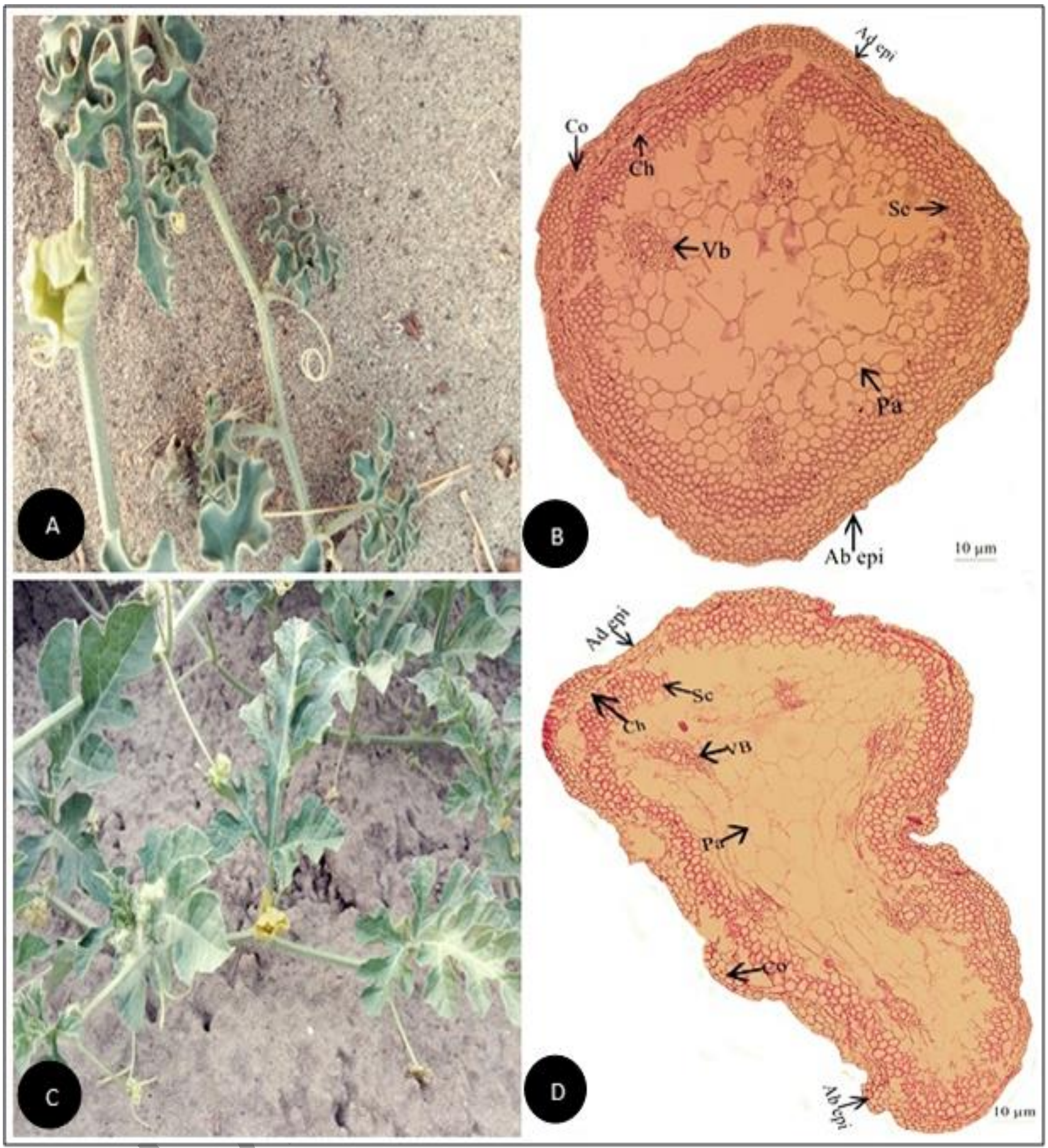


**Plate 7.** (**A**) Field pictorial view of *Citrullus colocynthis* (L.) Schrad. (**B**) Tendril cross-section of *Citrullus colocynthis* (L.) Schrad. (Scale bar = 10 µm) (**C**) Field pictorial view of *Citrullus lanatus* (Thunb.) Matsum. & Nakai (**D**) Tendril cross section of *Citrullus lanatus* (Thunb.) Matsum.& Nakai (Scale bar = 10 µm).Ad epi, Adaxial epidermis; Ab epi, Abaxial epidermis; Co, Collenchyma; Ch, Chlorenchyma; Sc, Sclerenchyma; Pa, Parenchyma; Vb, vascular bundle.

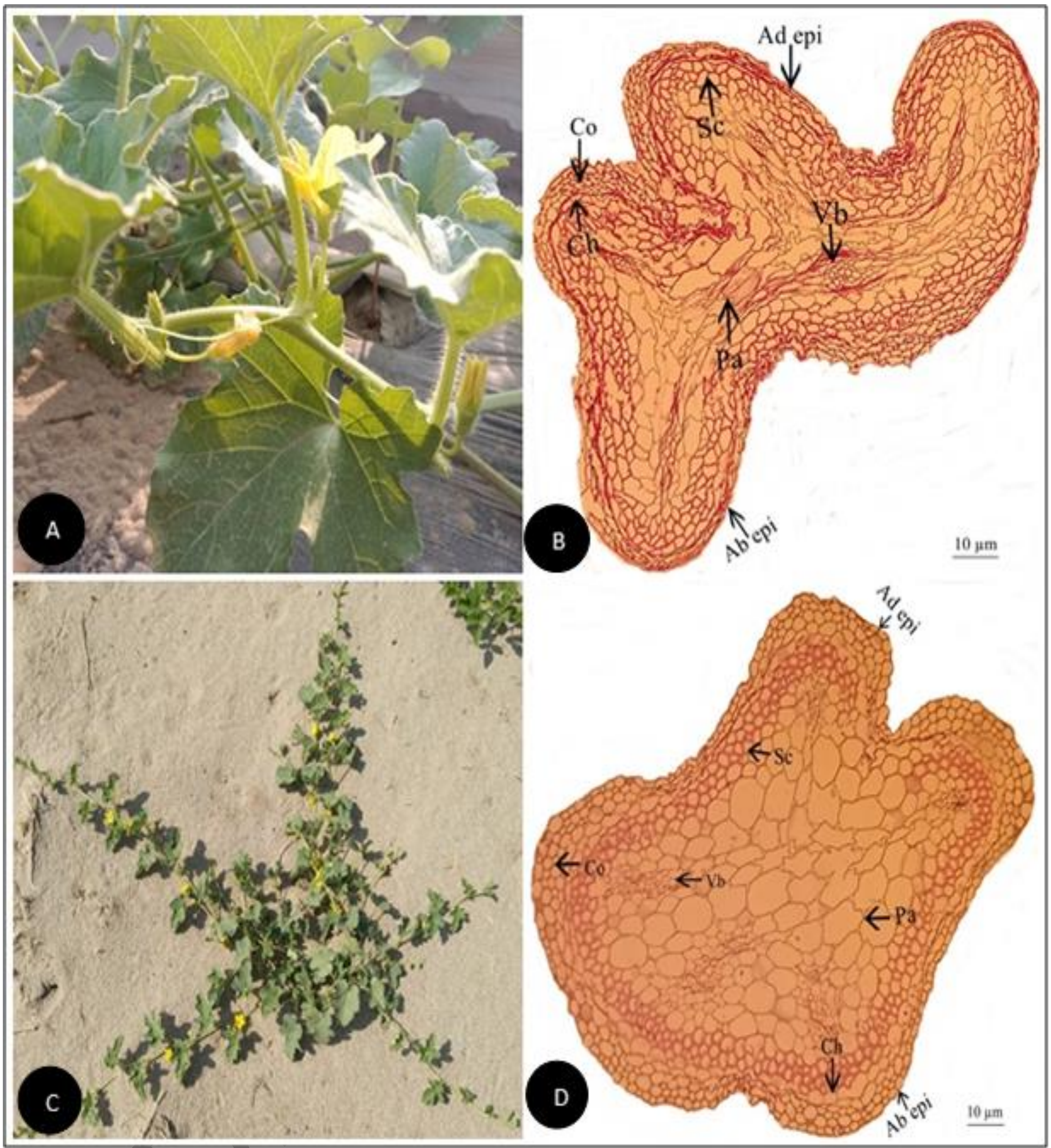


**Plate 8.** (**A**) Field pictorial view of *Cucumis melo* L. (**B**) Tendril cross-section of *Cucumis melo* L. (Scale bar = 10 µm) (**C**) Field pictorial view of *Cucumis melo subsp. agrestis* (Naudin) Pangalo (**D**) Tendril cross-section of *Cucumis melo subsp. agrestis* (Naudin) Pangalo (Scale bar = 10 µm). Ad epi, Adaxial epidermis; Ab epi, Abaxial epidermis; Co, Collenchyma; Ch, Chlorenchyma; Sc, Sclerenchyma; Pa, Parenchyma; Vb, vascular bundle.

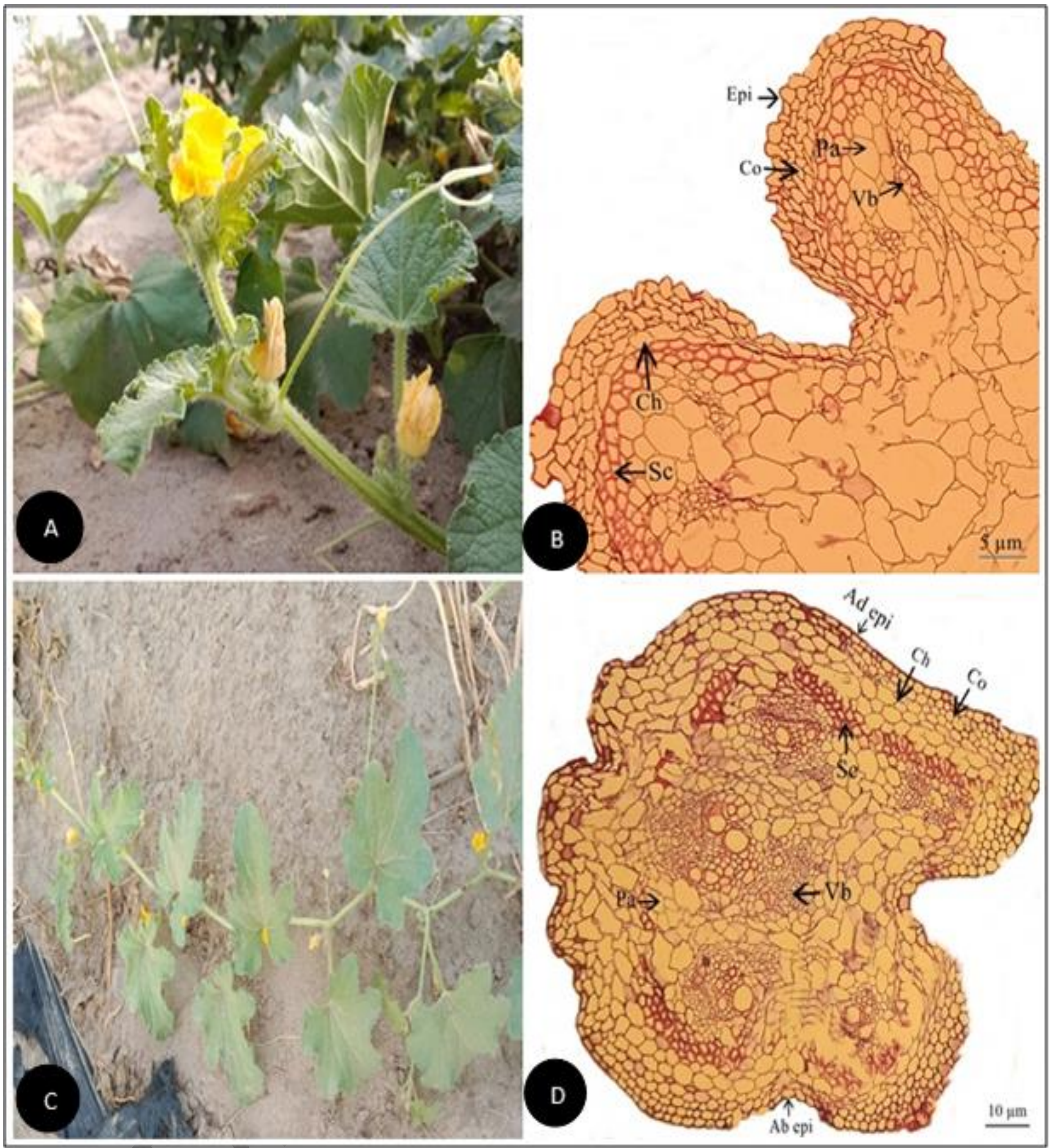


**Plate 9.** (**A**) Field pictorial view of *Cucumis melo var. flexuosus* (L.) Naudin (**B**) Tendril cross-section of *Cucumis melo* var. *flexuosus* (L.) Naudin (Scale bar = 10 µm) (**C**) Field pictorial view of *Cucumis melo var.cantalupensis* Naudin (**D**) Tendril cross-section of Cucumis melo var.cantalupensis Naudin (Scale bar = 10 µm). Ad epi, Adaxial epidermis; Ab epi, Abaxial epidermis; Co, Collenchyma; Ch, Chlorenchyma; Sc, Sclerenchyma; Pa, Parenchyma; Vb, vascular bundle.

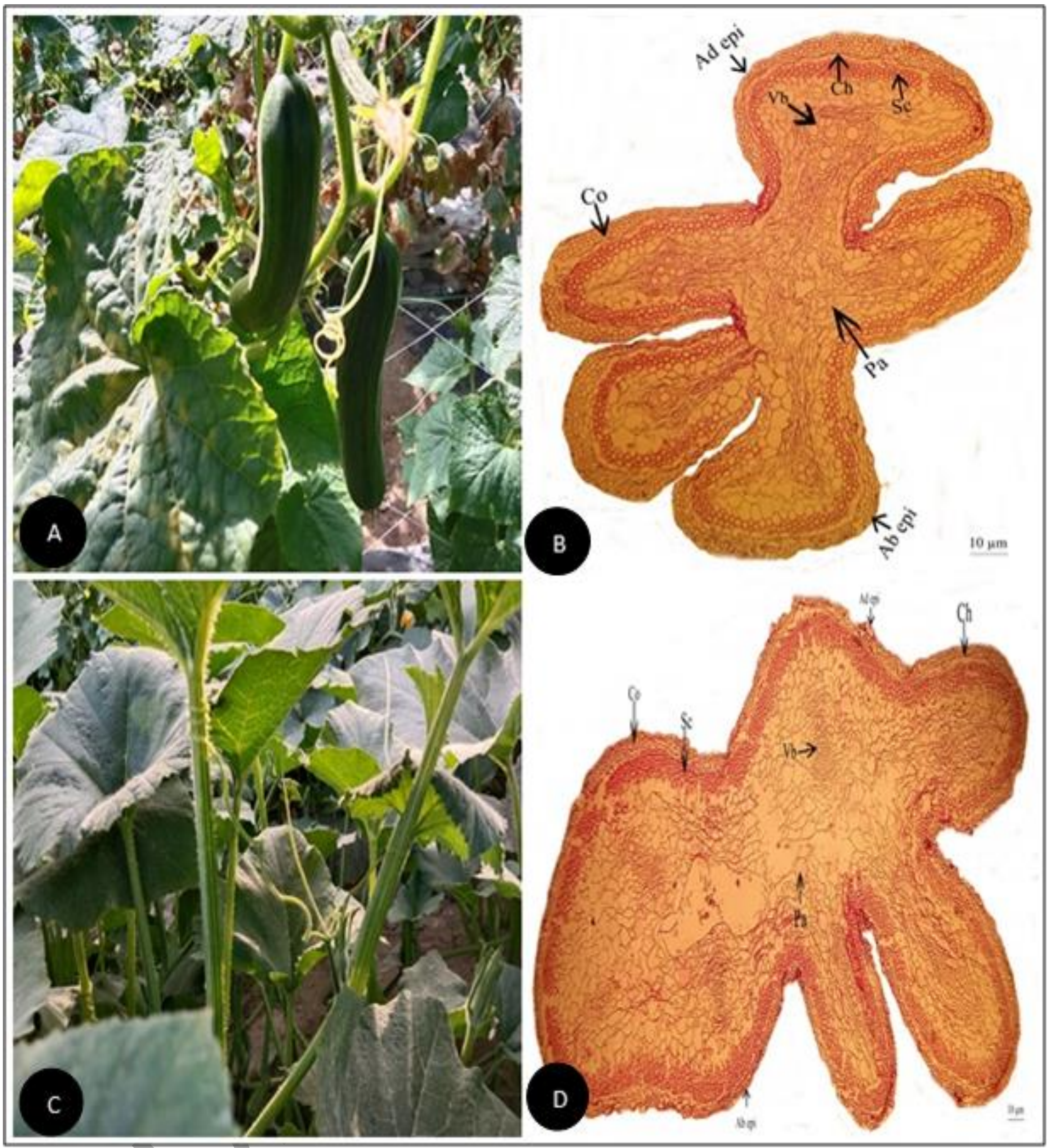


**Plate 10.** (**A**) Field pictorial view of *Cucumis sativus* L. (**B**) Tendril cross-section of *Cucumis sativus* L. (Scale bar = 10 µm) (**C**) Field pictorial view of *Cucurbita maxima* Duchesne (**D**) Tendril cross-section of *Cucurbita maxima* Duchesne (Scale bar = 10 µm). Ad epi, Adaxial epidermis; Ab epi, Abaxial epidermis; Co, Collenchyma; Ch, Chlorenchyma; Sc, Sclerenchyma; Pa, Parenchyma; Vb, vascular bundle.

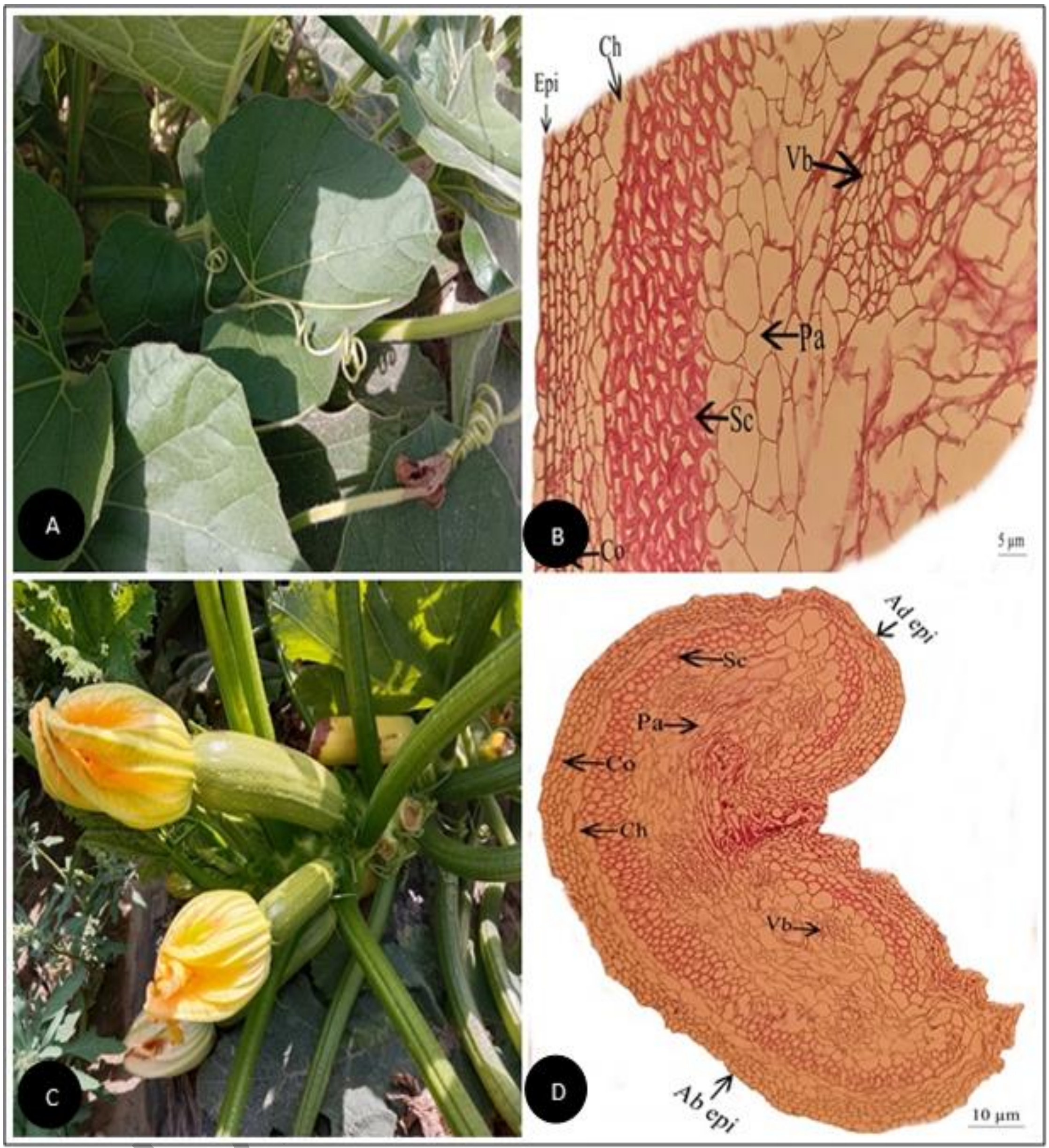


**Plate 11.** (**A**) Field pictorial view of *Cucurbita pepo* L. (**B**) Tendril cross-section of *Cucurbita pepo* L. (Scale bar = 5 µm) (**C**) Field pictorial view of *Cucurbita pepo var. cylindrica* (**D**) Tendril cross-section of *Cucurbita pepo var. cylindrica* (Scale bar = 10 µm). Ad epi, Adaxial epidermis; Ab epi, Abaxial epidermis; Co, Collenchyma; Ch, Chlorenchyma; Sc, Sclerenchyma; Pa, Parenchyma; Vb, vascular bundle.

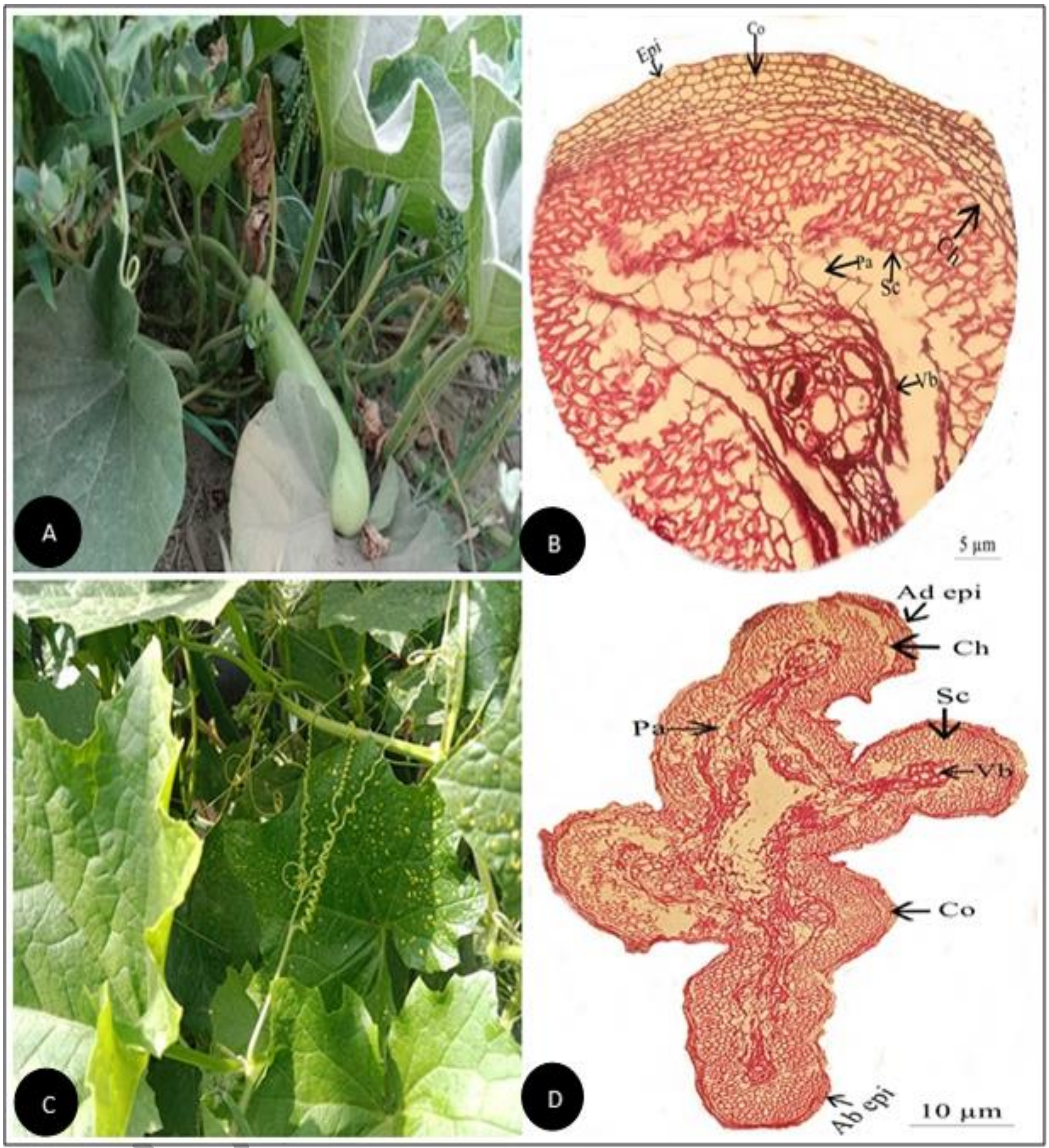


**Plate 12.** (**A**) Field pictorial view of *Lagenaria siceraria* (Molina) Standl. (**B**) Tendril cross-section of *Lagenaria siceraria* (Molina) Standl. (Scale bar = 5 µm) (**C**) Field pictorial view of *Luffa acutangula* (L.) Roxb (**D**) Tendril cross-section of *Luffa acutangula* (L.) Roxb (Scale bar = 10 µm). Ad epi, Adaxial epidermis; Ab epi, Abaxial epidermis; Co, Collenchyma; Ch, Chlorenchyma; Sc, Sclerenchyma; Pa, Parenchyma; Vb, vascular bundle.

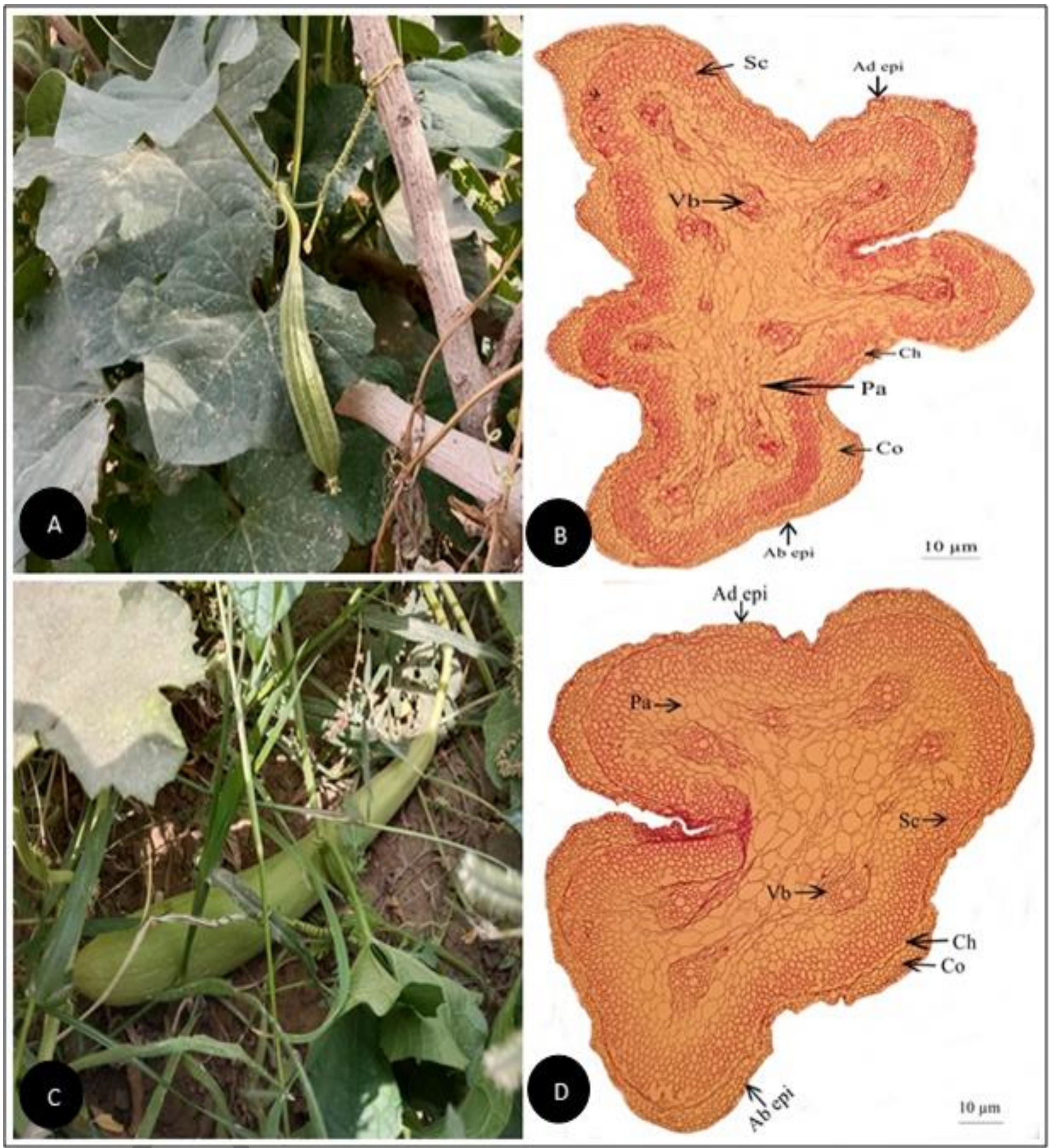


**Plate 13.** (**A**) Field pictorial view of *Luffa acutangula* var. *amara* C.B.Clarke (**B**) Tendril cross-section of *Luffa acutangula var. amara* C.B.Clarke (Scale bar = 10 µm) (**C**) Field pictorial view of *Luffa cylindrica*(L.) M.Roem (**D**) Tendril cross-section of *Luffa cylindrica*(L.) M.Roem (Scale bar = 10 µm). Ad epi, Adaxial epidermis; Ab epi, Abaxial epidermis; Co, Collenchyma; Ch, Chlorenchyma; Sc, Sclerenchyma; Pa, Parenchyma; Vb, vascular bundle.

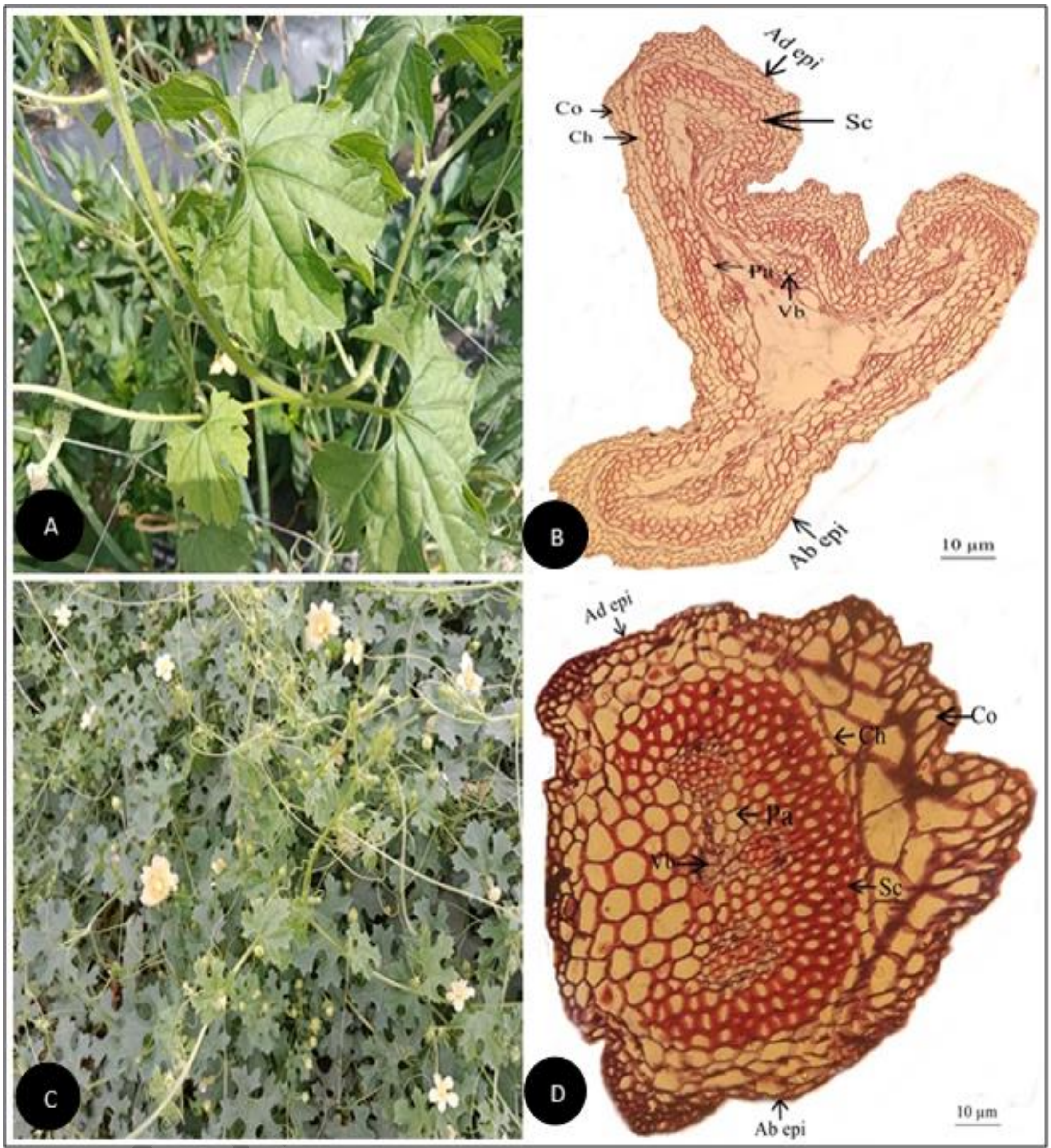


**Plate 14.** (**A**) Field pictorial view of *Momordica charantia* L. (**B**) Tendril cross-section of *Momordica charantia* L. (Scale bar = 10 µm) (**C**) Field pictorial view of *Momordica dioica* Roxb. ex Willd. (**D**) Tendril cross section of *Momordica balsamina L*. (Scale bar = 10 µm). Ad epi, Adaxial epidermis; Ab epi, Abaxial epidermis; Co, Collenchyma; Ch, Chlorenchyma; Sc, Sclerenchyma; Pa, Parenchyma; Vb, vascular bundle.

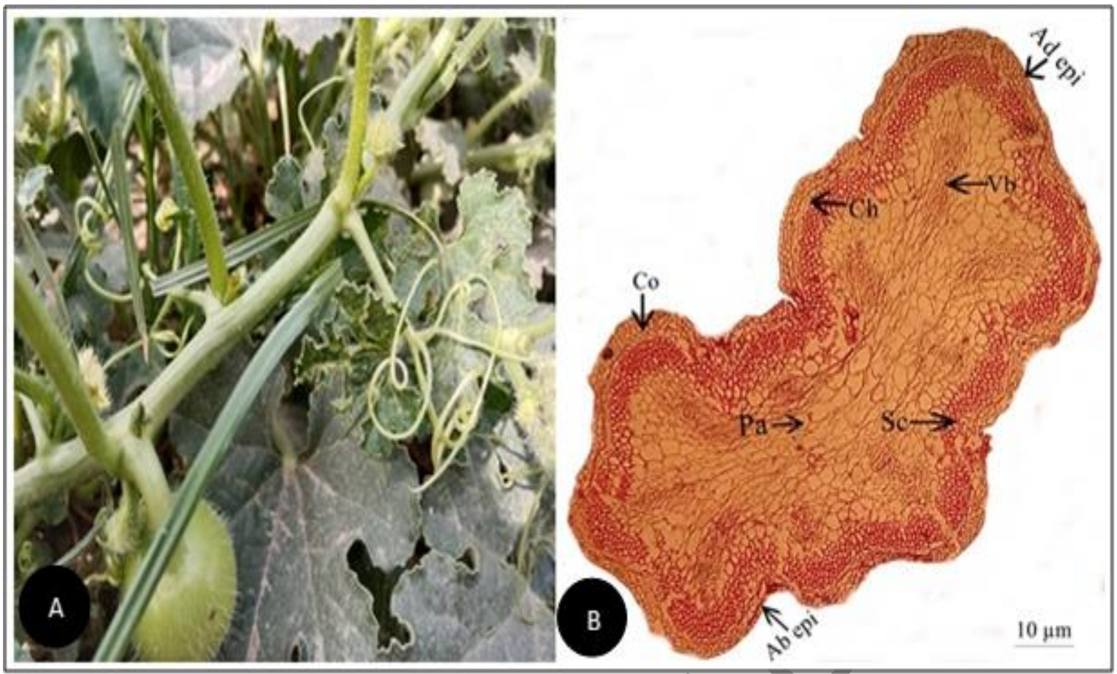


**Plate 15.** (**A**) Field pictorial view of *Praecitrullus fistulosus* (Stocks) Pangalo (**B**) Tendril cross section of *Praecitrullus fistulosus* (Stocks) Pangalo (Scale bar = 10 µm). Ad epi, Adaxial epidermis; Ab epi, Abaxial epidermis; Co, Collenchyma; Ch, Chlorenchyma; Sc, Sclerenchyma; Pa, Parenchyma; Vb, vascular bundle.

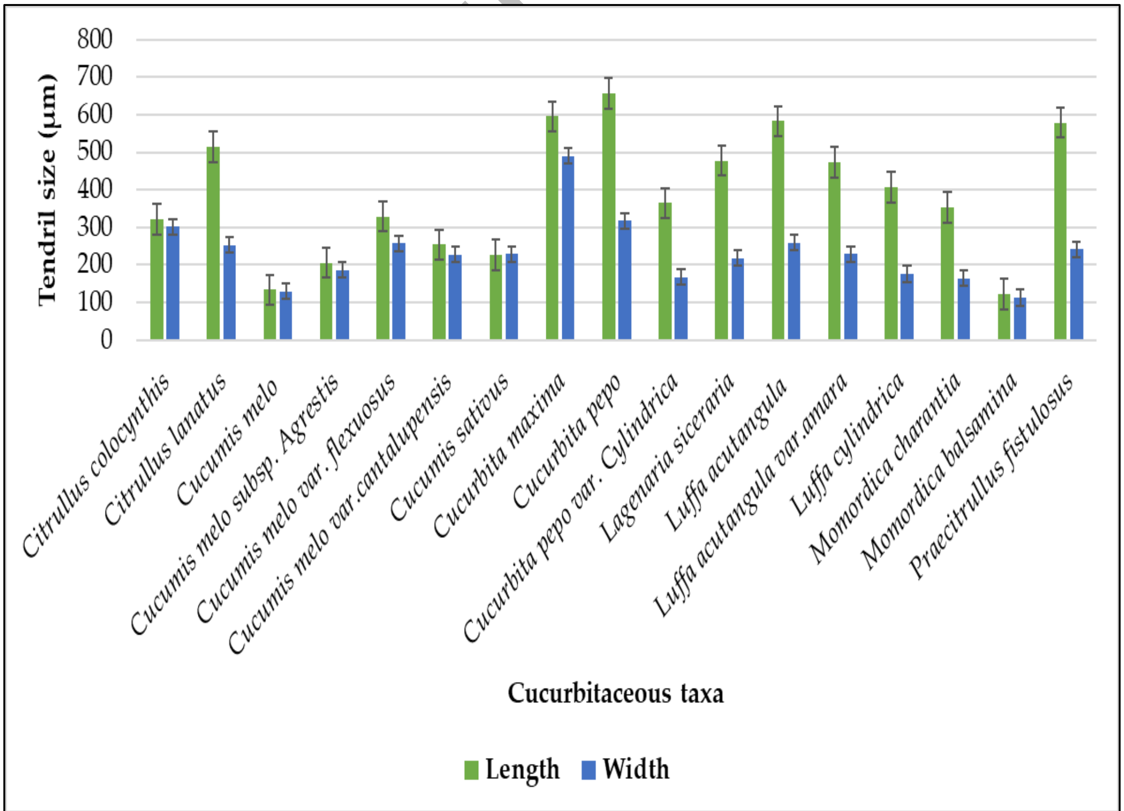


**Figure 2.** Mean tendril size variations among Cucurbitaceous taxa.

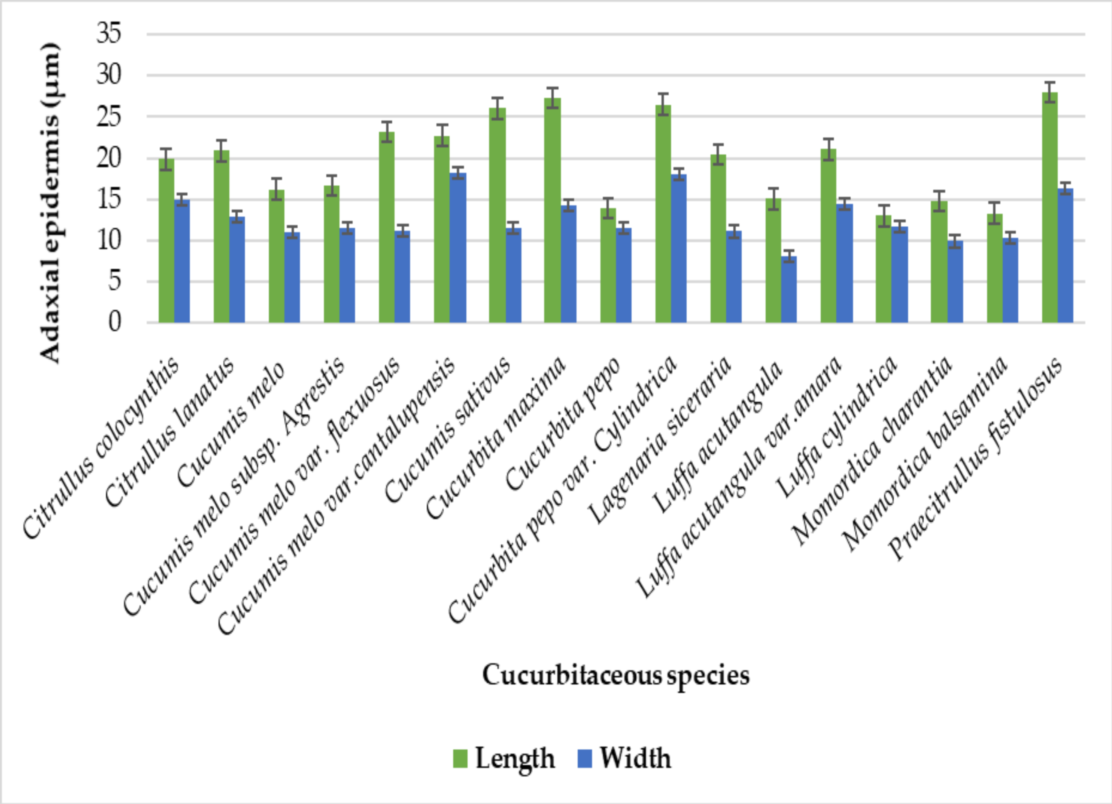


**Figure 3.** Mean epidermal cell size variations along Adaxial tendril surface.

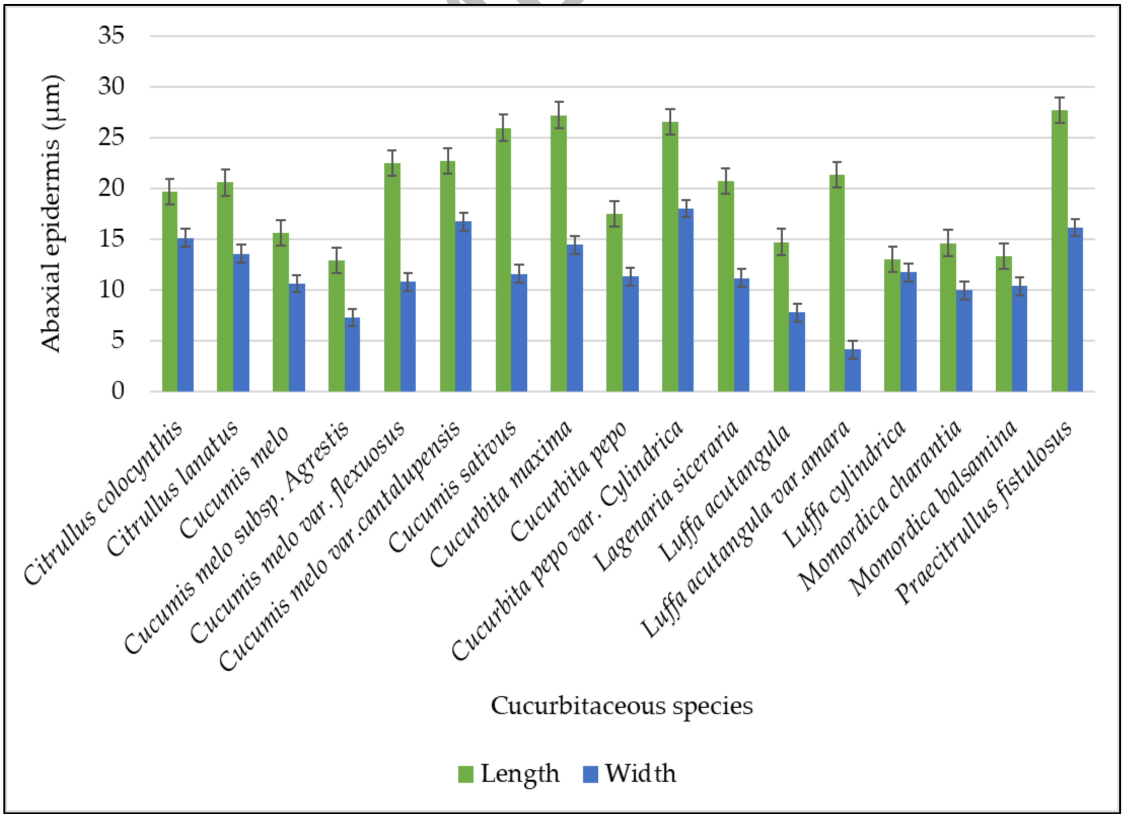


**Figure 4.** Variations among epidermal cell size on the abaxial tendril surface.

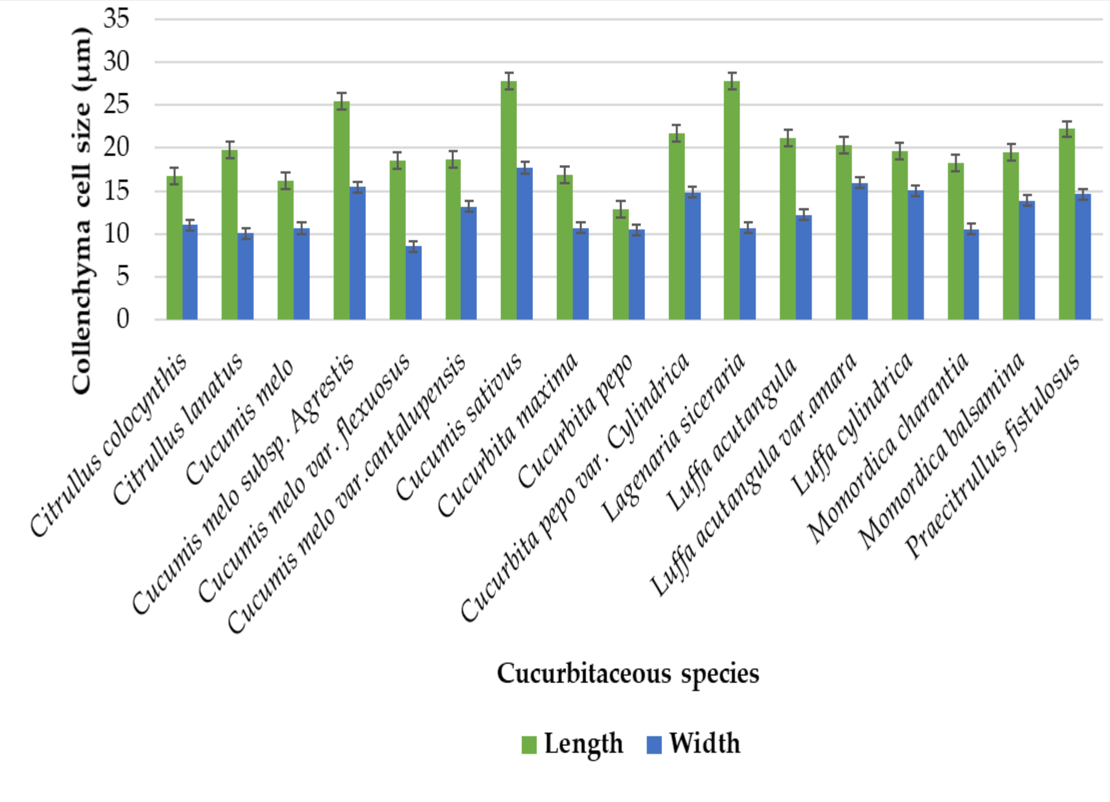


**Figure 5.** Average variations of collenchyma cell size on the surface of tendril

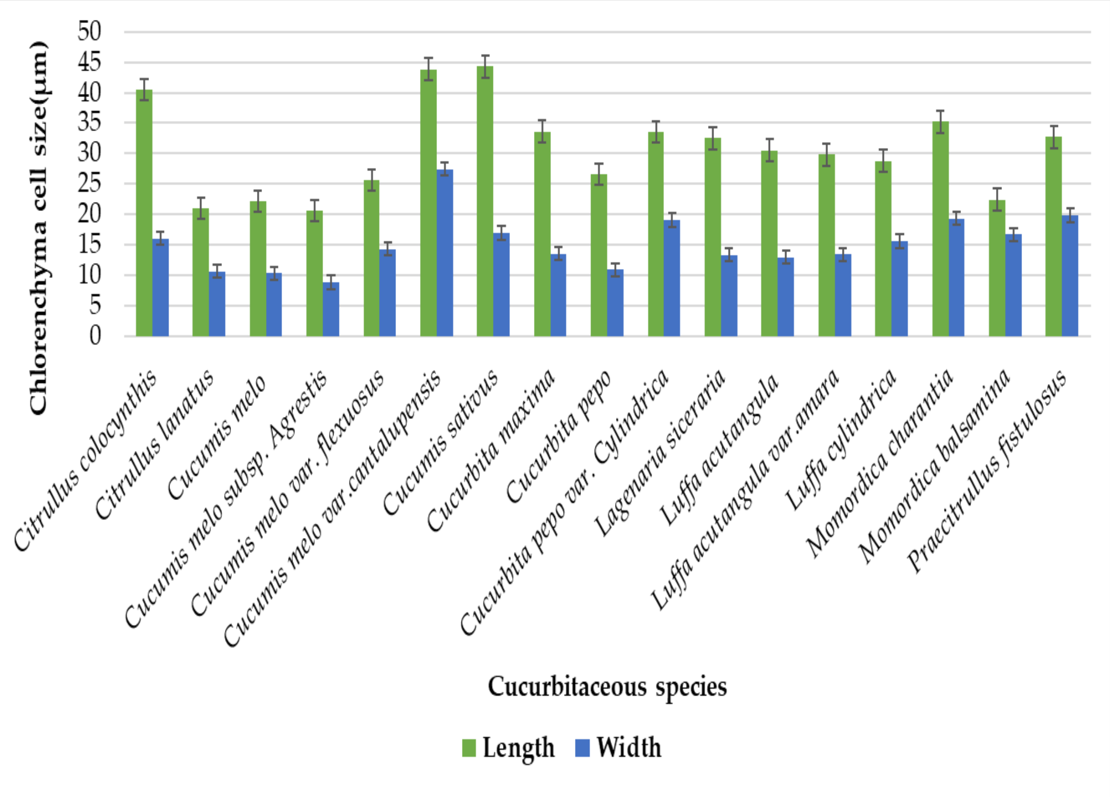


**Figure 6.** Mean chlorenchyma length and width on the surface of tendril.

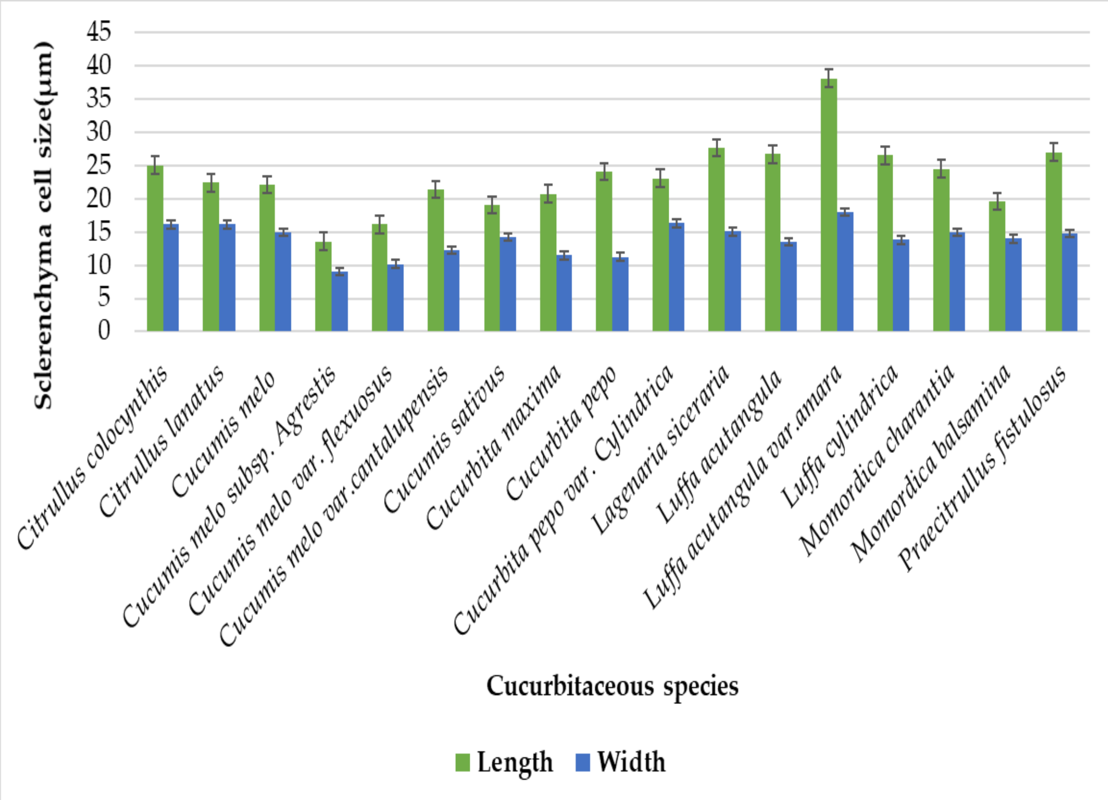


**Figure 7.** Average size of sclerenchyma length and width on tendril surface.

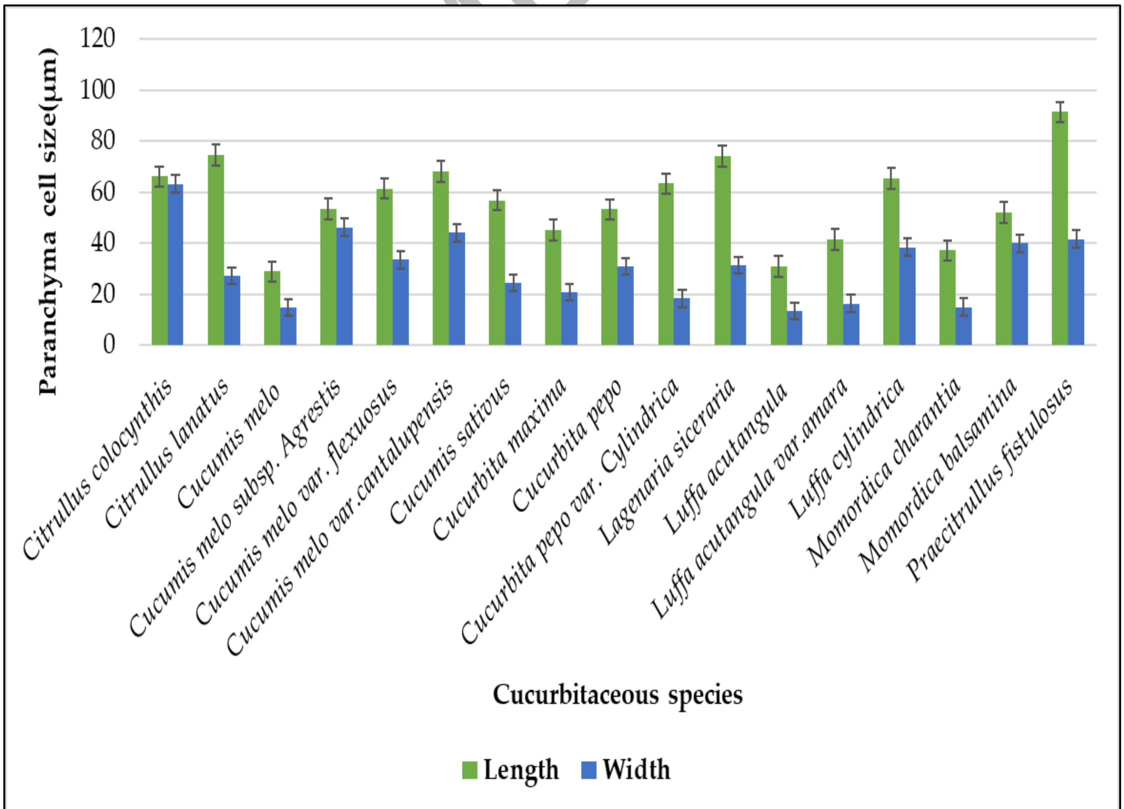


**Figure 8.** Mean parenchyma length and width on the surface of the tendril.

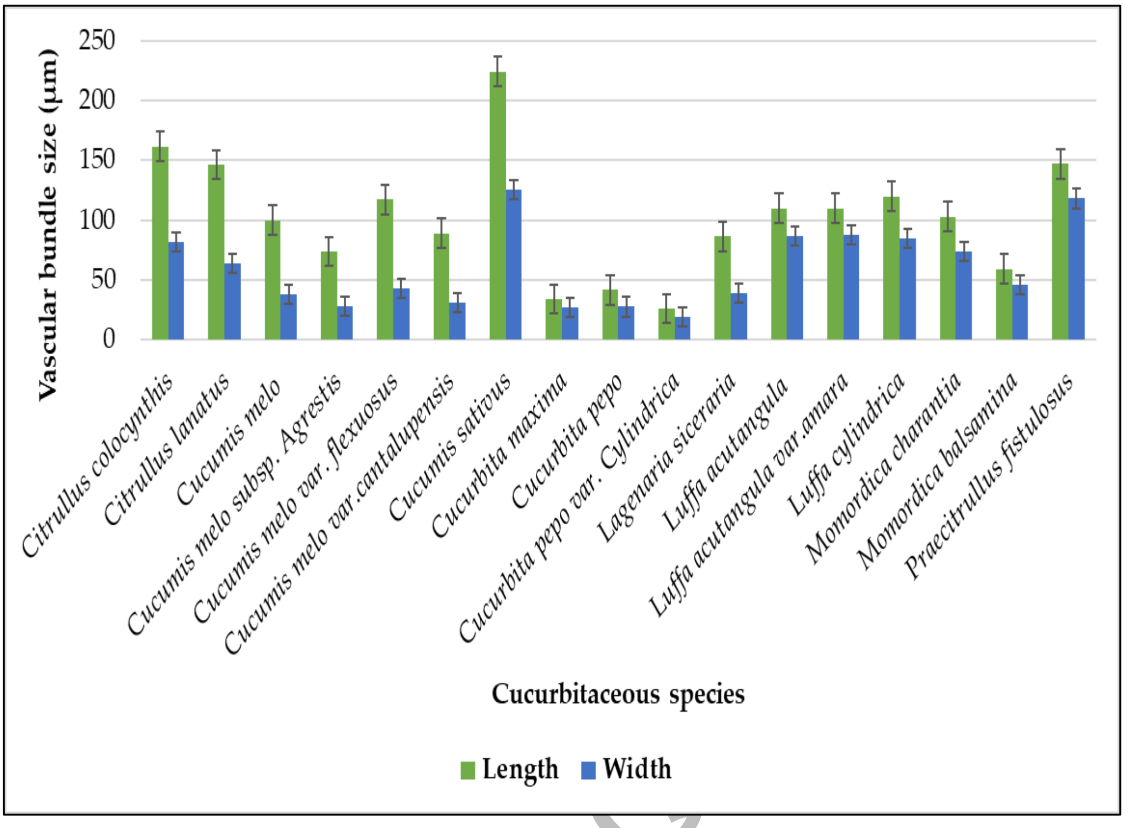


**Figure 9.** Graphical illustration showing vascular bundle size of tendril.

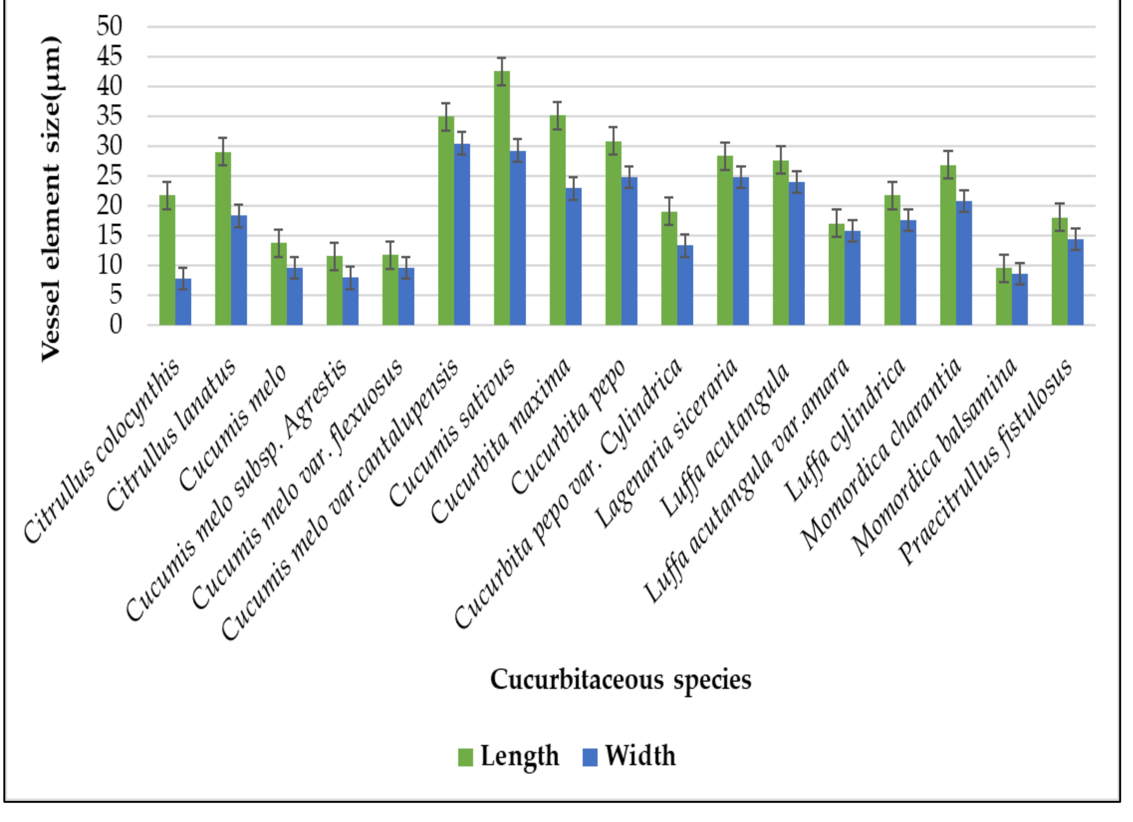


**Figure 10.** Graphical variations among tendril vessel elements.

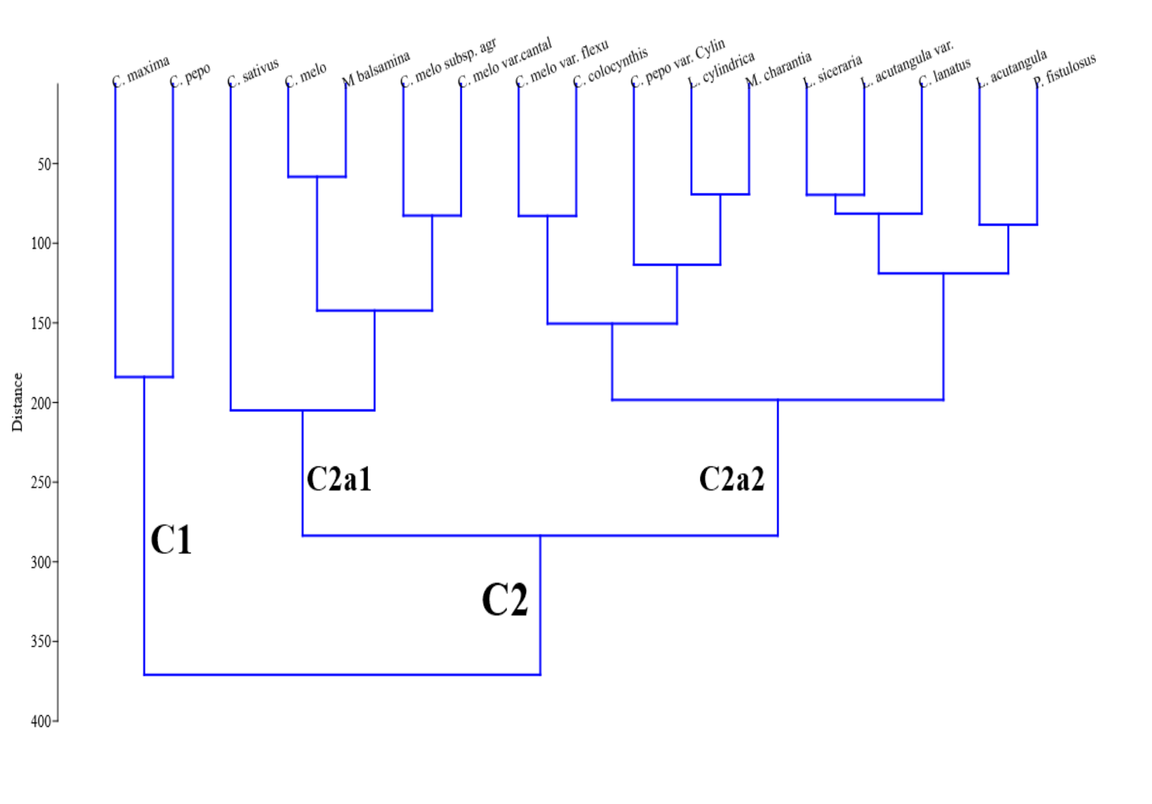


**Figure 11.** Cluster groupings of Cucurbitaceous taxa based on tendril features.

# Pollen Micromorphology of Cucurbitaceous Species

### 3.3. Pollen Micromorphology of Cucurbitaceous Species

#### 3.3.1 General description

The pollen of Cucurbitaceous taxa are monads, medium to large, iso-polar, circular, circular, semi-circular or triangular and semi-angular amb, prolate-spheroidal to oblate-spheroidal equatorial view shape, tricolporate, with raised, angular, prominent, wavy, elliptic, sunken and slit with distinct margins (Figure 18, 19 & 20; Table 6). The exine is aerolate, aerolate-perforate, reticulate, and echinate and scabrate. (Figure 21 to 26; Table 6).

#### 3.3.2 Pollen size and shape

Cucurbitaceous species have monads pollen unit. Pollen grains are medium or large (Table 2), with size range from 42.55 µm in *Cucurbita pepo* var. *cylindrica* to 93.8 µm in *Cucurbita maxima* (polar diameter) and from 42.5 µm in *Cucumis sativus* to 97.4 µm in *Cucurbita maxima* (equatorial diameter) as shown in Figure 12. The polar view diameter to equatorial distance ratio was calculated maximum for *Cucumis sativus* (1.2) to minimum for *Cucumis melo* (0.94) as shown in Figure 13.

The visualized Cucurbitaceous pollen represent prolate-spheroidal in *Citrullus colocynthis*, *Cucumis melo subsp. agrestis*, *Cucumis sativus*, *Cucurbita pepo*, *Lagenaria siceraria*, *Luffa acutangula*, *Luffa acutangula* var. *amara* and *Praecitrullus fistulosus*, oblate spheroidal in *Cucumis melo*, *Cucumis melo* var. *flexuosus*, *Cucumis melo* var. *cantalupensis*, *Cucurbita maxima*, *Cucurbita pepo* var. *cylindrica*, *Momordica charantia* and *Momordica balsamina* or spheroidal in *Citrullus lanatus* and *Luffa cylindrica* (Figure 18, 19 & 20). However, variations within the samples of each species were observed; for this reason, we present the variation and the predominant type for the shape of the pollen grains (Table 7).

#### 3.3.3 Colpi, exine stratification and mesocolpium distance

The pollen grains of all species are tricolporate, with angular, prominent, raised, wavy, elliptic and slit with distinct tapering ends. The maximum length of colpi was measured for *Cucurbita maxima* 30 µm and minimum for *Cucumis melo* var. *flexuosus* 4.7 µm. The highest value for colpi width was observed in *Lagenaria siceraria* 14.75 µm and lowest value was noted for *Cucurbita pepo* var. *cylindrica* 4.7 µm (Figure 14). The pollen grains present scabrate-reticulate exine in *Citrullus colocynthis*, scabrate-perforate in *Cucumis melo*, *Cucumis melo subsp. agrestis*, *Cucumis melo* var. *cantalupensis* and *Cucumis melo* var. *flexuosus*, echinate in *Cucurbita maxima* and *Cucurbita pepo* var. *cylindrica* reticulate in *Citrullus lanatus, Luffa cylindrica*, *Momordica charantia* and *Momordica balsamina*, gemmate-verrucate in *Cucumis sativus*, reticulate-gemmate in *Cucurbita pepo,* scabrate in *Lagenaria siceraria* and *Luffa acutangula* and scabrate micro-echinate in *Praecitrullus fistulosus*. The exine thickness ranged from 2.1 µm in *Cucumis melo* var. *flexuosus* to 5.9 µm in *Praecirullus fistulosus* (Figure 15). Mesocolpium distance between two colpi was measured highest for 59.7 µm in *Cucurbita maxima* and lowest 13.95 µm for *Cucurbita pepo* var. *cylindrical* (Figure 16).

### 3.3.4 Statistical analysis (UPGMA dendrogram and PCA)

The Cucurbitaceous species under examination were subjected to Cluster Analysis via Euclidian distance using quantitative measurement of pollen. The findings of the cluster analysis is presented in (Figure 17). There are two major clusters. The first cluster C-A comprise four species further subdivided into two sub-clusters. The first sub-cluster C-A1 comprises *L. acutangula*, *L. acutangula* var. *amara* and *P. fistulosus* is due to its unique reticulate sculptured exine. While second sub-cluster C-A2 with single species *C. maxima* with elongated, elliptical colpus. The second cluster C-B delineated into two sub-clusters; sub-cluster C-B1 comprises of seven species in which *C. sativus* and *C. colocynthis* show the least Euclidean distance due to common raised, angular with tapering ends colpus features. The sub-cluster C-B2 was categorized into six species with *C. lanatus* and *C. pepo* closely placed with minimum Euclidean distance count and shared prominent colpus.

The relevance of the factors in explaining the overall variation among the seven quantitative pollen traits among Cucurbitaceous taxa was determined by PCA analysis. Table 8 shows the values for the eigenvectors and the total cumulative variance, and Figure 18 displays the scatter plot of the morpho-palynological traits. The PCA explains 76.646% of the pollen characters variance noted for the first two principle axis. The first principal axis (PC1) accounted for 57.565% of the variation, with the equatorial view and mesocolpium distance being the most significant variables. Along PC1 the *C. melo* var. *cantalupensis*, *L. siceraria*, *M. charantia*, and *M. balsamina* were on the positive side of the axis. Whereas *C. melo* var. *flexuosus*, C. sativus, *C. colocynthis*, *C.*

*melo subsp. agrestis* and *C. lanatus* on the negative side. The exine thickness and polar diameter are the significant variables in the second component (PC2), which explains 19.081% of the variance. *C. pepo* var. *cylindrica, C. pepo*, *C. melo,* and *L. cylindrica* were lies on the positive side of the second principal axis and *C. maxima* and *P. fistulosus* on the negative side of PC2.

### 3.3.5 Taxonomic Key to Cucurbitaceous Pollen Identification

1 + Pollen spheroidal, reticulate ornamentation, prominent elongated colpi……………………………………………………………………..…..***C.lanatus***

- Elongated cylindrical colpus……………………………………………………**2**

2 + Pollen spherical, reticulate exine……………………………………......***L. cylindrica***

- Pollen oblate-spheroidal, prolate-spheroidal, raised wavy colpi…………….……**3**

3 + Oblate-spheroidal, scabrate-perforate mesocolpium………………..……..***C. melo***

- Psilate exine, angular margins colpi…………………………...………………**4**

4 + Scabrate-perforate mesocolpium, oblate-spheroidal shape………...........................................................................***C. melo* var. *flexuosus***

- Reticulate sculpturing, scabrate-perforate mesocolpium…………………..……..**5**

5 + Raised angular colpus margins, oblate-spheroidal pollen……..........................................................................***C. melo* var. *cantalupensis***

- Echinate exine, oblate-spheroidal pollen…………………………………….…………**6**

6 + Baculate-echinate mesocolpium, elongated elliptical colpi……….…...***C. maxima***

- Reticulate ornamentation, oblate-spheroidal pollen…………..............................**7**

7 + Scabrate-echinate mesocolpium, short angular tapered colpi……………………………………………………..……..***C. pepo* var. *cylindrica***

- Reticulate exine, oblate-spheroidal pollen……………………………………….**8**

8 + Reticulate mesocolpium, short wavy colpus margins………………....***M. charantia***

- Short sunken angular colpi margins……………………………………….……..**9**

9 + Reticulate sculptured exine and mesocolpium……………………...***M. balsamina***

**-** Reticulate ornamentation, raised angular colpus……………………………….**10**

10 + Scabrate-reticulate mesocolpium, prolate-spheroidal pollen...……***C. colocynthis***

**-** Psilate exine, raised elongated colpus margins……………………………**.....11**

11 + Scabrate-perforate mesocolpium, prolate-spheroidal pollen……………………………………………………….***.C. melo subsp. agrestis***

- Psilate sculpturing, angular colpi margins…………………………………….**12**

12 + Gemmate-verrucate mesocolpium……………………………..…...***C. sativus***

**-** Sunken angular colpus margins, psilate exine………………………………**..13**

13 + Reticulate gemmate mesocolpium……………………………………….***C. pepo***

- Reticulate exine sculpturing…………………………………………… …**14**

14 + Scabrate mesocolpium, slit like colpi margins……………………..***.L. siceraria***

- Exine psilate, wavy colpus margins…………………………….………….**15**

15 + Scabrate-verrucate mesocolpium……………………….…..…. ***L. acutangula***

**-** Reticulate exine, slit like prominent colpi…………………………………….**16**

16 + Perforated mesocolpium…………………………..***L. acutangula* var. *amara***

- Reticulate ornamentation, short raised margins colpus……………………….**..17**

17 + Scabrate micro-echinate mesocolpium…………………………..***.P. fistulosus***

**Table 6.** Qualitative pollen micromorphological traits of Cucurbitaceous pollen.

| Sr. No | Cucurbitaceous species | Pollen size | Pollen shape | Aperture condition | Colpi/Pore | Ornamentations | Spine | Colpi orientation | Mesocolpium |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *Citrullus colocynthis* (L) Schrad. | Medium | Prolate - spheroidal | Tricolporate | P | Reticulate | A | Raised, angular with tapering ends | Scabrate-reticulate |
| 2 | *Citrullus lanatus* (Thunb.) Matsum.& Nakai | Medium | Spheroidal | Tricolporate | P | Reticulate | A | Prominent, elongated with tapering ends | Reticulate |
| 3 | *Cucumis melo* L. | Medium | Oblate - spheroidal | Tricolporate | P | Reticulate | A | Short, angular, and wavy margins | Scabrate-perforate |
| 4 | *Cucumis melo subsp. agrestis* (Naudin) Pangalo | Medium | Prolate - spheroidal | Tricolporate | P | Psilate | A | Raised elongated with prominent margins | Scabrate-perforate |
| 5 | *Cucumis melo* var. *flexuosus* (L.) Naudin | Medium | Oblate - spheroidal | Tricolporate | P | Psilate | A | Short angular with margins distinct | Scabrate-perforate |
| 6 | *Cucumis melo* var.*cantalupensis* Naudin | Medium | Oblate - spheroidal | Tricolporate | P | Reticulate | A | Raised Angular and prominent margins | Scabrate-perforate |
| 7 | *Cucumis sativus* L. | Medium | Prolate - spheroidal | Tricolporate | P | Psilate | A | Raised, angular with tapering ends | Gemmate-verrucate |
| 8 | *Cucurbita maxima* Duchesne | Large | Oblate - spheroidal | Tricolporate | P | Echinate | P | Elongated, elliptical with prominent margins | Baculate-echinate |
| 9 | *Cucurbita pepo* L. | Medium | Prolate - spheroidal | Tricolporate | P | Psilate | A | Prominent short, sunken and angular | Reticulate-gemmate |
| 10 | *Cucurbita pepo* var. *cylindrica* | Medium | Oblate - spheroidal | Tricolporate | P | Reticulate | P | Short, angular with tapering ends | Scabrate-echinate |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 11 | *Lagenaria siceraria* (Molina) Standl. | Medium | Prolate - spheroidal | Tricolporate | P | Reticulate | A | Sunken, Slit like with pointed ends | Scabrate |
| 12 | *Luffa acutangula* (L.)Roxb | Large | Prolate - spheroidal | Tricolporate | P | Psilate | A | Short sunken and wavy margins | Scabrate-verrucate |
| 13 | *Luffa acutangula* var.*amara* C.B.Clarke | Large | Prolate - spheroidal | Tricolporate | P | Reticulate | A | Raised, Slit like, and prominent margins | Perforate |
| 14 | *Luffa cylindrica*(L) M.Roem | Medium | Spheroidal | Tricolporate | P | Reticulate | A | Elongated cylindrical and prominent margins | Reticulate |
| 15 | *Momordica charantia* L. | Medium-Large | Oblate - spheroidal | Tricolporate | P | Reticulate | A | Short, angular with wavy margins | Reticulate |
| 16 | *Momordica balsamina* L. | Medium | Oblate - spheroidal | Tricolporate | P | Reticulate | A | Short, sunken and angular | Reticulate |
| 17 | *Praecitrullus fistulosus* (Stocks) Pangalo | Large | Prolate - spheroidal | Tricolporate | P | Reticulate | A | Short, raised with distinct margins | Scabrate and micro-echinate |

**Keynotes:** P = Present; A = Absent

**Table 7.** Quantitative attributes of Cucurbitaceous Pollen.

| Sr. No | Cucurbitaceous species | PD (µm) Min-Max=M±SE | ED (µm) Min-Max=M±SE | P/E ratio | CL (µm) Min-Max=M±SE | CW (µm) Min-Max=M±SE | ET (µm) Min-Max=M±SE | MC (µm) Min-Max=M±SE |
|---|---|---|---|---|---|---|---|---|
| 1 | *Citrullus colocynthis* (L) Schrad. | 40.50-60.25=48.50±3.47 | 39-51.25=44.85±2.27 | 1.08 | 3.75-8.75=6.10±.92 | 4.50-9=6.9±.73 | 2-3.50=2.85±.26 | 21.25-33.50=26.55±2.09 |
| 2 | *Citrullus lanatus* (Thunb.) Matsum.& Nakai | 46-65=56.15±3.38 | 47.50-63.25=55.70±2.92 | 1.00 | 6.25-13.50=10.20±1.48 | 8.75-12.50=10±.65 | 4.25-6=5.15±.32 | 23.75-35.75=27.50±2.19 |
| 3 | *Cucumis melo* L. | 42.75-55.75=47.40±4.99 | 46.50-59=50.35±5.045 | 0.94 | 7.25-11=8.85±1.53 | 9.25-11.50=10.25±.88 | 2.25-4=3.10±.72 | 13.50-20.25=16.80±2.61 |
| 4 | *Cucumis melo subsp. agrestis* (Naudin) Pangalo | 45-61.50=50.45±2.96 | 43.50-52.75=47.75±1.93 | 1.05 | 4.75-7.50=6.20±.45 | 6.50-10.25=8±.71 | 2-4=2.85±.33 | 18.50-28.25=22.05±1.70 |
| 5 | *Cucumis melo* var. *flexuosus* (L.) Naudin | 42.50-55.25=47.60±2.14 | 40.25-56.50=48.55±2.68 | 0.98 | 4-5.50=4.70±.26 | 7.50-12=9.75±.79 | 1-3=2.10±.34 | 30-42.75=34.95±2.49 |
| 6 | *Cucumis melo* var.*cantalupensis* Naudin | 43.25-62.50=52.50±3.51 | 45.25-62.75=55.20±3.05 | 0.95 | 14.50-23.75=18.50±1.61 | 5.25-9.50=7±.72 | 3.50-5.75=4.65±.37 | 21.50-28.25=25.40±1.30 |
| 7 | *Cucumis sativus* L. | 45-60=51.35±2.59 | 32.75-48.75=42.50±2.87 | 1.20 | 3.75-7=4.95±.55565 | 5-7.75=6.25±.48 | 1.75-3.50=2.75±.30 | 21.25-28=24.10±1.17 |
| 8 | *Cucurbita maxima* Duchesne | 62.50-121=93.80±9.51 | 79.25-125.25=97.40±7.88 | 0.96 | 23.75-35.75=30±2.07 | 11.50-20.25=14.70±1.50 | 4-7=5.50±.49 | 45.25-74.50=59.70±5.60 |
| 9 | *Cucurbita pepo* L. | 48.75-61=54.45±2.20 | 46.50-60=51.4±2.30 | 1.05 | 2.30-16.25=12.10±1.14 | 8.50-11.25=10.10±.50 | 3.75-5=4.4±.23 | 17-25.25=25.25±1.77 |
| 10 | *Cucurbita pepo* var. *cylindrica* | 38.50-48.25=42.55±1.93 | 37-51.25=44.55±44.55 | 0.95 | 6.25-8.25=7.15±.35 | 3.50-5.75=4.70 | 1.50-2.75=2.10±.23 | 11.50-17.50=13.95±1.18 |
| 11 | *Lagenaria siceraria* (Molina) Standl. | 47.75-71=59.05±3.90 | 50.25-65=56.95±2.80 | 1.03 | 7-12.50=10.45±.99 | 8.75-20.25=14.75±2.28 | 2.25-4.25=3.25±.37 | 17.50-23.50=19.95±1.05 |
| 12 | *Luffa acutangula* (L.)Roxb | 62.50-95=79.75±5.89 | 47.50-90=74.10±7.37 | 1.07 | 5-9=7.45±.67 | 7-10.25=8.20±.61 | 2.25-4=3.10±.32 | 45.25-60.25=52.85±3.12 |
| 13 | *Luffa acutangula* var.*amara* C.B.Clarke | 61-85=72.85±4.87 | 53.50-76.25=67.20±3.89 | 1.08 | 4.75-10.75=7.60±1.02 | 7.25-10=8.60±.45 | 2.25-3.75=2.90±.26 | 35.75-47.75=42.45±2.03 |
| 14 | *Luffa cylindrica*(L) M.Roem | 38-48.50=43.20±1.79 | 39.75-48.50=42.60±1.53 | 1.01 | 14.50-26=18.20±2.05 | 5-9.50=6.90±.93 | 2-3.25=2.70±.21 | 15.75-23.75=19.65±1.43 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 15 | *Momordica charantia* L. | 48.50-71=61.20±3.86 | 50.25-76=62.70±4.70 | 0.97 | 10.75-20=15.75±1.73 | 9.50-14=11.75±.75 | 2.25-4=3.35±.30 | 23.50-30.75=26.85±1.31 |
| 16 | *Momordica balsamina* L. | 46-63.50=55.65±3.24 | 45.25-62.75=52.50±3.02 | 1.06 | 16.50-29=22.30±2.32 | 8.75-12=10.05±.55 | 2-3.50=2.85±.26 | 17.25-25.75=21.10±1.62 |
| 17 | *Praecitrullus fistulosus* (Stocks) Pangalo | 73.50-98.50=83.70±4.82 | 60-92.75=75.50±5.92 | 1.1 | 13.50-25.25=18.80±2.34 | 7.50-12.75=10.20±.94 | 4.50-7=5.90±.40 | 6-62.75=41.90±9.81 |

**Keywords:** PD = Polar diameter, ED = Equatorial diameter, CL = Colpi length, CW = Colpi width, ET = Exine thickness, MC = Mesocolpium, SE = Standard error, Min = Minimum, Max = Maximum, M = Mean, µm = Micrometer

**Table 8.** Principal component analysis (PCA) % variance loadings for the Cucurbitaceous pollen.

| PC | Eigenvalue | % variance |
|---|---|---|
| 1 | 4.02956 | 57.565 |
| 2 | 1.3357 | 19.081 |
| 3 | 0.656474 | 9.3782 |
| 4 | 0.544982 | 7.7855 |
| 5 | 0.355604 | 5.0801 |
| 6 | 0.075847 | 1.0835 |
| 7 | 0.001831 | 0.026155 |

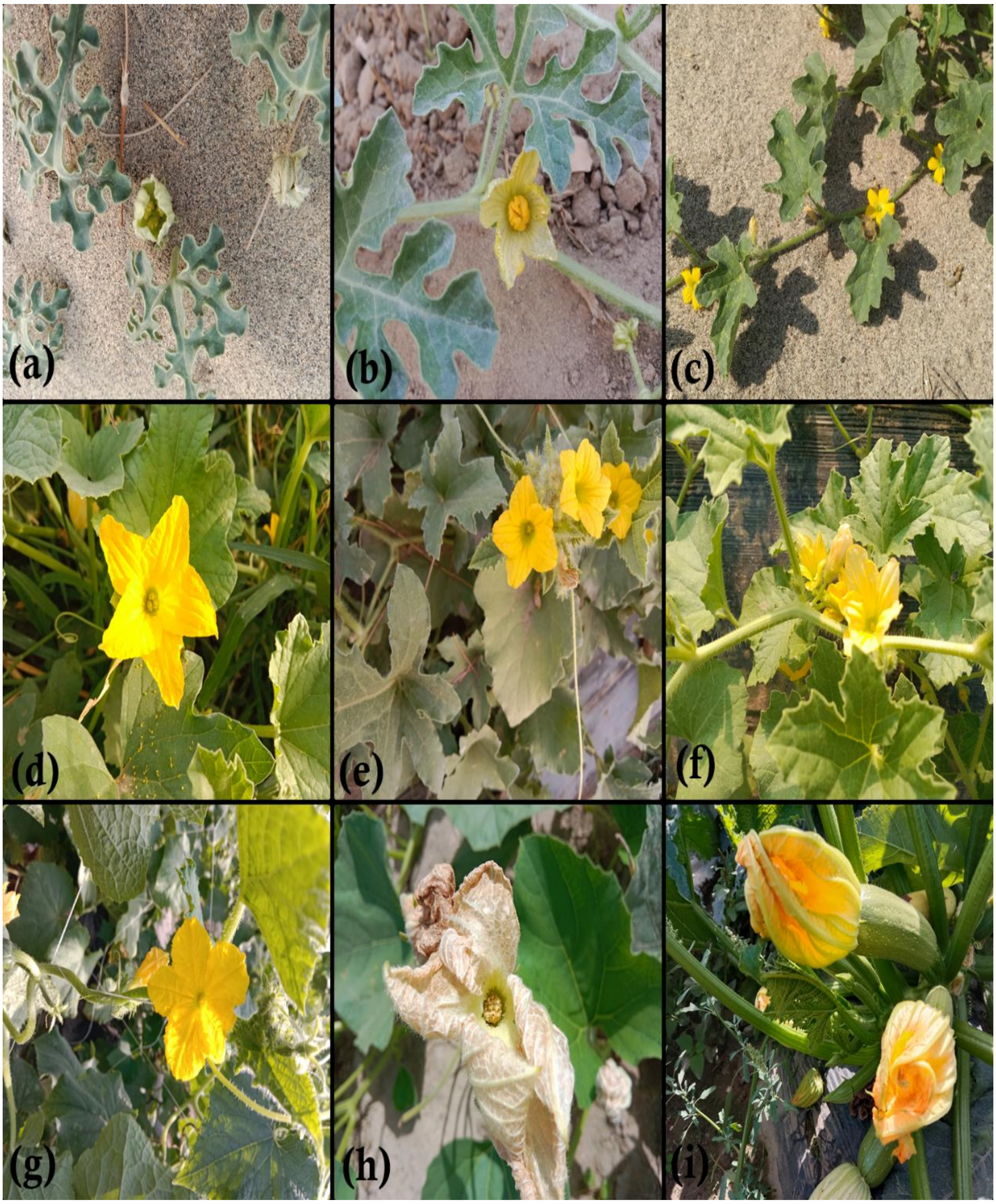


**Figure 16.** Field pictorial photographs of Cucurbitaceous species (a) *Citrullus colocynthis*; (b) *Citrullus lanatus*; (c) *Cucumis melo*; (d) *Cucumis melo subsp. agrestis*; (e) *Cucumis melo* var. *flexuosus*; (f) *Cucumis melo* var. *cantalupensis*; (g) *Cucumis sativus*; (h) *Cucurbita maxima*; (i) *Cucurbita pepo*.

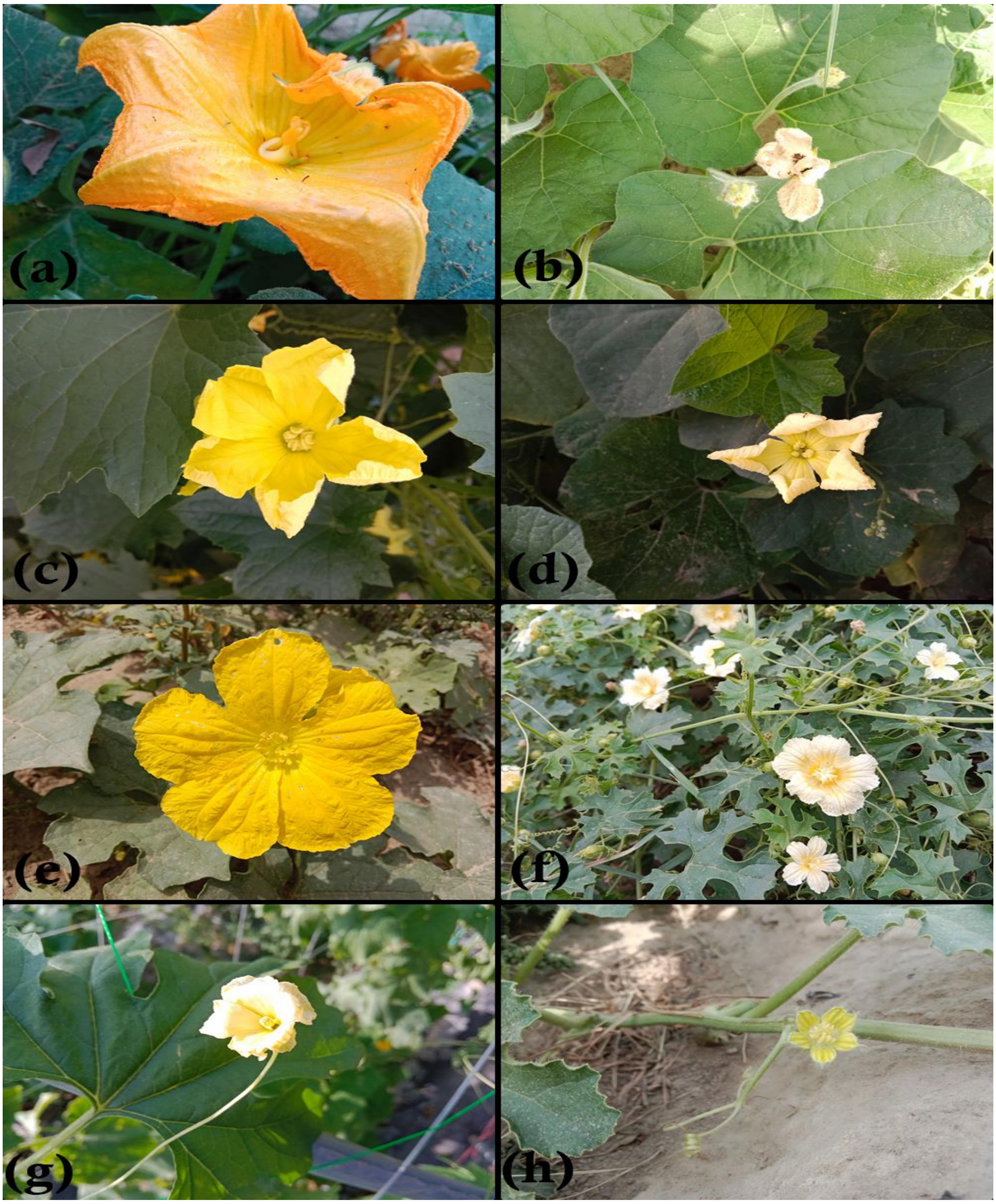


**Figure 17.** Field pictorial photographs of Cucurbitaceous species (a) *Cucurbita pepo* var. *cylindrica*; (b) *Lagenaria siceraria*; (c) *Luffa acutangula*; (d) *Luffa acutangula* var. *amara*; (e) *Luffa cylindrica*; (f) *Momordica charantia*; (g) *Momordica balsamina*; (h) *Praecitrullus fistulosus*.

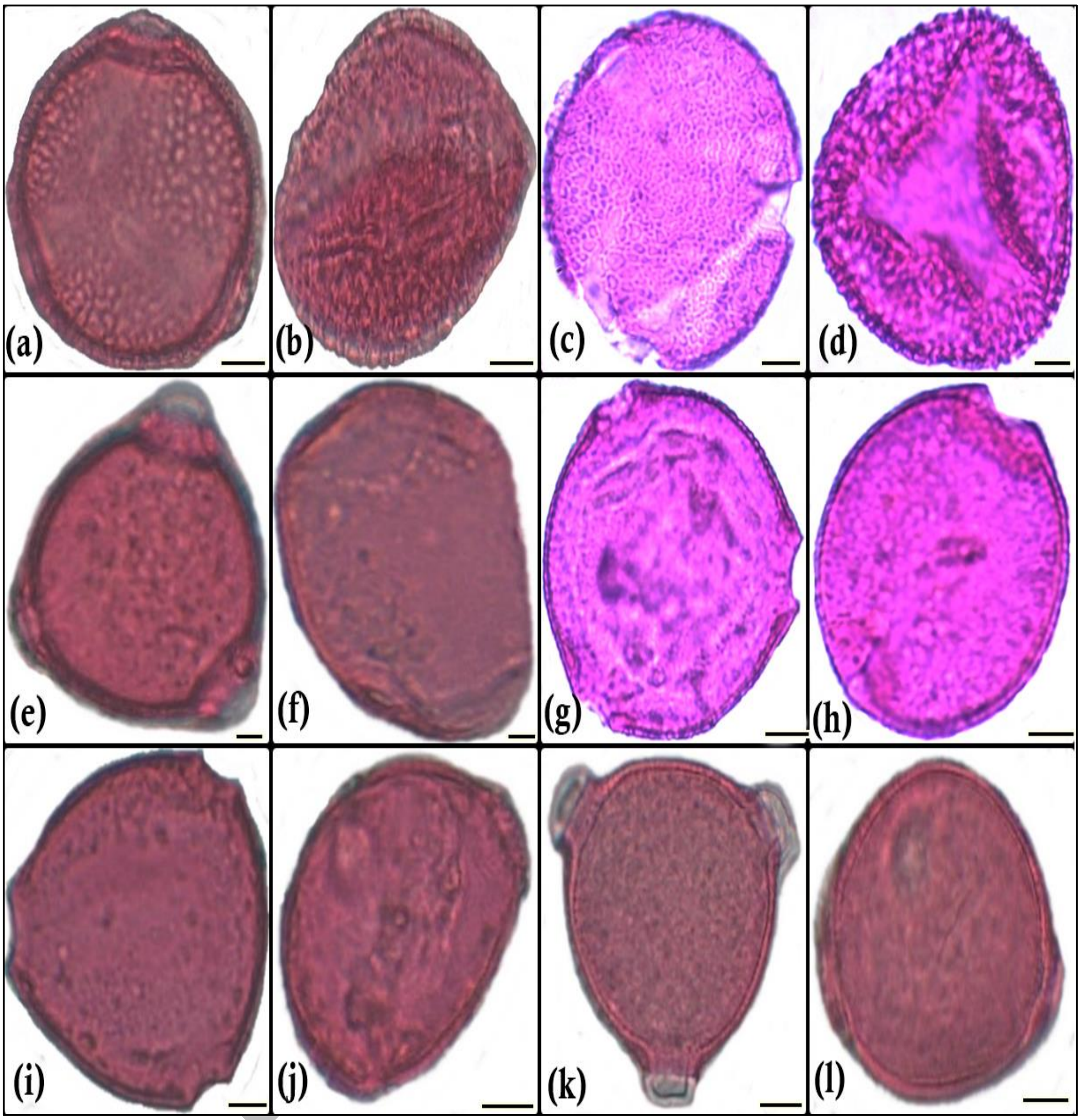


**Figure 18.** Light microphotographs of Cucurbitaceous showing polar and equatorial view of pollen taken at 40X magnification scale bar = 10µm (a-b) *Citrullus colocynthis*; (c-d) *Citrullus lanatus*; (e-f) *Cucumis melo*; (g-h) *Cucumis melo subsp. agrestis*; (i-j) *Cucumis melo* var. *flexuosus*; (k-l) *Cucumis melo* var. *cantalupensis*.

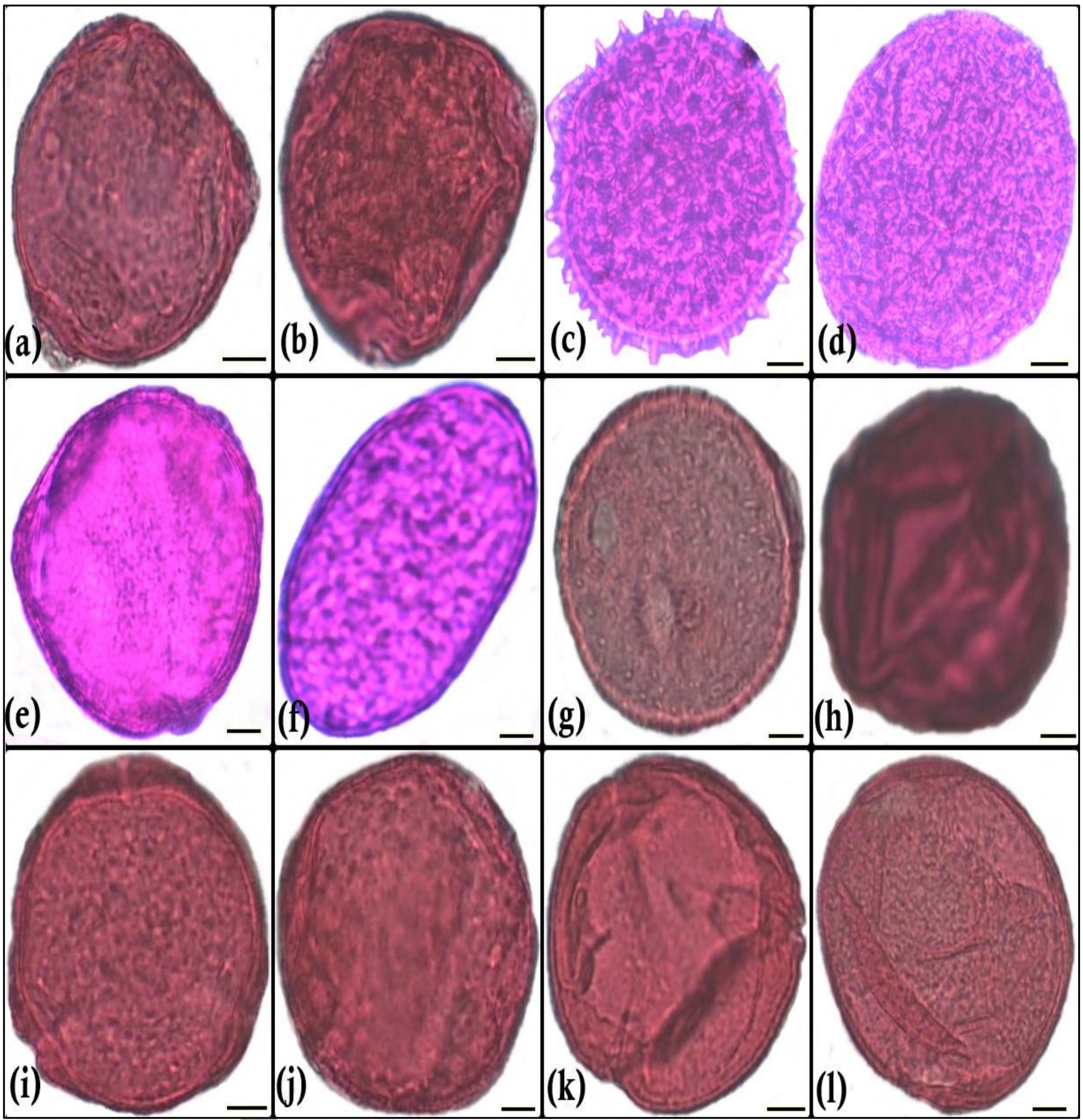


**Figure 19.** Light microphotographs of Cucurbitaceous showing polar and equatorial view of pollen taken at 40X magnification scale bar 10 µm (a-b) *Cucumis sativus*; (c-d) *Cucurbita maxima*; (e-f) *Cucurbita pepo*; (g-h) *Cucurbita pepo var. cylindrica*; (i-j) *Lagenaria siceraria*; (k-l) *Luffa acutangula*.

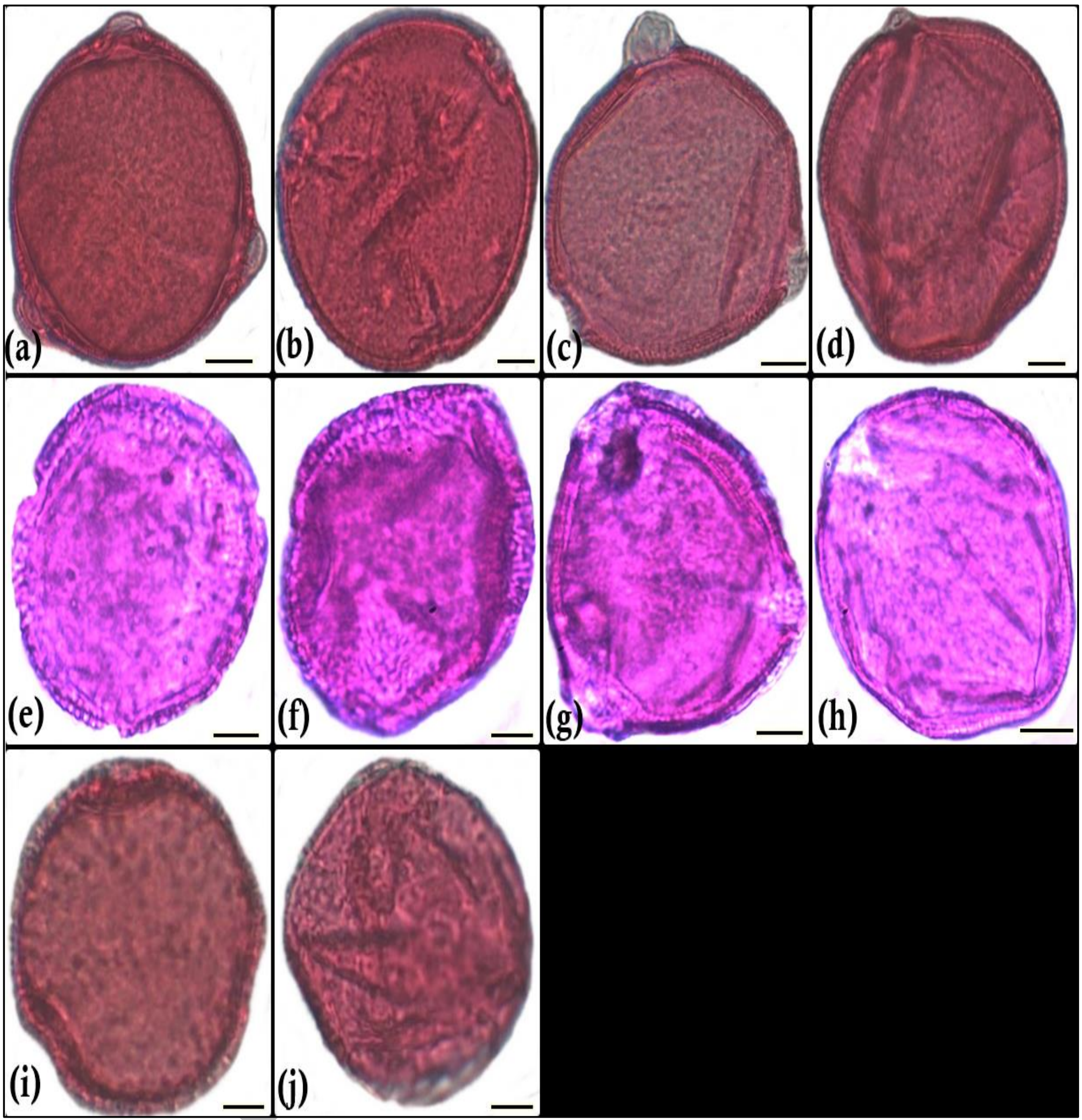


**Figure 20.** Light microphotographs of Cucurbitaceous showing polar and equatorial view of pollen taken at 40X magnification scale bar = 10 μm (a-b) *Luffa acutangula* var. *amara*; (c-d) *Luffa cylindrica*; (e-f) *Momordica charantia*; (g-h) *Momordica balsamina*; (i-j) *Praecitrullus fistulosus*.

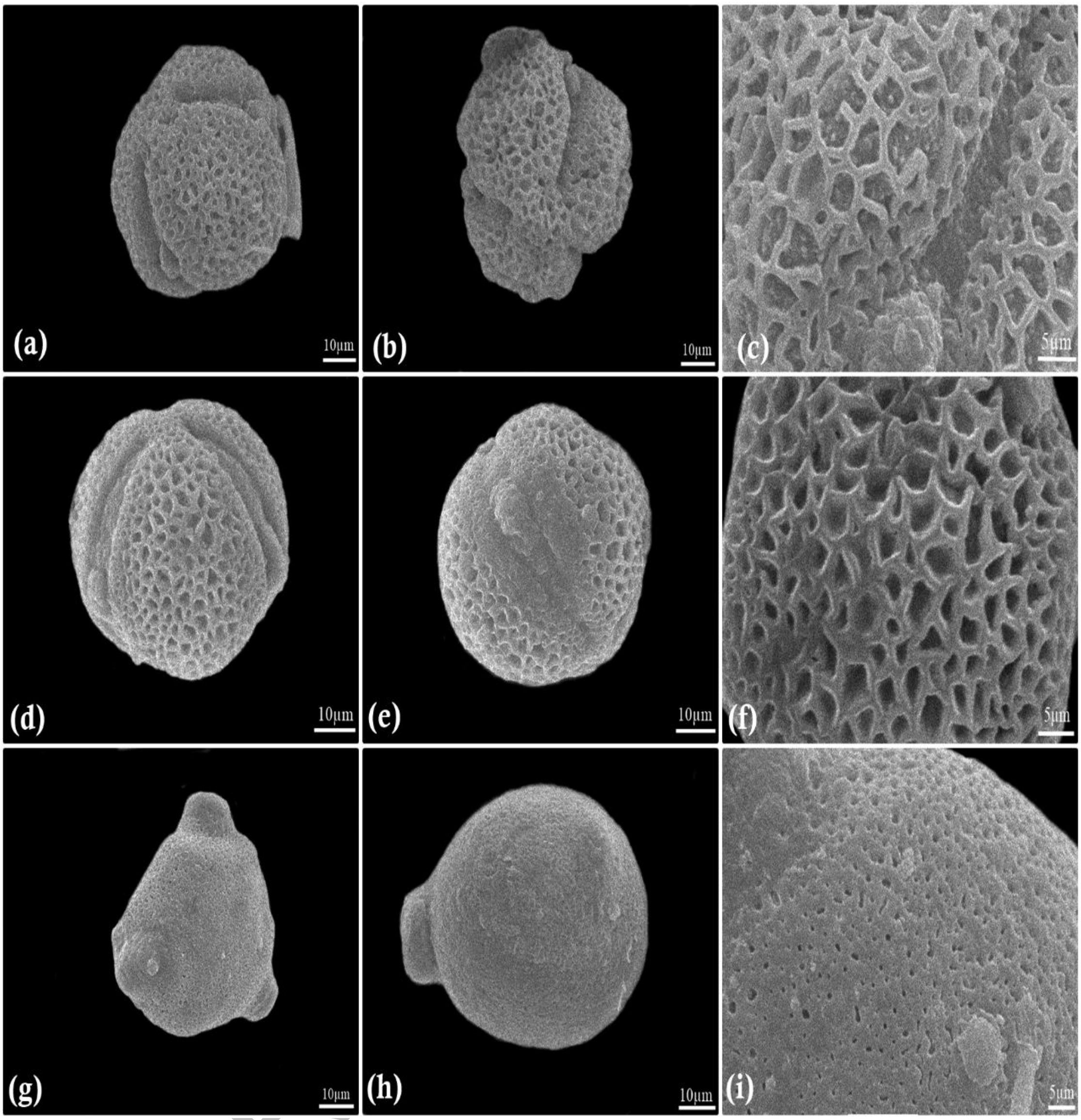


**Figure 21.** Scanning electron photomicrographs of pollen grains of Cucurbitaceae. (a-c) *Citrullus colocynthis*; (d-f) *Citrullus lanatus*; (g-i) *Cucumis melo*.

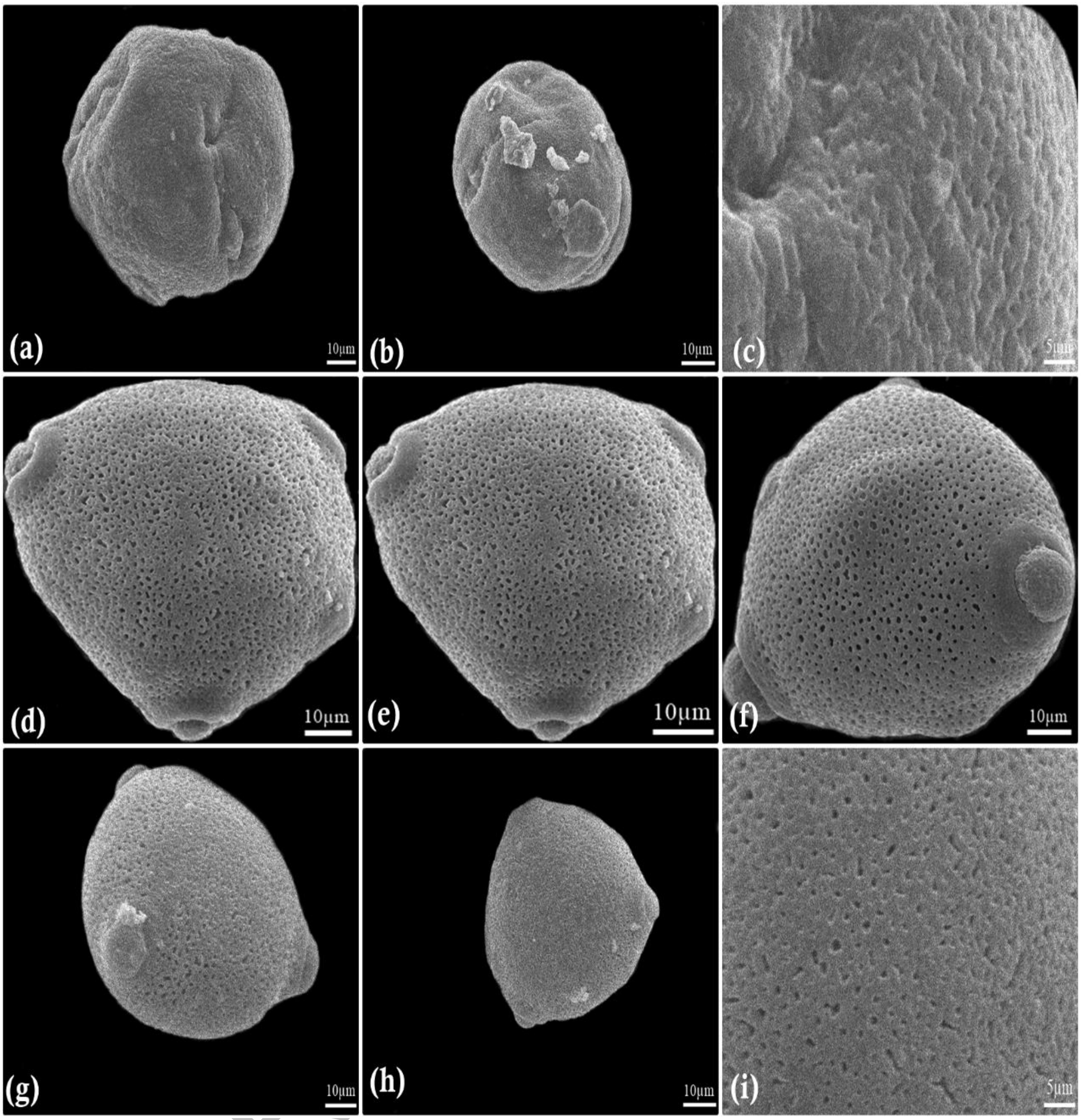


**Figure 22.** Scanning electron photomicrographs of pollen grains of Cucurbitaceae. (a-c) *Cucumis melo subsp. agrestis*; (d-f) *Cucumis melo* var. *flexuosus*; (g-i) *Cucumis melo* var. *cantalupensis*.

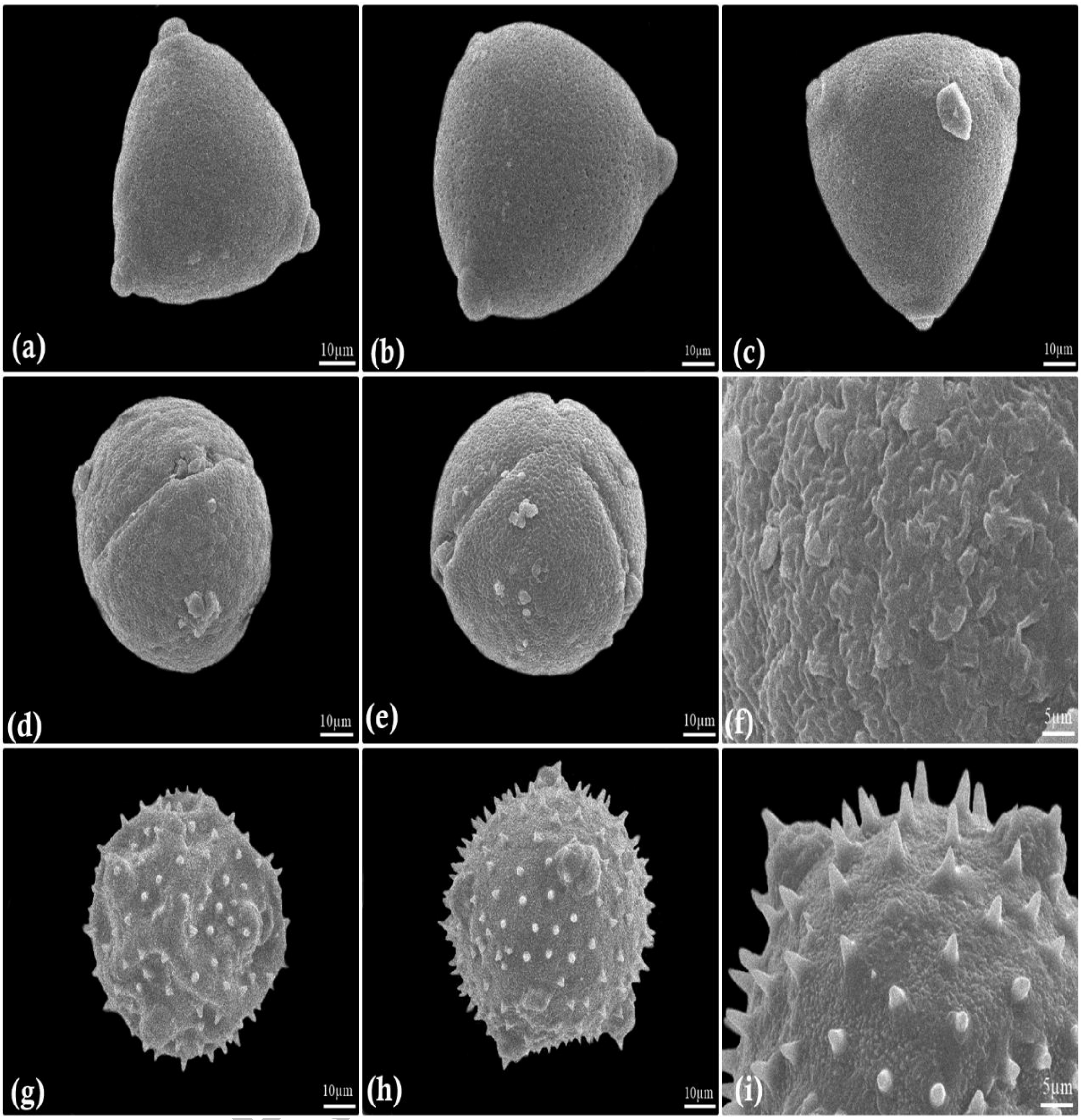


**Figure 23.** Scanning electron photomicrographs of pollen grains of Cucurbitaceae. (a-c) *Cucumis sativus*; (d-f) *Cucurbita maxima*; (g-i) *Cucurbita pepo*.

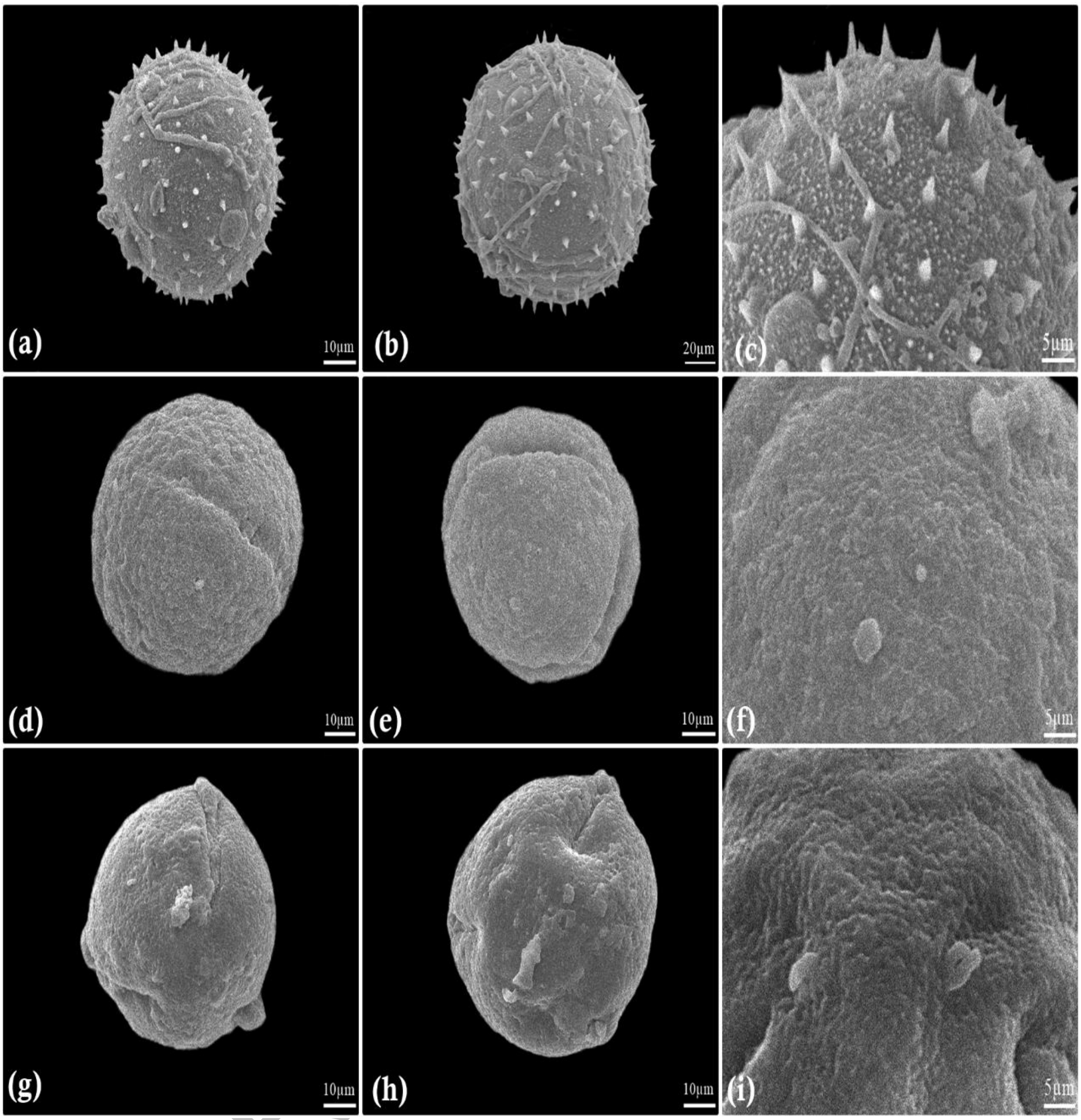


**Figure 24.** Scanning electron photomicrographs of pollen grains of Cucurbitaceae. (a-c) *Cucurbita pepo var. cylindrica*; (d-f) *Lagenaria siceraria*; (g-i) *Luffa acutangula*.

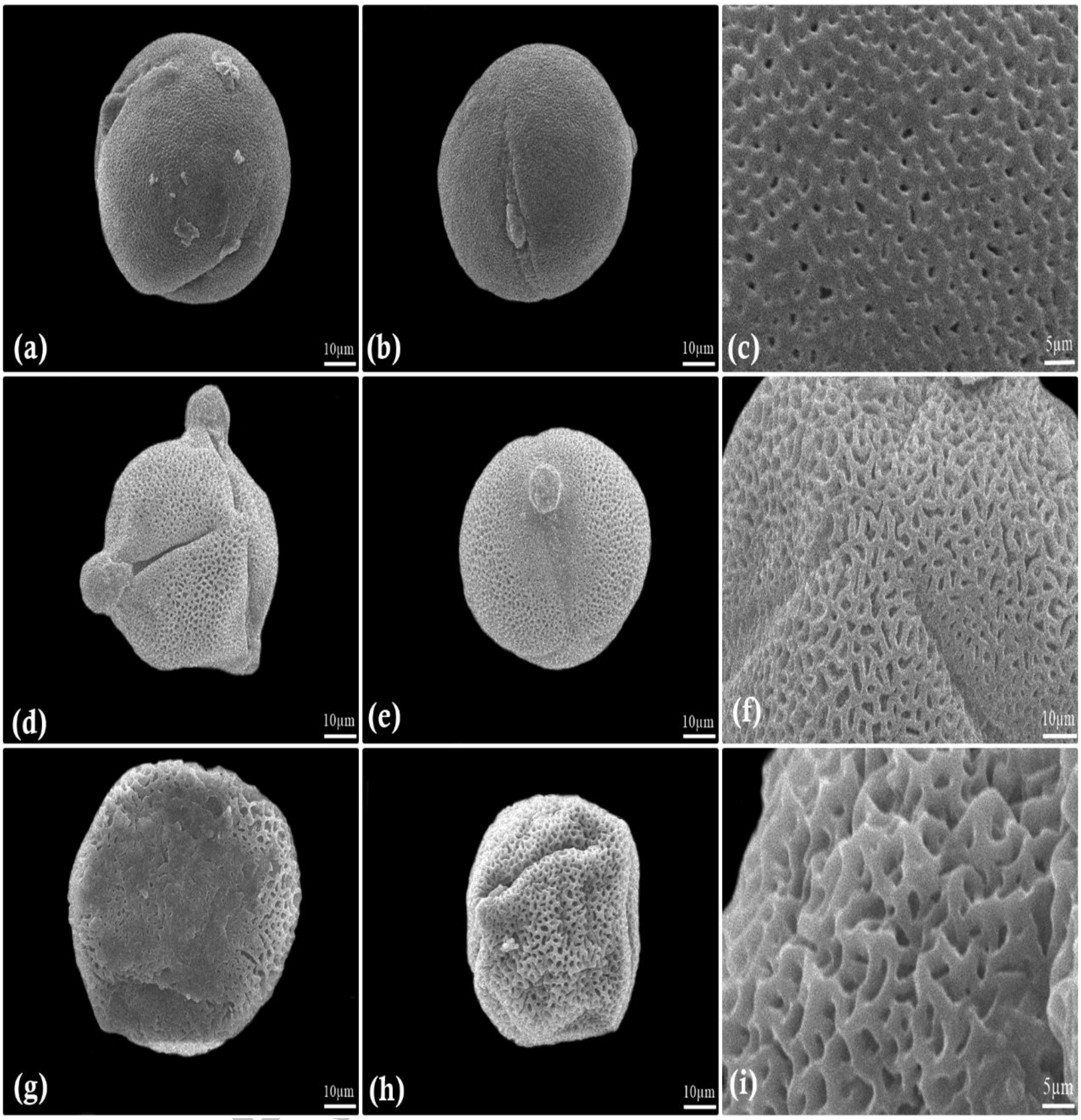


**Figure 25.** Scanning electron photomicrographs of pollen grains of Cucurbitaceae. (a-c) *Luffa acutangula* var. *amara*; (d-f) *Luffa cylindrica*; (g-i) *Momordica charantia*.

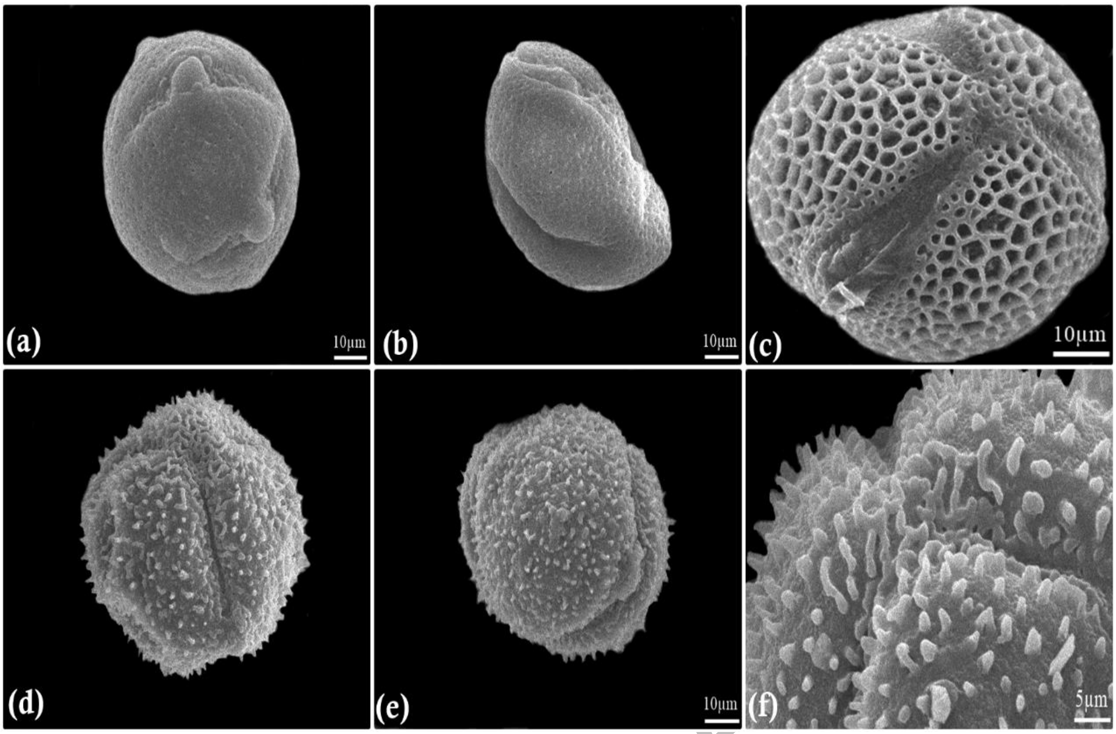


**Figure 26.** Scanning electron photomicrographs of pollen grains of Cucurbitaceae. (a-c) *Momordica balsamina*; (c-d) *Praecitrullus fistulosus*.

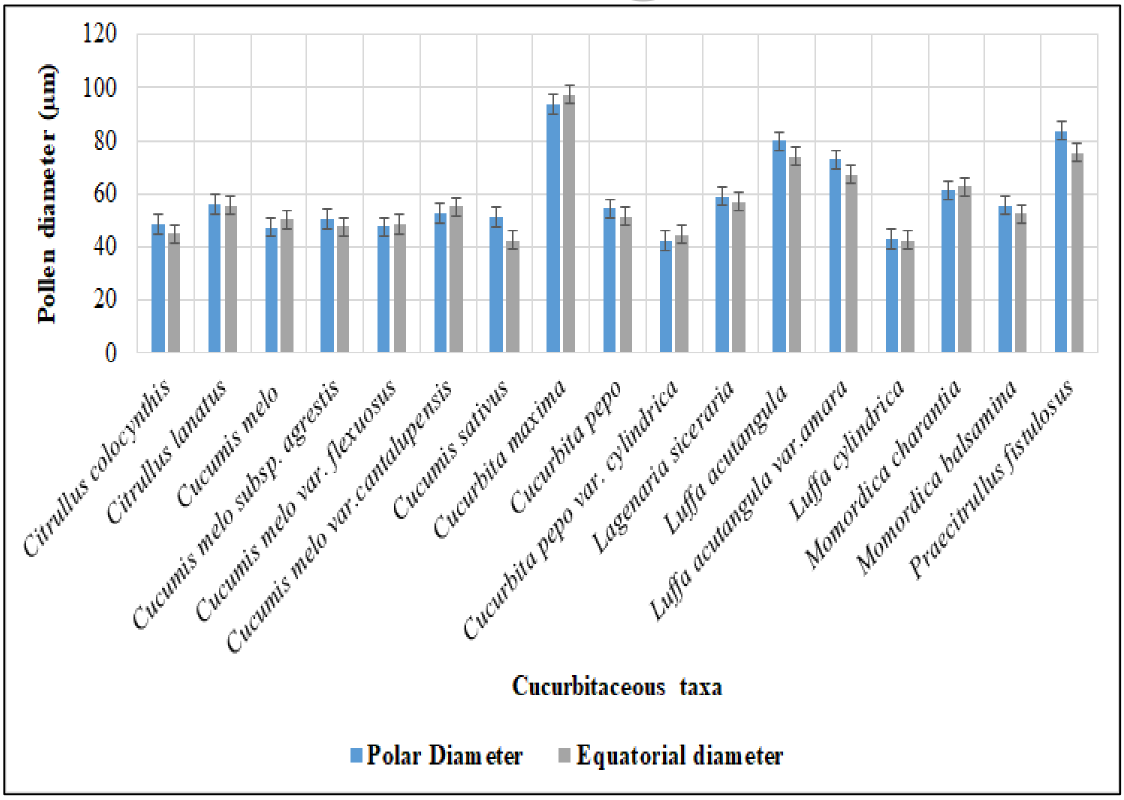


**Figure 12.** Mean pollen size variability among Cucurbitaceous taxa.

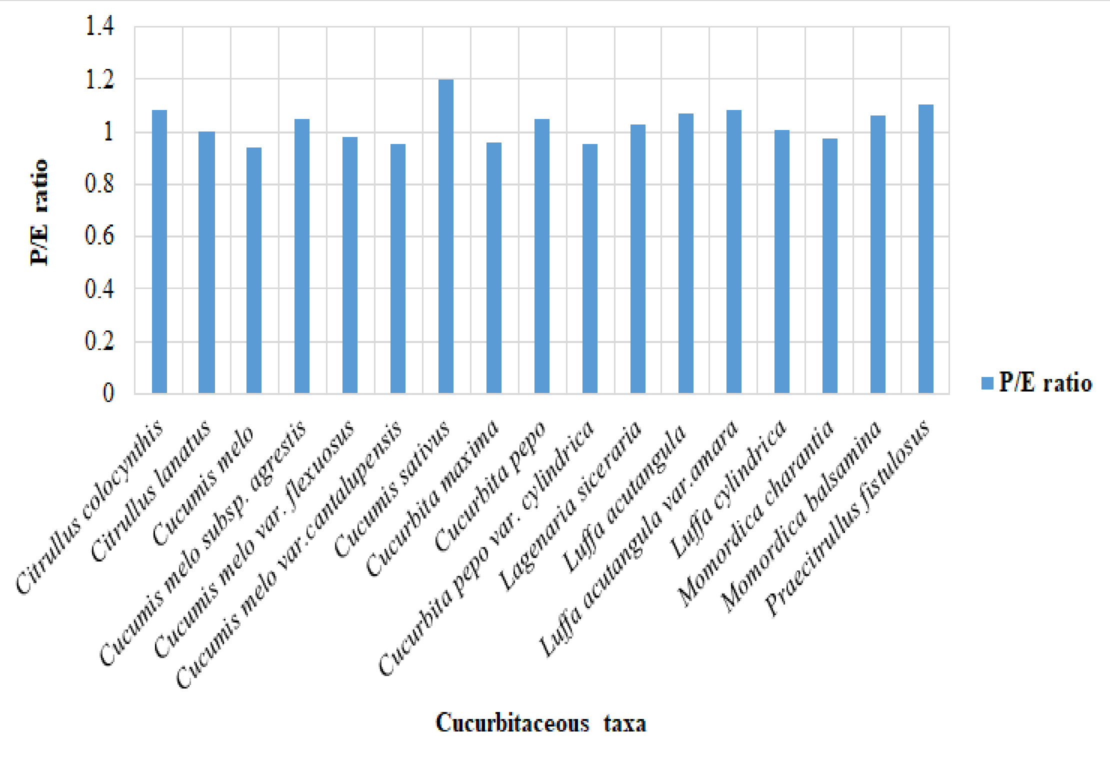


**Figure 13.** Polar to equatorial diameter ratio among Cucurbitaceous taxa.

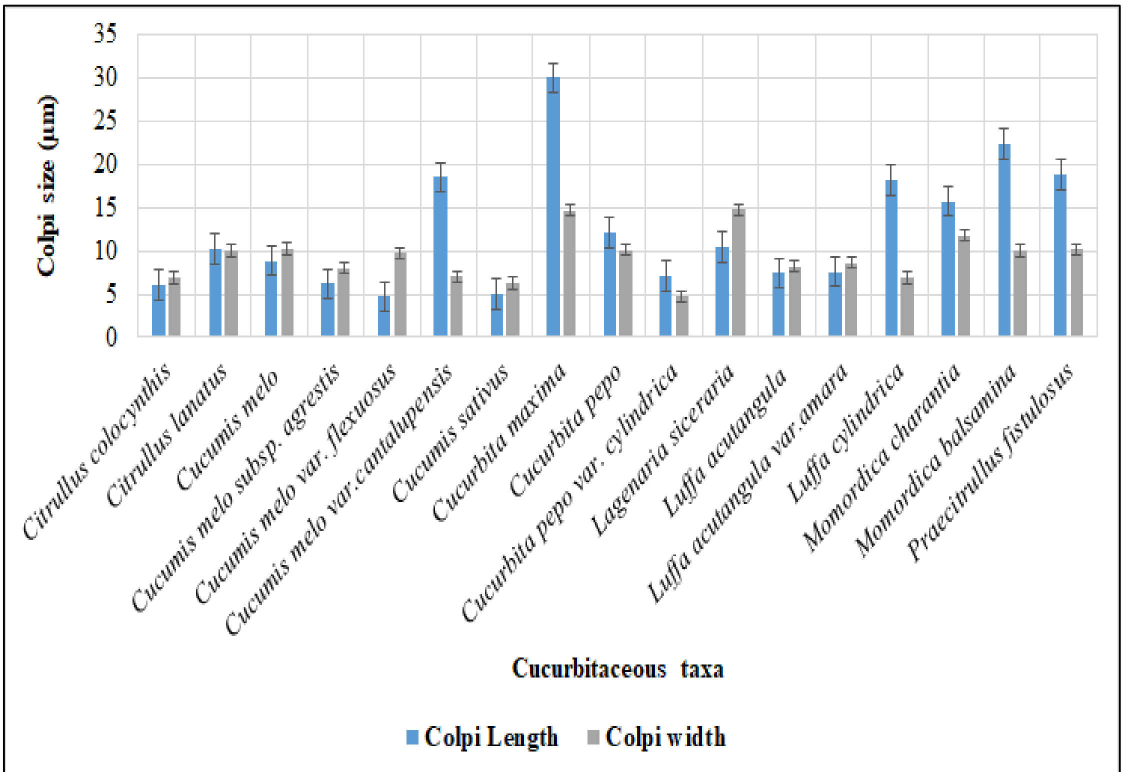


**Figure 14.** Average variations in colpi size among Cucurbitaceous taxa.

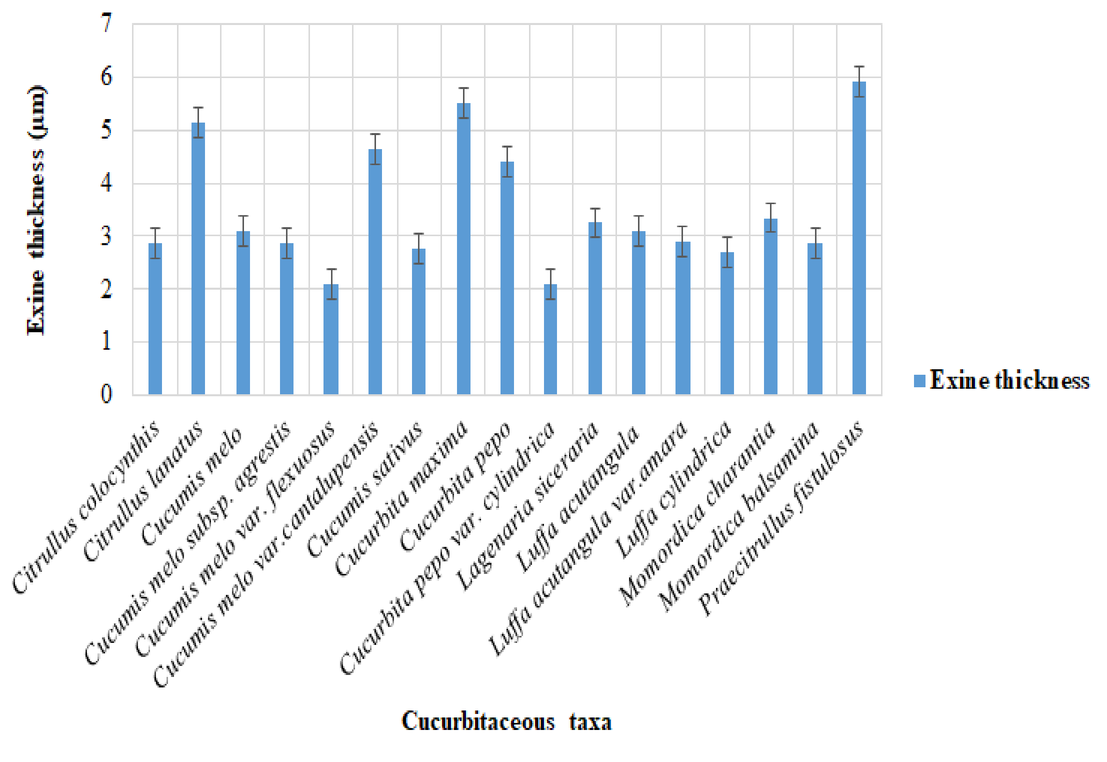


**Figure 15.** Mean exine thickness measurements among Cucurbitaceous taxa.

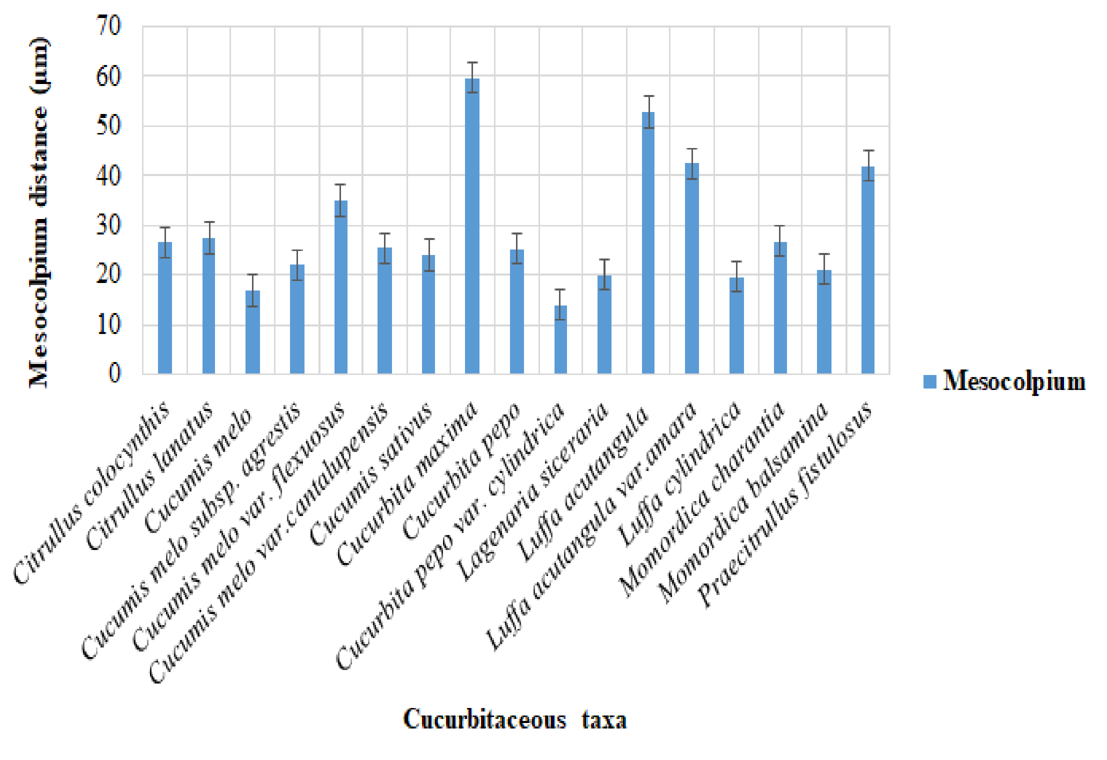


**Figure 16.** Means mesocolpium distance variability among Cucurbitaceous taxa.

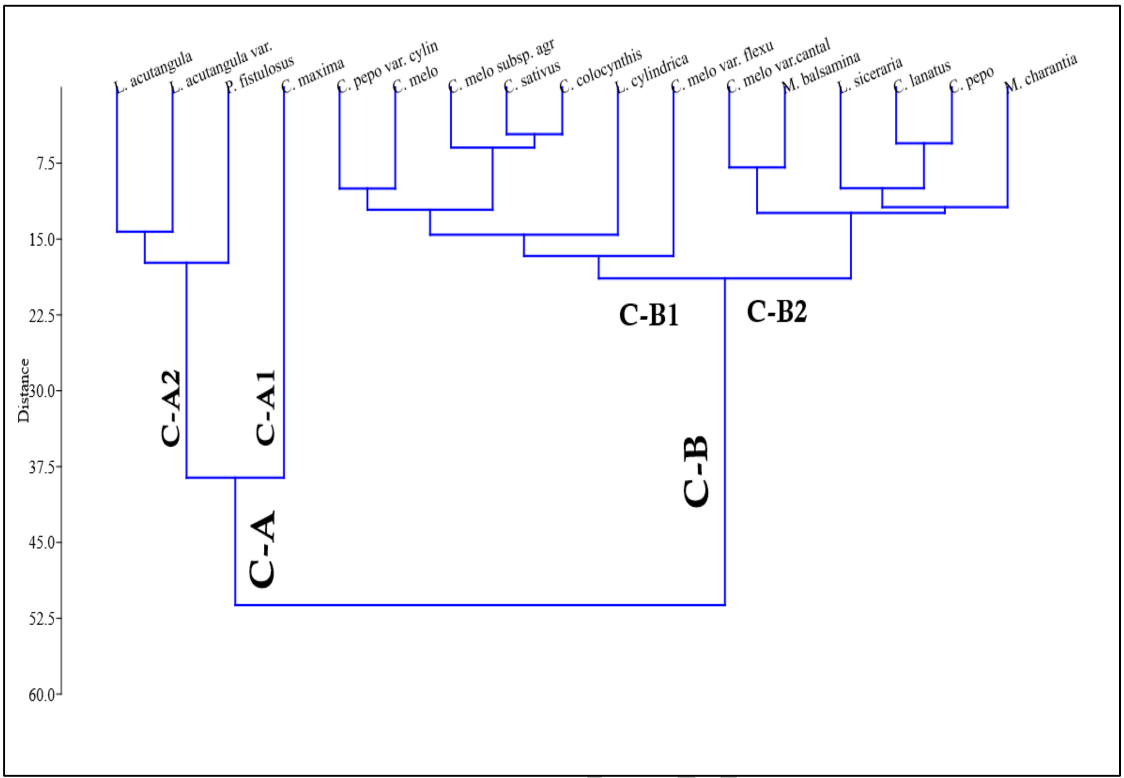


**Figure 17.** UPGMA of Cucurbitaceous taxa based on pollen quantitative characters.

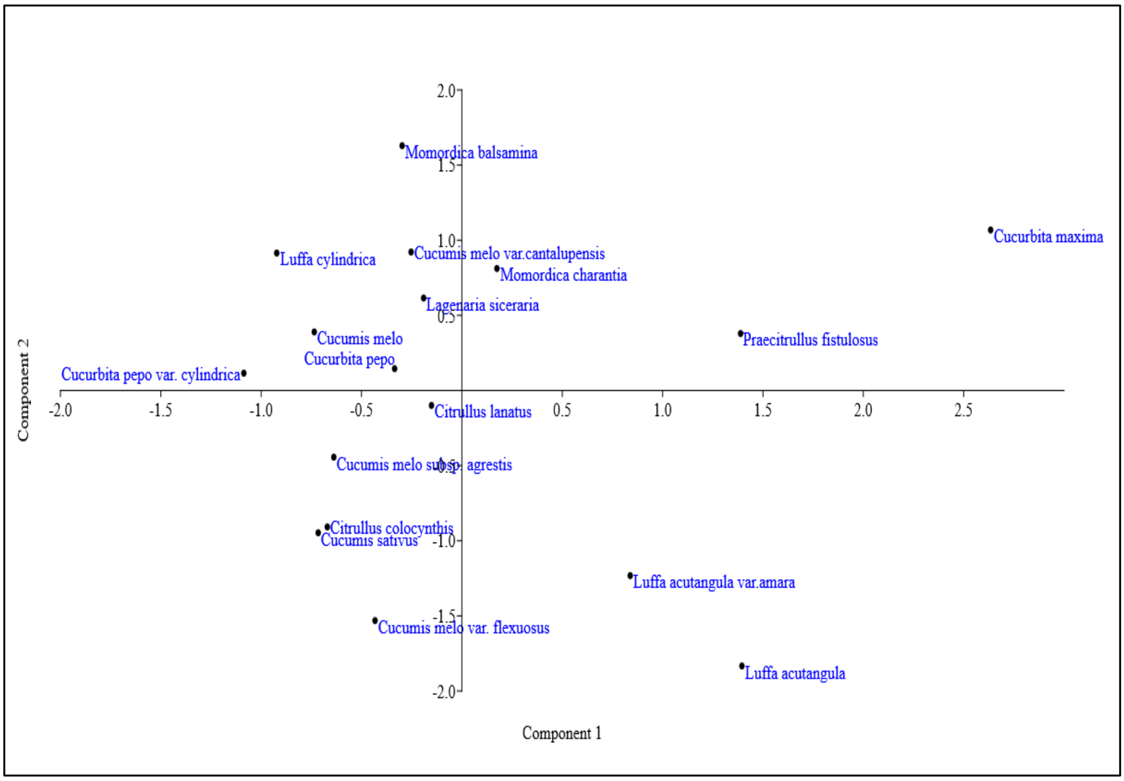


**Figure 18.** Principal component analysis (PCA) of the Cucurbitaceous pollen traits.

## 3.3.6 Discussion

In this study, we examined eight taxa containing representatives from the Cucurbitaceous flora that have economic and medicinal value to the local populace. The taxonomy gave critical pollinic features that allowed them to be identified. Pollen research in the Cucurbitaceae family is uncommon. Previous study has been undertaken by Ham and Preusapan (2006) on pollen in the genus *Zehneria*, Teppner (2004) on pollen in the genera *Lagenaria* and *Cucurbita*, and Perveen and Qaiser (2008) on pollen in Pakistani Cucurbitaceous taxa. Van Der Ham et al. (2010) conducted a literature survey on the palynology of Cucurbitaceae, focusing on the subfamilies. They define the Cucurbitoideae subfamily as having small to extremely large pollen grains, oblate to prolate format, pantoapertured, brevicolpated operculated, exine typically tectate, perforated, and reticulated as the most prevalent ornamentation. *Citrullus lanatus* and *Citrullus colocynthis* both have conical-shaped grains with a psilate surface. *Cucumis melo* and *Cucumis sativus* pollen is tricolpate and triangular psilate, but *Cucurbita maxima* pollen is globular with stigmatic peculiarities. *Lagenaria siceraria* pollen is spherical with significant symmetry and contains psilate exine (Kouonon et al., 2019).

Six native Brazilian species were examined by Lutz et al. in 2021 while taking pollen morphotype diversity into account. *Momordica charantia* L., one of the species, was also examined in this study, and the traits discovered there were identical to those discovered in our study in terms of dispersal units, number and kind of aperture, and exine peculiarities. However, the size of pollen measured in the current study ranged from medium to large, which differed from previous work. Perveen and Qaiser (2008) palynologically characterized nine Cucurbitaceae species in Pakistan, categorizing them into seven taxa. The authors classified three pollen types, one of which, *Momordica charantia*, was identical to the authors' in terms of size and sculpturing type.

Our findings reveal that the form and polarity of pollen grains, the number of colpi, and the sculpturing of pollen grains from *Cucurbita pepo* are similar to descriptions of pollen from other Cucurbitaceae species. However, pantoporate and echinate grains have been previously documented for species (Srivastava and Sharma, 2016), demonstrating differences from the intricate tricolporate and reticulate-gemmate type sculpturing found in this work. The pollen of *Citrullus colocynthis* was described by Akhtar et al. (2019) as circular-elliptic with coarsely reticulate ornamentation and a thick exine; nevertheless, the findings of our study reveal elaborate scabrate-reticulate tectate grains. Perveen and Qaiser (2008) examined the pollen of Cucurbitaceous species from Pakistan and found that *Citrullus colocynthis* grains have similar microstructural properties to the grains we examined in this study.

Oblate-spheroidal grains and reticulate rugulate exine sculptured features characterize *Cucumis melo*. Regarding the circular elliptical shape and the finely reticulated exine tectum, Akhtar et al., (2019) and Perveen and Qaiser (2008) findings diverged from the aforementioned information. Levi et al. (2010) described the triangular, tricolpate, and smooth surface grains in *Cucumis melo* in a different study. Ullah et al., (2021) found similar findings on Cucumis melo pollen as prospective honeybee forage flora, with only a few changes. Usma et al., (2022) recently completed palynological observations of weeds including *Cucumis melo subsp. agrestis* pollen with perforated mesocolpium and exine characteristics, whereas the current study found scabrate-perforate tectum grains. Ullah et al., (2021) described pollen surface traits of *Cucumis melo subsp. agrestis* var. *agrestis*, including reticulate exine sculpturing, a sunken interapertural area, and tricolporate pollen.



Levi et al., (2010) explanation of *Praecitrullus fistulosus* spherical pollen grains with hairy walls contrasted with our findings' descriptions of prolate-spheroidal, scabrate, and micro-echinate sculptural features. In Praecitrullus, there were three-zonocolporate and spinescent exine surfaces (Sridhar and Singh, 2019). Pollen size variation within *Citrullus lanatus* has been recorded. The full degree of pollen size and shape diversity within and among *Citrullus spp.* has not been determined (Jarret et al., 2017). *Citrullus lanatus* exine was visualized as reticulate sculptured patterns in the current study, which was consistent with studies done by Parveen and Qaiser (2008) but differed from the findings of Awasthi (1962) in terms of exine thickness.

Biate et al. (2019) discovered a favorable link between agromorphological and pollen features in *Cucumis sativus* when they examined sub-oblate to oblate-spheroidal, sub-triangular, elliptical, trizonoporate, and reticulated exine patterns. *Cucumis sativus* pollen was found to be gemmate-verrucate, tricolporate, and prolate-spheroidal in this study. In another study (Lakhsmi, 2013), oblate-type grains in *Cucumis sativus* were observed. According to Tin et al., (2020) from Myanmar, pollen with a triporate aperture and an obscurely reticulate stratification differ from our findings. Jones and Pearce (2015) reported scabrate-granulate tectum characteristics on *Cucumis sativus*, but the current study reported gemmate-verrucate type sculpturing.

Previous research revealed that *Luffa* pollen was spheroidal, trilobate, circular tricolporate, colpi thin sexine surrounding pores slightly thickened, sculpture finely reticulate to striate-reticulate heterogeneous-brochate (Tin et al., 2020; Yang et al., 2018), so while our study showed that the pollen of *Luffa acutangula* scabrate verrucate pollen ornamentation. Myint (2019) evaluated tricolporate, oblate-spheroidal, big, and rounded exine patterns in *Luffa aegyptiaca,* which was consistent with our findings. Yang et al., (2018) from Southern China performed palynology of wild crop plants including *Luffa cylindrica* showing spheroidal, trilobate, tricolporate, and circular with thickened finely reticulate pore sculpturing. Naik et al., (2016) observation on pollen morphotypes of *Luffa cylindrical* also revealed reticulated pollen exine stratification similar to present work.

Abd-El Maksou and Nassar (2013) compare the pollen morphology of *Lagenaria siceraria* as spheroidal, 3-colporate and glebulate tectum. However current study revealed prolate-spheroidal and reticulate sculptured pollen surfaces. While Akhtar et al., (2019) described the morpho-palynology of *Lagenaria siceraria* and showed that it was radial symmetrical, compound aperturate with three pores and colpi, finely reticulation with perforations was analyzed on an exine surface. Hulse (2018) analyzed apolar, spherical, and echinate projected exine in *Cucurbita maxima* at higher magnification using scanning microscopy from Central America to be used as a reference involved in paleopalynology. *Cucurbita maxima* show 3-4-5 zonoporoate grains is not consistent with a study by (Lakshmi, 2013) which indicated that the pollen in these two species is 10-12 zonoporoate. Saklani et al., (2018) from Himachal Pradesh utilized scanning microscopy to find echinate sculptured pollen in *Cucurbita maxima*.

The pollen micromorphological variability identifies the eurypalynous state of the Cucurbitaceae family. The explanatory taxonomic revisions have been performed along with authenticated high-resolution pollen micrographs for accurate identification.

# Conclusion & Future Recommendations

## 4. Conclusion

This is the first comprehensive documentation with respect to taxonomic characteristics of tendril and pollen (LM & SEM) of family Cucurbitaceae, 17 species belonging to 07 genera from district Bhakkar Pakistan. Through microscopic magnifications, the anatomical morphometry of tendril micromorphological features in Cucurbitaceous taxa exhibited variations. Tendril microanatomical is crucial to consider the shape of the tendril outline, vascular bundle arrangements, sclerenchyma layers, and the morphology of the collenchyma and epidermal cells. The largest collenchyma cell was found in *Cucumis sativus* (27.85 µm), whereas the longest sclerenchyma cell was found in *Luffa acutangula* var. *amara* (38.05 µm). Palyno-morphology of Cucurbitaceous species explained significant taxonomic information about the micromorphological qualitative features. The pollen type prolate-spheroidal was dominant and the pollen shape in the polar view was circular. Quantitatively mesocolpium distance measured a maximum of 59.7 µm for *Cucurbita maxima*. Diverse types of exine stratification were found significant taxonomically to accurately identify and delimit the Cucurbitaceous species. The echinate exine peculiarities of diverse types baculate-echinate in *Cucurbita maxima*, scabrate-echinate in *Cucurbita pepo var. cylindrica,* and scabrate-microechinate in *Praecitrullus fistulosus*. The findings show that the taxonomic identification of Cucurbitaceous species and their relationships will benefit from quantitative anatomical tendril features through clustering (UPGMA and PCA) analysis. It is concluded from this study that the Palyno-anatomy of Cucurbitaceae is very helpful in the identification and delimitation of species.

## 5. Future Recommendation

- Phylogenetic analysis using advanced analytical techniques will be needed to better understand the placement of Cucurbitaceous species in the evolutionary lineages.
- Advanced molecular, chromatographic, and spectroscopic methodologies will be used for the identification of Cucurbitaceous medicinal species to incorporate them into the pharmaceutical industry.
- Exploration of the diversity of Cucurbitaceae and their potential in drug development in the herbal industry and their propagation, preservation, and conservation will be practiced.
- Efforts are needed to develop a floral catalog of Cucurbitaceous family which will be helpful for research communities of allied disciplines and the herbal industry for socio-economic uplift of local community specifically the elimination of diseases.

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# Tendril Anatomy: A Tool for Correct Identification among Cucurbitaceous taxa

**Naveed Abbas [1], Muhammad Zafar [1,*], Mushtaq Ahmad [1,2], Ashwaq T. Althobaiti [3], Mohamed Fawzy Ramadan [4], Trobjon Makhkamov [5], Yusufjon Gafforov [6,7], Khislat Khaydarov [8], Muhammad Kabir [9], Shazia Sultana [1], Salman Majeed [1,10] and Tajalla Batool [1]**

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**Abstract:** This research examined the histological micro-structure of tendril vasculature in cucurbitaceous taxa. In this research, the tendril anatomy of 17 taxa of Cucurbitaceae categorized into seven genera, including *Cucumis* (five species), *Cucurbita* and *Luffa* (three species each), Citrullus and Momordica (two species each) while Lagenaria and Praecitrullus (one species each), collected from different areas of the Thal desert were examined via microscopic imaging to explore its taxonomic significance. Tendril transverse sections were cut with a Shandon Microtome to prepare slides. The distinctive characteristics of taxonomic value (qualitative and quantitative) include tendril and vascular bundle shape, variation in the number of vascular bundles, tendril diameter length, layers of sclerenchyma, and shape of collenchyma and epidermal cells. Tendril shapes observed are irregular, slightly oval-shaped, slightly C shaped, angular (4-angled, 6-angled, or polygonal), and star shaped. Quantitative measurements were taken to analyze the data statistically using SPSS software. *Cucurbita pepo* had a maximum tendril diameter length of 656.1 µm and a minimum in *Momordica balsamina of* 123.05 µm. The highest number of vascular bundles (12) were noticed in *Luffa acutangula* var.*amara. Angular* type was prominent in collenchyma, and irregular shape was dominant in sclerenchyma cells. A maximum of seven to nine sclerenchyma layers were present in *Lagenaria siceraria* and a minimum of two or three layers in *Cucumis melo* subsp. *agrestis*, *Cucumis melo* var. *flexuosus*, and *Cucumis melo* var.*cantalupensis.* Epidermis cells also show great variations with a rectangular shape being dominant. Statistical UPGMA dendrogram clustering of tendril vasculature traits shows that histological sections studied with microscopic techniques can be used to identify species and will play a vital role in future taxonomic and phylogenic linkages.

**Keywords:** anatomy; Cucurbitaceae; micromorphology; parenchyma; vessel elements