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# Anatomical modification of Cyperaceae family from Rasool Headworks, District Mandi Bahauddin

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## ABSTRACT

Among monocots Cyperaceae family is third largest family having 109 genera containing 5500 species. Its distribution is worldwide including pine forests and mountainous areas. On economic and ethnobotanical scale almost 10% species of this family are specially used at local or regional level. Anatomical study of these species can be helpful in various fields as systematics, cytogenetics, and taxonomy. Characteristics including size and shape of vascular tissue, thickness, and nature of epidermis, within vascular bundles and cortex presence of sclerenchyma and moderation in bulliform cells were some annalistic characters which were distinguished among closely related species of this family. Through double standard staining technique by free hand sectioning permanent slides were prepared. Using ocular microscope transverse sections of leaf were observed and later photographed using camera equipped compound micrometer. As herbarium sheet plant material was preserved for future records. Statistical analysis including multivariate (cluster) and variance was used for comparison of mean using LSD (least significant difference) for determining the differences between different species of Cyperaceae family.

**Key words:** Anatomical, Cyperaceae family

## INTRODUCTION

Among monocots Cyperaceae is third wide-ranging family. (Lunkai *et al.*, 2010). It consists of 109 genera including 5500 species (Muasya *et al*., 2009). Its distribution pattern is worldwide, most species are weeds (Kukkonen, 2001). Nearly 35% within genera are monotypic among them plant species of Cyperus 686 and of Carex are 1,757. On economic and ethnobotanical scale almost 10% species of this family are specially used at local or regional level (Simpson and Ignlis, 2001). In Pakistan 179 species having 22 genera of family Cyperaceae are reported, most of them are weedy species (Kukkonen, 2001). Habitat of these species varies from grime salinities to hyperregulate water, but most are present in wetland, or poor soils (Khan and Qaiser, 2006). For example, *Fimbristylis dichotoma* Vahl (Kranz) and *Cyperus laxus* L. (non-Kranz) were found at the edges of forests in subtropical and tropical regions (Alves *et al.*, 2009), and regarded as weed of respective areas due to great distributional range (Bryson and Carter, 2008). Anatomical study of these species can be helpful in various fields as systematics, cytogenetics, and taxonomy (Chase *et al*., 2000). Cyperaceae family absent within arid areas (annual rainfall less than 250mm). Species mostly inhabit within areas with maximum winter rainfall. Here species widespread from tropic to sub tropic and temperate to Sub Antarctic level. Within Japan wet shade habitats are covered by the distribution of C3 cyperaceae species (Ueno and

Takeda, 1992). Cyperaceae species contain epidermal cells that are larger on adaxial surface as compared to abaxial side (Sharma and Mehra, 1972). Within most well-developed taxa bulliform cells are present in midrib portion of leaf (Sonnenberg and Botha, 1992). Within epidermis of several species' silica bodies are reported (Starr and Ford, 2001).

Among Cyperaceae species leaves are mostly sub basal or basal, without or with ligules, having sheath, usually glabrous blades, margins, and apex barded; reduced into several groups. Silica bodies generally within plants (Ball *et al*., 2002). The flowers of Cyperacae are covered by spirally or distichously arranged spiklets or glumes with or without stolons or rhizomes. Achenes or nuts are indehiscent fruits. Sedges resemble grasses but to differentiated them; grasses are right up hollow from ground, sedges contain edges while round are rushes (Mishra and Chauhan, 2013). The stem having spongy, solid, or hollow internodes. Triangular shape young stems, oval, flatted, or cylindrical rarely (Parsad and Krishnamuthy, 2008).

In Cyperaceae main underground stem is rhizome (Metcalfe, 1971), even within several species including *Cyperus esculentus* and *Cyperus rotundus*, stem system is composed of branches combining to form a strong network underground also having monopodial or sympodial growth and reduced internodes (Gifford and Bayer, 1995). Division of Cyperaceae is into two sub families the Cyperoideae and the Caricoideae. Further subfamilies sub divided into twelve groups having 122 genera. They include: *Bisboekeleraceae, Arthostylideae, Abildgaardieae, Hypolytreae, Arthostylideae, Cariceae, Scirpeae, Cryptangieae, Schoeneae, Trilepideae, Sclerieae* and *Rhynchosporeae*. Physiologicaly Cyperaceae family classified into C3 and C4 species. Meanwhile, on presence and arrangement of vascular sheath C4 species are further subdivide within four types (Bruhl and Perry,1995). In Cyperoideae flowers are monocotyledonous having superior gynoecium, trimerous androecium, trimperous perianth (Rudall and Bateman, 2004) or they may be originated from this pattern (Vrijdaghs *et al*., 2009). In cyperaceae taxonomical data is more emphasized (Silveira and Longhi-Wagner, 2010), while development of anatomical characters are confined to limited species that are having economic consequences, e.g. *Cyperus papyrus (Menezes et al. 2005)*. Generally anatomical development studies description is restricted to one organ as; rhizmoes in *Cyperus esculentus, Scleria (Lima and Menezes, 2009).* In certain species, anatomical attributes of several plant organs are represented as an adaptative response in relation with habitat ecology (Grigore and Toma, 2007). Within severe conditions like drought and salinity Cyperaceae family native species have evolved specific leaf anatomical characteristic. The variation in the photosynthetic pathways is due to ultra-structural and anatomical characters including ecological and physiological differences (Soros and Bruhl, 2000).

Plants has great economic importance all over the world. Members of the family are well known in view of horticultural use (Simpson and Inglis, 2001). Sedges can be used in various forms including animal poisons, food flavoring, fibers, drinks, foods, in making processes of various objects which include boats, clothing, ropes, perfumes, shoes, paper, mats and medicines (Simpson and Inglis, 2001). Rhizomes, tubers, foliage, and seeds of sedges are important as domesticated forage or animal feeds (Abad *et al.,* 2000). These are also used in control of soil erosion, soil fertility, improvement, and vegetation of barren areas (Simpson and Inglis, 2001).

**OBJECTIVES**

- To explore Cyperaceae family diversity

- To explore biodiversity of District Mandi Bahauddin for future planning in association with conservation of exotic and native flora
- To identify relative anatomy for ecological and taxonomical significances

## MATERIALS AND METHODS

A survey was conducted on Head Rasool barrage to collect the different plant sample of family Cyperaceae. Sample were brought in ice bags to laboratory. To study anatomical features different plant parts including leaf, root and stem were selected for free hand sectioning. To check anatomical properties plant material used include petiole root and leaves. For anatomy of root, one cm from abundant root base was chosen. Similarly leaves containing one cm along midrib from leaf center were used. For selection material was preserved in FFA (Formalin Acetic Acid) solution, which contain acetic acid 10%, distilled water 35%, formalin 5% and ethyl alcohol 50%. For durable preservation material was preserved in Acetic Alcohol solution (three parts of ethyl alcohol and one-part of acetic acid). For transverse section slide preparation Double-stained technique was utilized. From permanent slides comparative anatomy of root, leaves and stem was studied. Using Carl-Ziess camera microscope photographs was taken from permanent slides. Anatomical features examined during examination includes:

**Leaf Anatomy**

Epidermal cell area, Sclerenchyma thickness, Aerenchyma area, Cortical thickness, Cortical cell area, Metaxylem area, Phloem area, Vascular bundle thickness, Vascular bundle area, Bulliform cell thickness, Bulliform cell area

**Stem anatomy**

Epidermal cell area, Sclerenchyma thickness, Aerenchyma area, Cortical thickness, Cortical cell area, Pith area, Metaxylem area, Phloem area, Vascular bundle thickness, Vascular bundle area

**Root anatomy**

Epidermal thickness, Endodermis thickness, Epidermal cell area, Aerenchyma area, Cortical thickness, Cortical cell area, Metaxylem area, Phloem area, Vascular bundle thickness, Vascular bundle area, Pericycle thickness

**Statistical analysis**

Statistical analysis including multivariate (cluster) and variance was used for comparison of mean using LSD (least significant difference) for determining the differences between different species of Cyperaceae family.

## RESULTS AND DISCUSSION

**Anatomical Studies**

Rasool Headwork, district Mandi Bahauddin was thoroughly explored for floral record of Cyperaceae family. Different species of sedges were collected. Anatomical characters of plant leaf were sectioned and photographed. Data of various anatomical attributes presented below.

**Leaf Anatomy**

**Epidermal cell area ($\mu m^2$):** Results of epidermal cell area were highly significant. *Cyperus articulatus* and *Eriophorum comosum* showed maximum epidermal cell area. It was closed followed by *Cyperus conglomeratus*. Moderate cell area was recorded among *Fimbristylis miliacea, Cyperus compressus* and *Cyperus alternifolia* followed by *Cyperus rotundus* and *Cyperus flavescens*. While *Pycreus flavidus*, *Cyperus nutans*, *Cyperus difformis* and *Cyperus*

*longus* showed minimum epidermal cell area. In comparison with *Cyperus flavescens* and *Cyperus rotundus*, *Cyperus rotundus* showed more significant results.

**Sclerenchyma thickness (µm)**

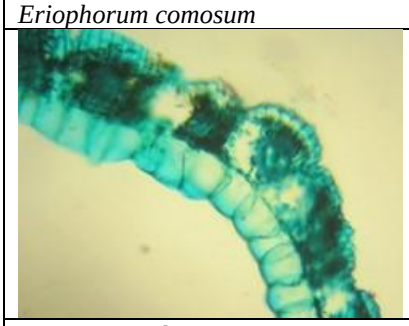
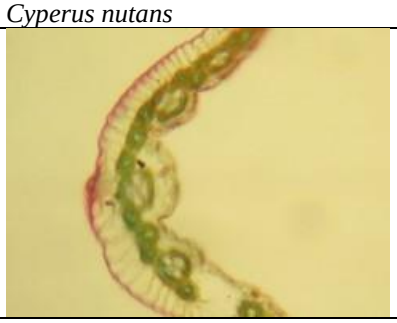
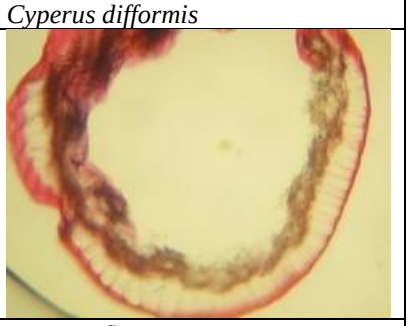
Highly significant results of sclerenchyma thickness were recorded among all species. *Cyperus articulatus* showed thickest sclerenchyma followed by Fimbristylis dichotoma, Fimbristylis miliacea, *Cyperus compressus*, *Cyperus flavescens* and *Cyperus conglomeratus*. *Cyperus alternifolia* and *Cyperus longus* showed much resembled results. On the other hand, *Cyperus difformis*, *Cyperus esculentus*, *Fimbristylis complanata* and *Cyperus nutans* showed minimum thickness in sclerenchyma.

**Aerenchyma area (µm²)**

Recorded results for aerenchyma area were highly significant. *Fimbristylis miliacea* showed maximum aerenchyma area followed by *Cyperus difformis*. *Cyperus compressus*, *Cyperus esculentus*, *Cyperus flavescens* and *Cyperus nutans* showed moderate aerenchyma area. Meanwhile minimum results were seen within *Cyperus rotundus*, *Cyperus conglomeratus*, *Cyperus articulatus* and *Eriophorum comosum*. Among *Cyperus nutans* and *Fimbristylis dichotoma*, *Cyperus nutans* showed highly significant result.

**Cortical thickness (µm)**

*Cyperus compressus* showed maximum cortical thickness. *Pycreus flavidus*, *Fimbristylis dichotoma*, *Cyperus longus*, *Cyperus flavescens*, *Cyperus esculentus*, *Cyperus difformis* and *Fimbristylis complanata* showed intermediate cortical thickness. While

| | | |
|---|---|---|
|  |  |  |
| *Eriophorum comosum* | *Cyperus nutans* | *Cyperus difformis* |
|  |  |  |
| *Cyperus esculentus* | *Cyperus longus* | *Cyperus flavescens* |

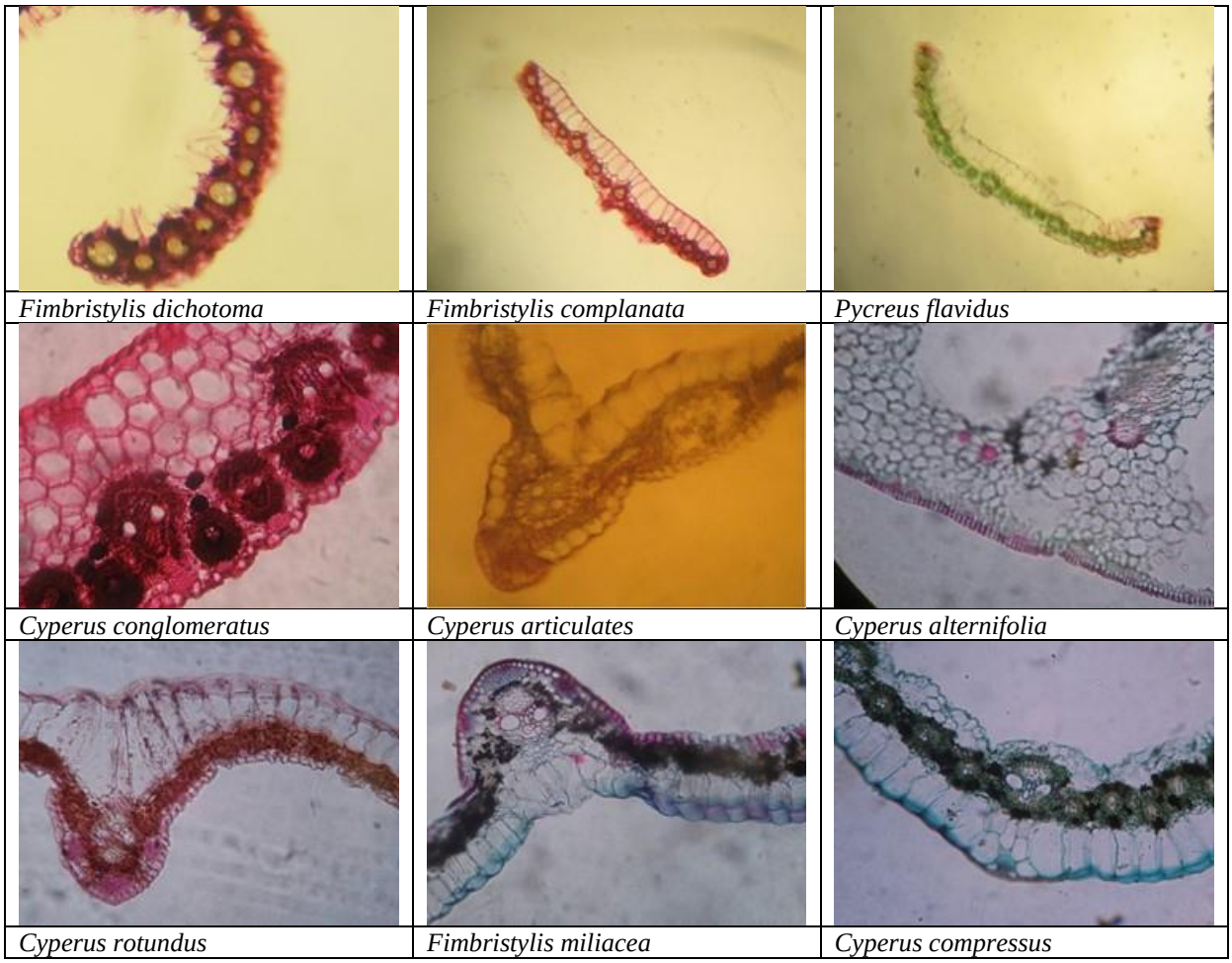



**Plate.4.3. Leaf transverse section of Cyperaceae species collected from Rasool Headworks.**

**Table 1. Analysis of variance (ANOVA) for leaf epidermal cell area (µm²) of some Cyperaceae species**

| Source | df | SS | MS | F-Value | P-Value |
|---|---|---|---|---|---|
| **Species** | 14 | 1953248 | 139518 | 10.24 | 0.000 |
| **Error** | 30 | 408913 | 13630 | | |
| **Total** | 44 | 2362161 | | | |

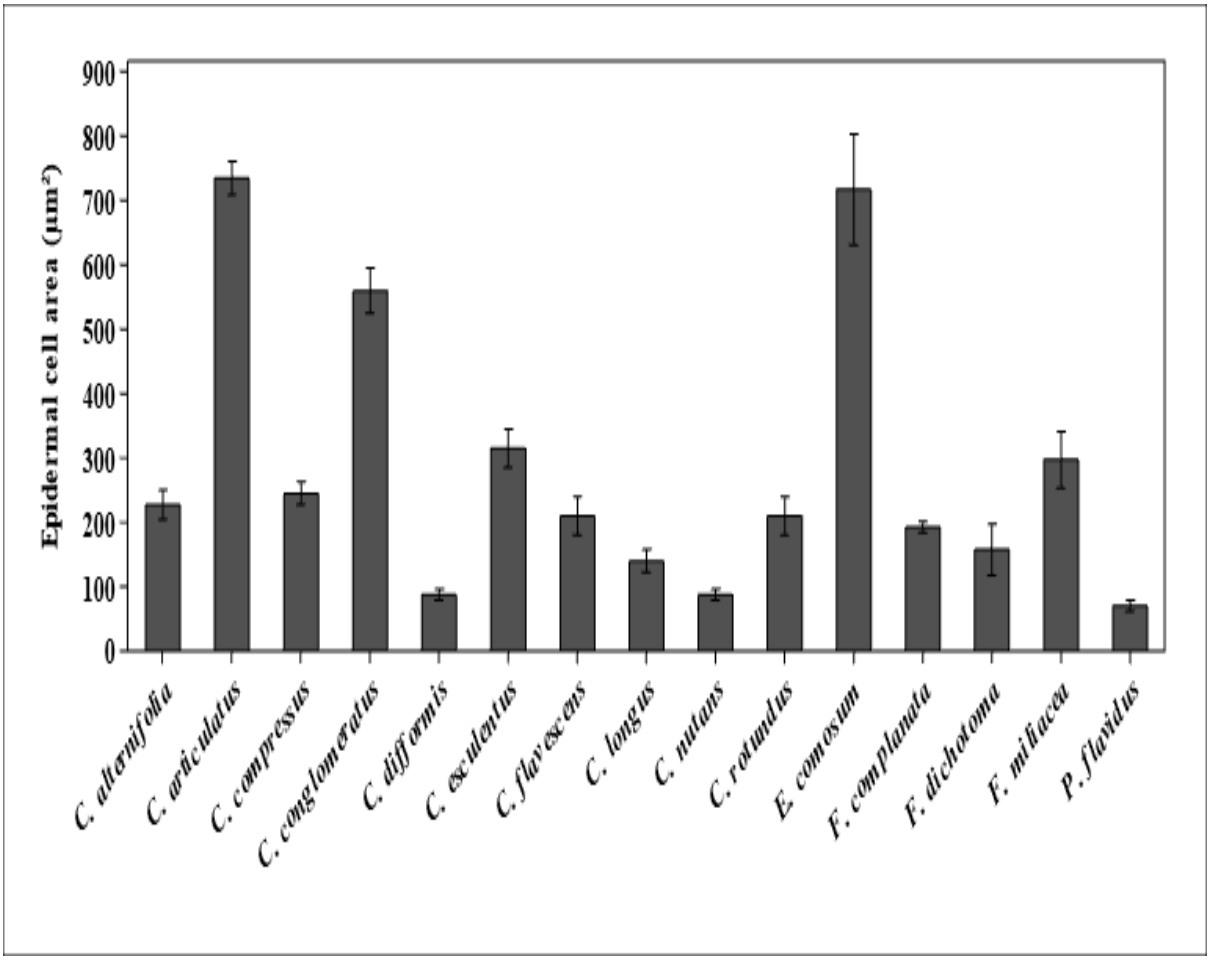



**Fig. 1. Leaf anatomical characteristics (Epidermal cell area) of some Cyperaceae species collected from Rasool Headworks, District Mandi Bahauddin.**

**Table 2. Analysis of variance (ANOVA) for leaf Sclerenchyma thickness (µm) of some Cyperaceae species**

| Source | df | SS | MS | F-Value | P-Value |
|---|---|---|---|---|---|
| **Species** | 14 | 1370.6 | 97.90 | 3.47 | 0.002 |
| **Error** | 30 | 845.5 | 28.18 | | |
| **Total** | 44 | 2216.1 | | | |

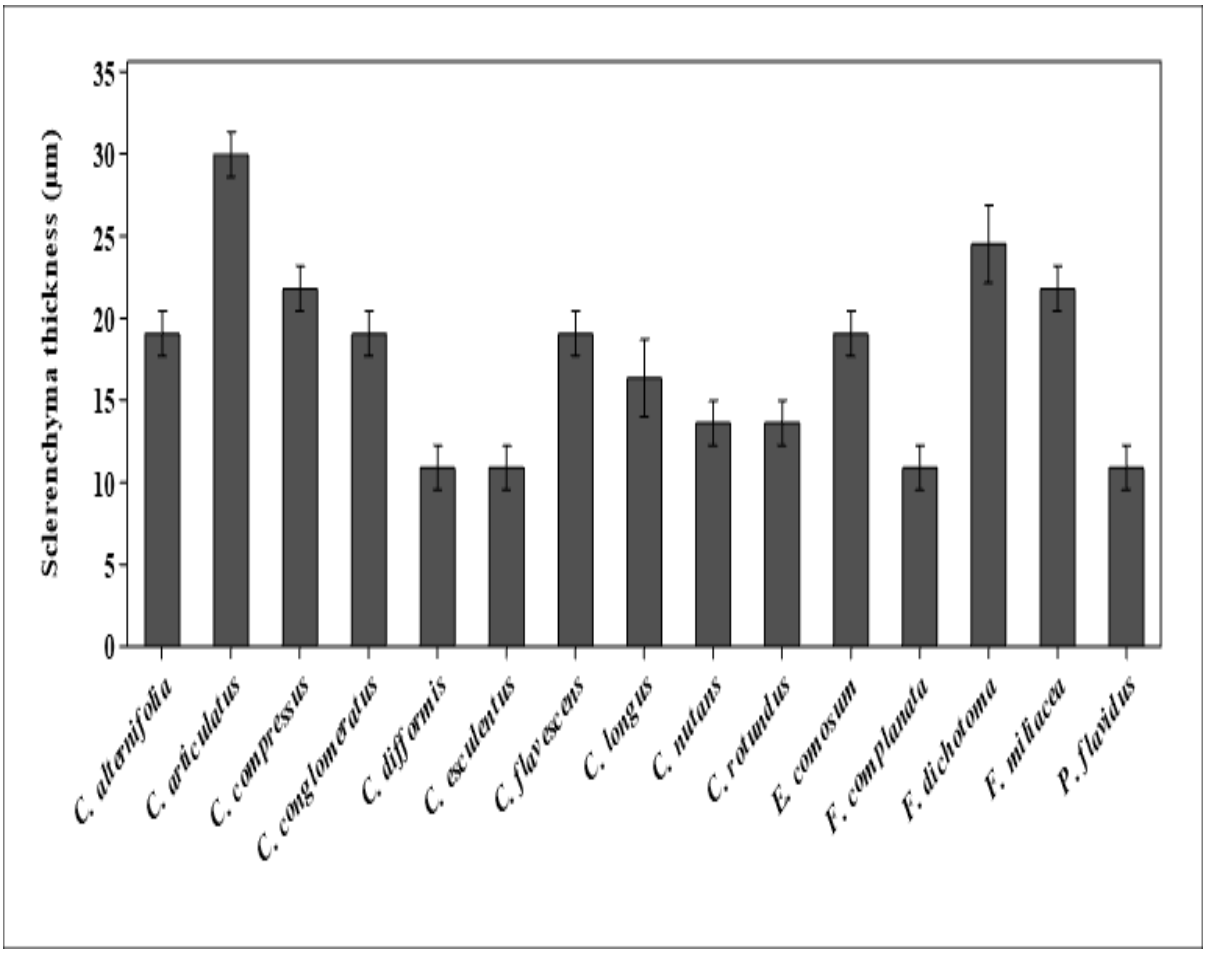



**Fig.2. Leaf anatomical characteristics (Sclerenchyma thickness) of some Cyperaceae species collected from Rasool Headworks, District Mandi Bahauddin.**

**Table 3. Analysis of variance (ANOVA) for leaf aerenchyma area (µm²) of some Cyperaceae species**

| SOV | df | SS | MS | F-Value | P-Value |
|---|---|---|---|---|---|
| **Species** | 14 | 4462961 | 318783 | 6.38 | 0.000 |
| **Error** | 30 | 1499960 | 49999 | | |
| **Total** | 44 | 5962920 | | | |

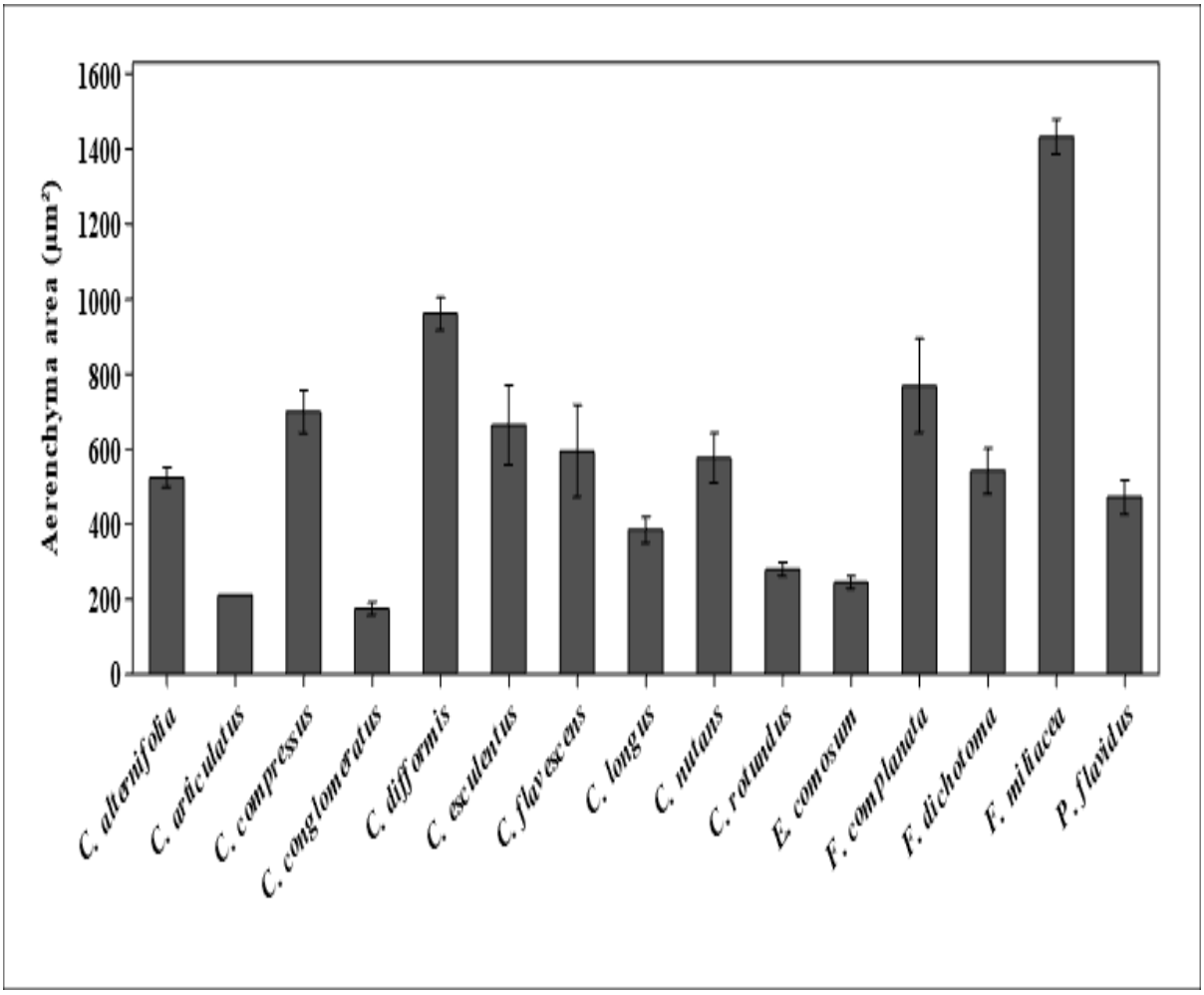



**Fig.3. Leaf anatomical characteristics (Aerenchyma area) of some Cyperaceae species collected from Rasool Headworks, District Mandi Bahauddin.**

**Table 4. Analysis of variance (ANOVA) for leaf cortical thickness (µm) of some Cyperaceae species**

| SOV | df | SS | MS | F-Value | P-Value |
|---|---|---|---|---|---|
| **Species** | 14 | 1522 | 108.71 | 1.74 | 0.098 |
| **Error** | 30 | 1869 | 62.30 | | |
| **Total** | 44 | 3391 | | | |

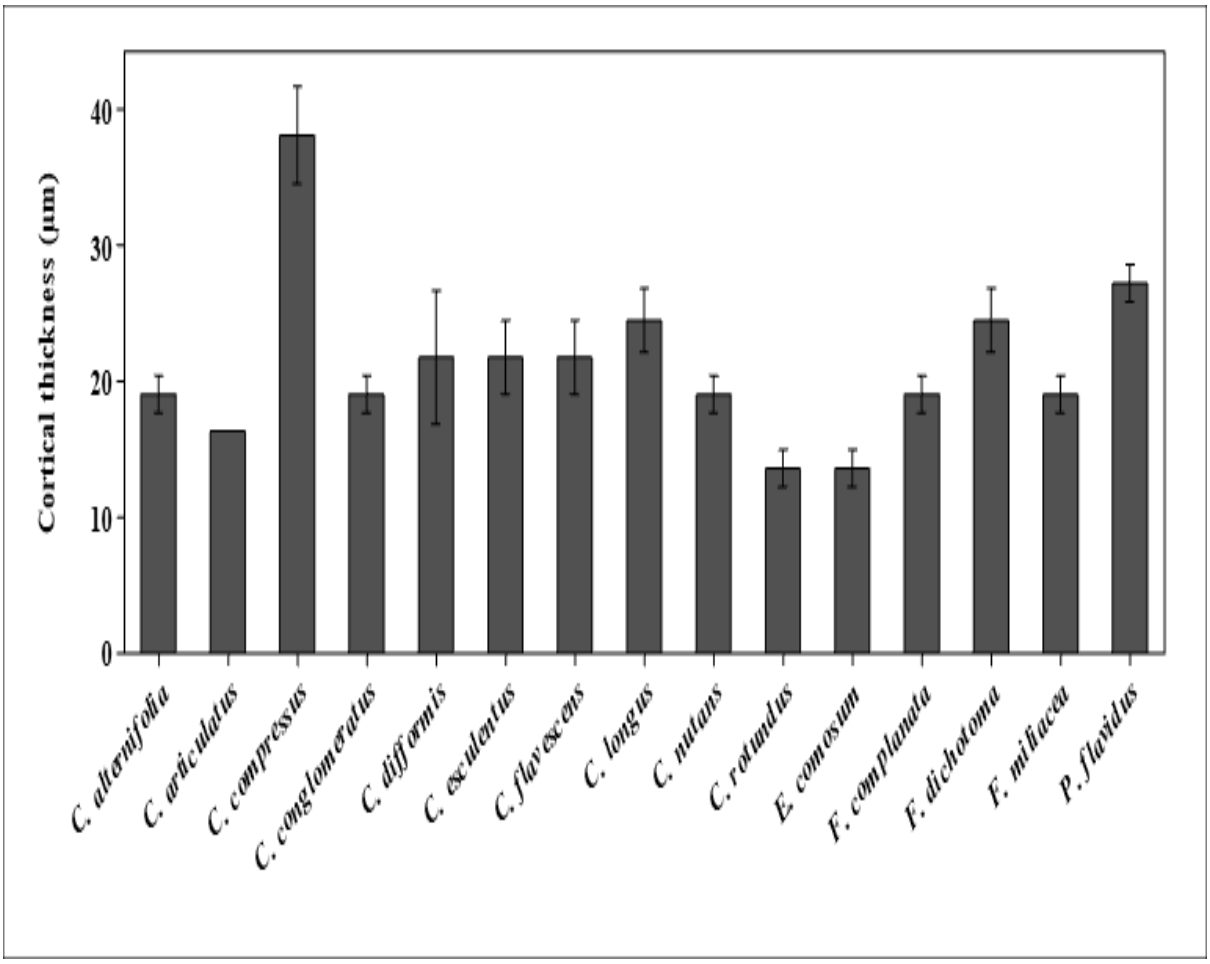



**Fig.4. Leaf anatomical characteristics (Cortical thickness) of some Cyperaceae species collected from Rasool Headworks, District Mandi Bahauddin.**

*Cyperus rotundus* and *Eriophorum comosum* showed minimum cortical thickness. Among *Cyperus longus* and *Cyperus flavescens, Cyperus longus* showed highly significant results in comparison with *Cyperus flavescens.*

**Cortical cell area (µm²)**

Highly significant results were seen among species regarding cortical cell area. *Pycreus flavidus* showed maximum cortical cell area. *Cyperus compressus* and *Cyperus flavescens* were showing almost maximum results. *Fimbristylis dichotoma, Cyperus alternifolia, Cyperus longus, Cyperus difformis* and *Cyperus esculentus* showed moderate cortical cell area. Minimum results were shown among *Eriophorum comosum* and *Fimbristylis complanata*. By comparing *Cyperus difformis* and *Cyperus nutans* highly significant results recorded in *Cyperus difformis.*

**Metaxylem area (µm²)**

Results for metaxylem area were highly significant. *Fimbristylis miliacea* showed maximum metaxylem area followed by *Cyperus esculentus. Cyperus flavescens, Cyperus difformis, Fimbristylis dichotoma, Cyperus compressus, Eriophorum comosum* and *Cyperus conglomeratus* showed moderate metaxylem area. *Cyperus nutans, Fimbristylis*

*complanata* and *Pycreus flavidus* showed minimum metaxylem area. More significant results regarding metaxylem area were recorded in *Cyperus alternifolia* than *Cyperus conglomeratus.*

**Phloem area (µm²)**

Highly significant results were seen regarding phloem area. *Fimbristylis complanata* and *Fimbristylis dichotoma* showed maximum phloem area. *Cyperus longus* and *Cyperus difformis* showed resembled phloem area but more significant result in *Cyperus longus*. *Pycreus flavidus, Cyperus articulatus, Cyperus conglomeratus, Cyperus rotundus, Cyperus compressus* and *Eriophorum comosum* showed moderate phloem area.

**Vascular Bundle thickness (µm)**

Records showed highly significant results for vascular bundle thickness. *Cyperus articulatus* showed maximum vascular bundle thickness, closely followed by *Cyperus*

**Table 5. Analysis of variance (ANOVA) for leaf cortical cell area (µm²) of some Cyperaceae species**

| SOV | df | SS | MS | F-Value | P-Value |
|---|---|---|---|---|---|
| **Species** | 14 | 2148720 | 153480 | 11.01 | 0.000 |
| **Error** | 30 | 418082 | 13936 | | |
| **Total** | 44 | 2566801 | | | |

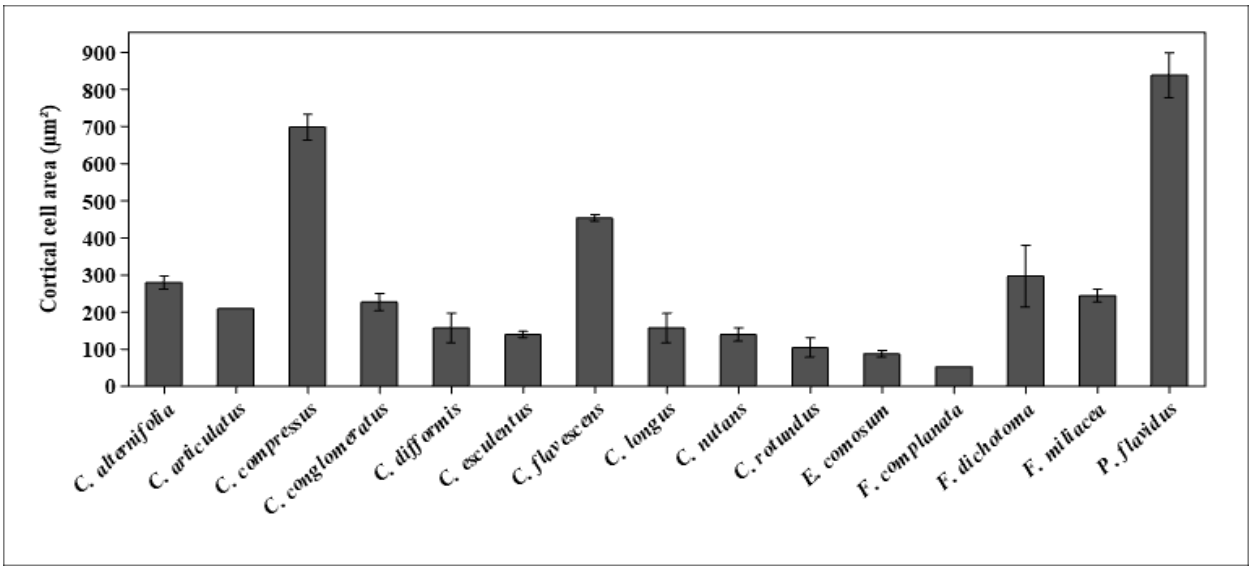



**Fig. 5. Leaf anatomical characteristics (Cortical cell area) of some Cyperaceae species collected from Rasool Headworks, District Mandi Bahauddin.**

**Table 6. Analysis of variance (ANOVA) for leaf metaxylem cell area (µm²) of some Cyperaceae species**

| SOV | df | SS | MS | F-Value | P-Value |
|---|---|---|---|---|---|
| **Species** | 14 | 3683519 | 263109 | 32.61 | 0.000 |
| **Error** | 30 | 242047 | 8068 | | |
| **Total** | 44 | 3925567 | | | |

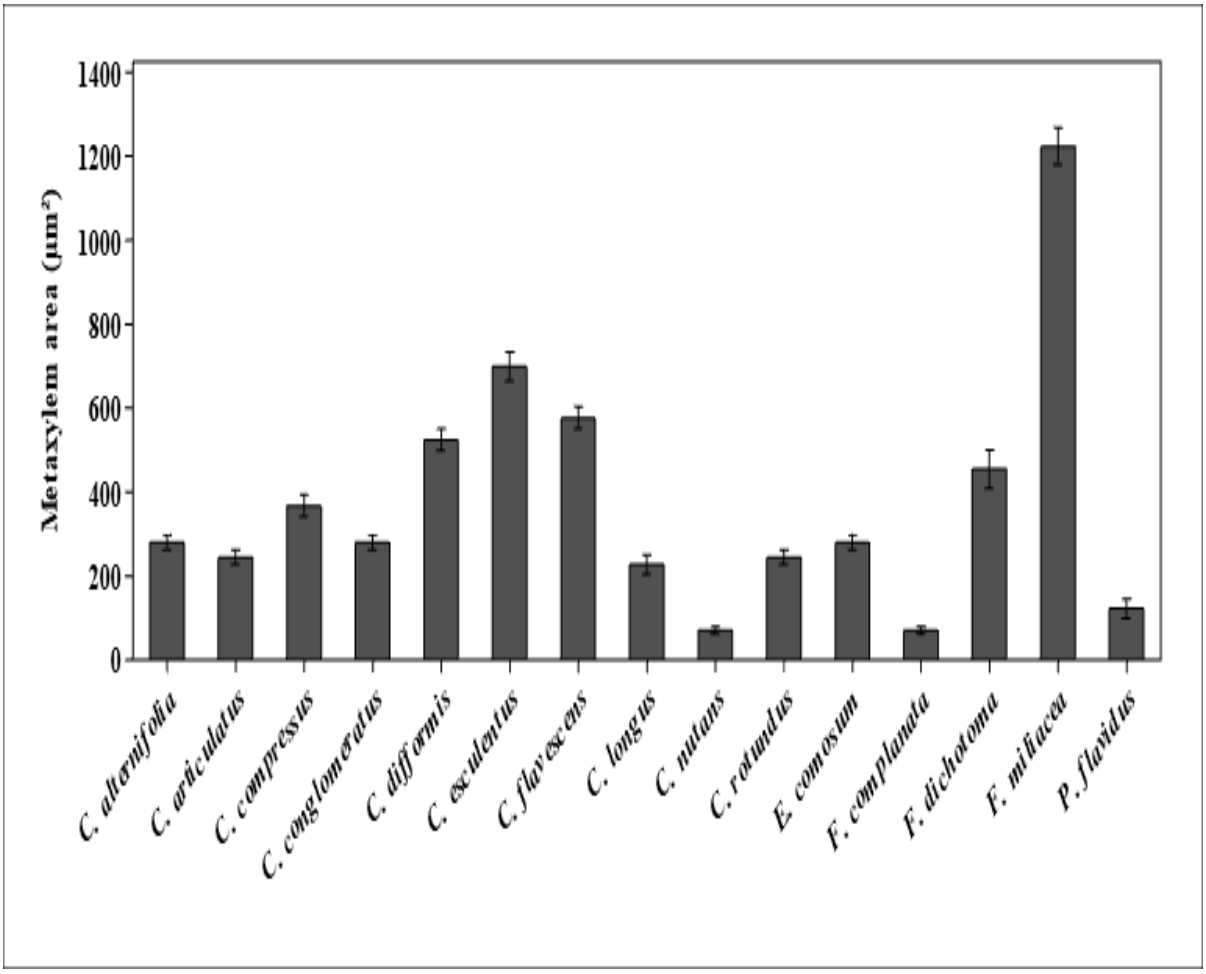



**Fig.6. Leaf anatomical characteristics (Metaxylem area) of some Cyperaceae species collected from Rasool Headworks, District Mandi Bahauddin.**

**Table 7. Analysis of variance (ANOVA) for leaf phloem cell area (µm²) of some Cyperaceae species**

| SOV | df | SS | MS | F-Value | P-Value |
|---|---|---|---|---|---|
| **Species** | 14 | 128358 | 9168 | 5.17 | 0.000 |
| **Error** | 30 | 53177 | 1773 | | |
| **Total** | 44 | 181535 | | | |

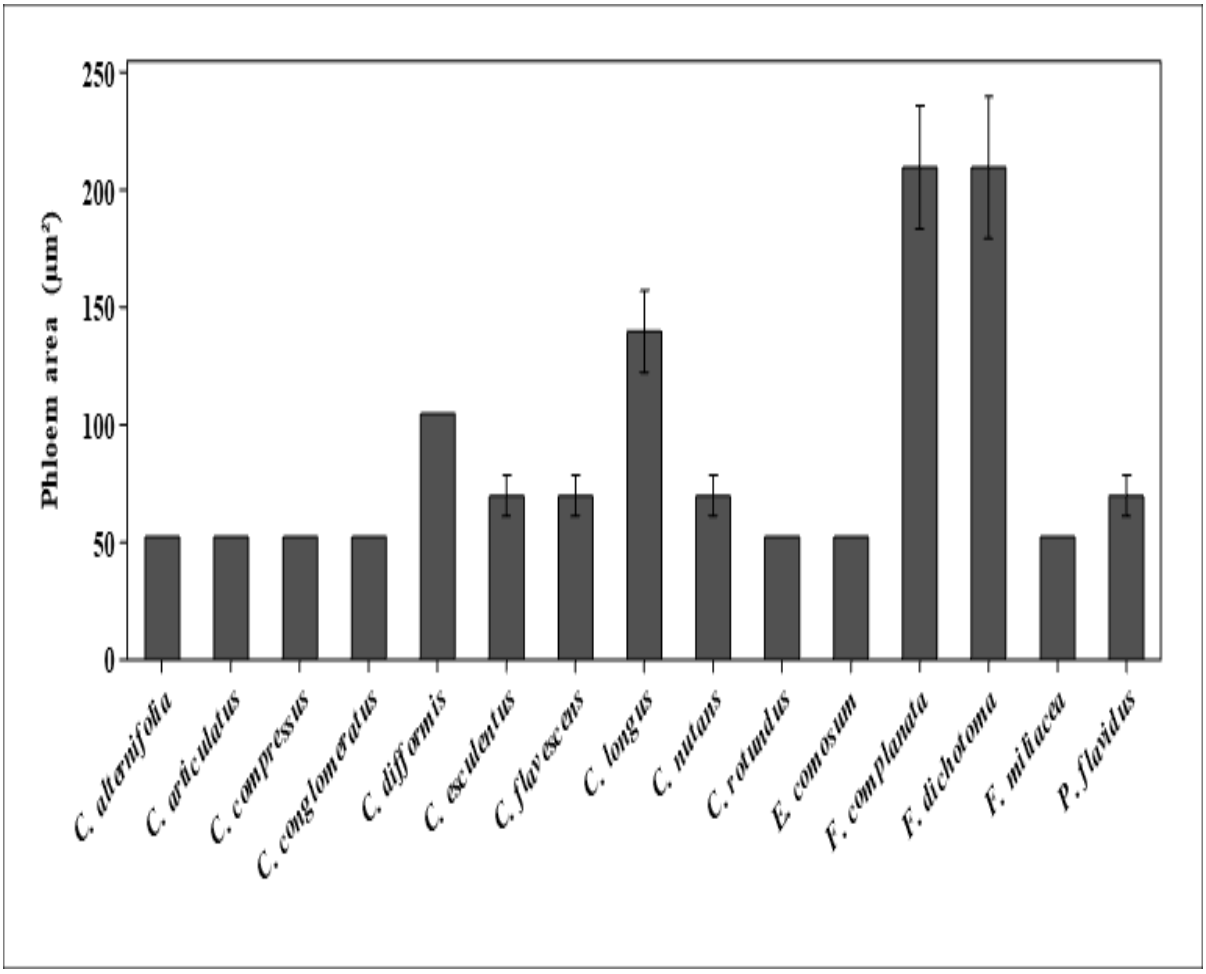



**Fig.7. Leaf anatomical characteristics (Phloem area) of some Cyperaceae species collected from Rasool Headworks, District Mandi Bahauddin.**

**Table 8. Analysis of variance (ANOVA) for leaf vascular bundle thickness (µm) of some Cyperaceae species**

| SOV | df | SS | MS | F-Value | P-Value |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| **Species** | 14 | 13994 | 999.5 | 8.53 | 0.000 |
| **Error** | 30 | 3515 | 117.2 | | |
| **Total** | 44 | 17509 | | | |

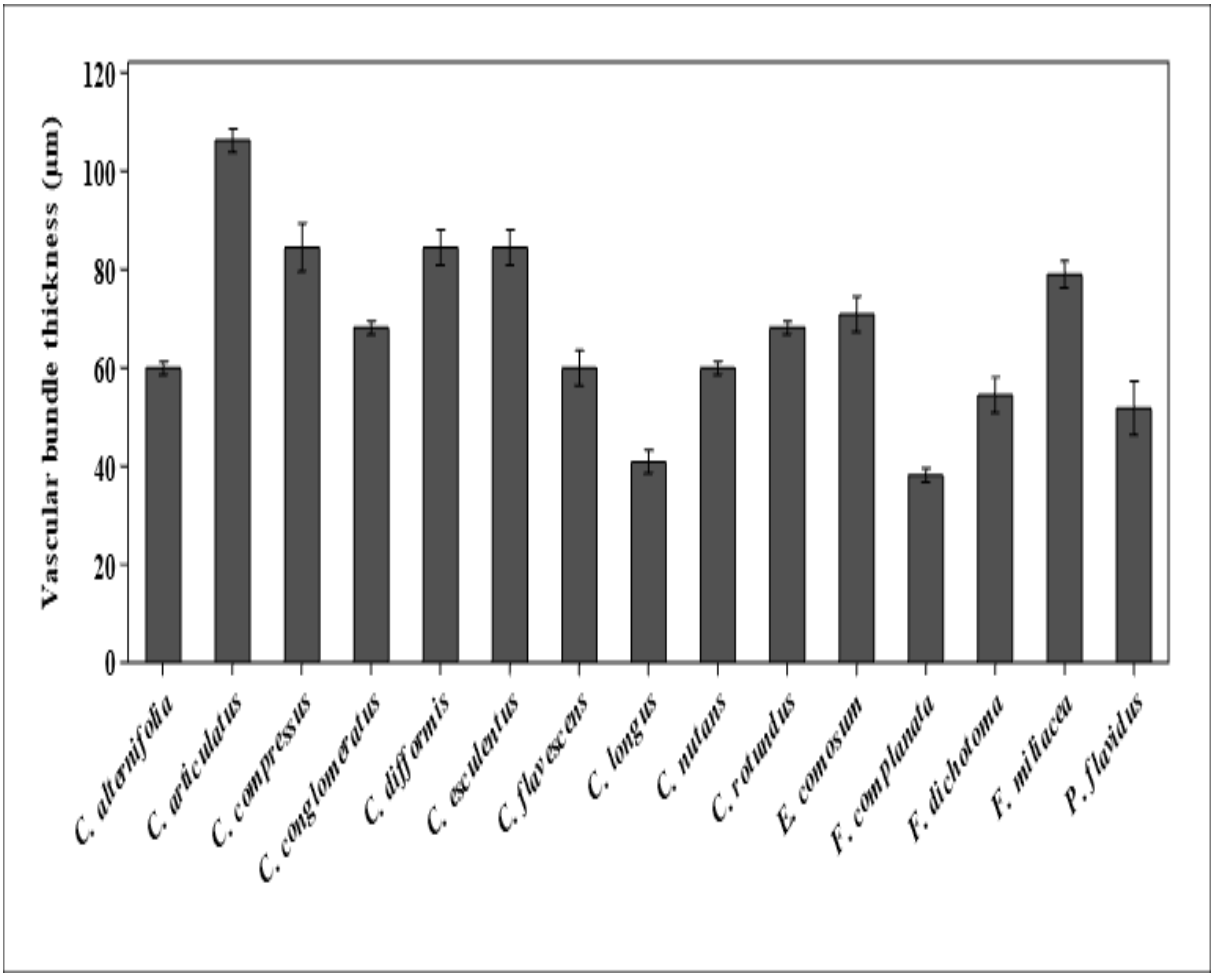



**Fig. 8. Leaf anatomical characteristics (Vascular bundle thickness) of some Cyperaceae species collected from Rasool Headworks, District Mandi Bahauddin.**

*difformis*, *Cyperus esculentus* and *Cyperus compressus*. While minimum vascular bundle thickness was recorded in *Fimbristylis complanata* and *Cyperus longus*. Among *Cyperus compressus* and *Cyperus esculentus* more significant result for vascular bundle thickness was recorded in *Cyperus esculentus.*

**Vascular bundle area (µm²)**

*Cyperus articulatus* showed maximum vascular bundle area and minimum vascular bundle was seen among *Cyperus longus, Fimbristylis complanata, Cyperus difformis* and *Pycreus flavidus*. *Fimbristylis miliacea, Eriophorum comosum, Cyperus rotundus, Cyperus conglomeratus* and *Cyperus esculentus* showed moderate results. Among *Cyperus conglomeratus* and *Cyperus rotundus,* more significant results regarding vascular bundle recorded in *Cyperus rotundus*.

**Bulliform cell thickness (µm)**

Species records showed highly significant results for bulliform cell thickness. *Cyperus articulatus* showed maximum bulliform cell thickness closely followed by *Cyperus flavescens*. *Cyperus compressus, Cyperus rotundus, Cyperus esculentus* and *Cyperus nutans* showed moderate bulliform cells thickness. Minimum recorded among *Fimbristylis complanata* and *Fimbristylis dichotoma*. *Pycreus flavidus* showed more significant results than *Cyperus difformis*.

**Bulliform cell area (µm²)**

Bulliform cell area results were highly significant. Maximum bulliform cell area was recorded in *Cyperus articulatus* closely followed by *Cyperus esculentus. Cyperus longus, Cyperus rotundus, Cyperus flavescens, Pycreus flavidus* and *Eriophorum comosum* showed moderate bulliform cell area. While minimum results recorded among *Fimbristylis dichotoma* and *Cyperus alternifolia. Cyperus longus* showed more significant results for bulliform cell area as compared to *Cyperus rotundus.*

**Table 9. Analysis of variance (ANOVA) for leaf vascular bundle area (µm²) of some Cyperaceae species**

| SOV | df | SS | MS | F-Value | P-Value |
|---|---|---|---|---|---|
| **Species** | 14 | 134151890 | 9582278 | 28.79 | 0.000 |
| **Error** | 30 | 9984450 | 332815 | | |
| **Total** | 44 | 144136340 | | | |

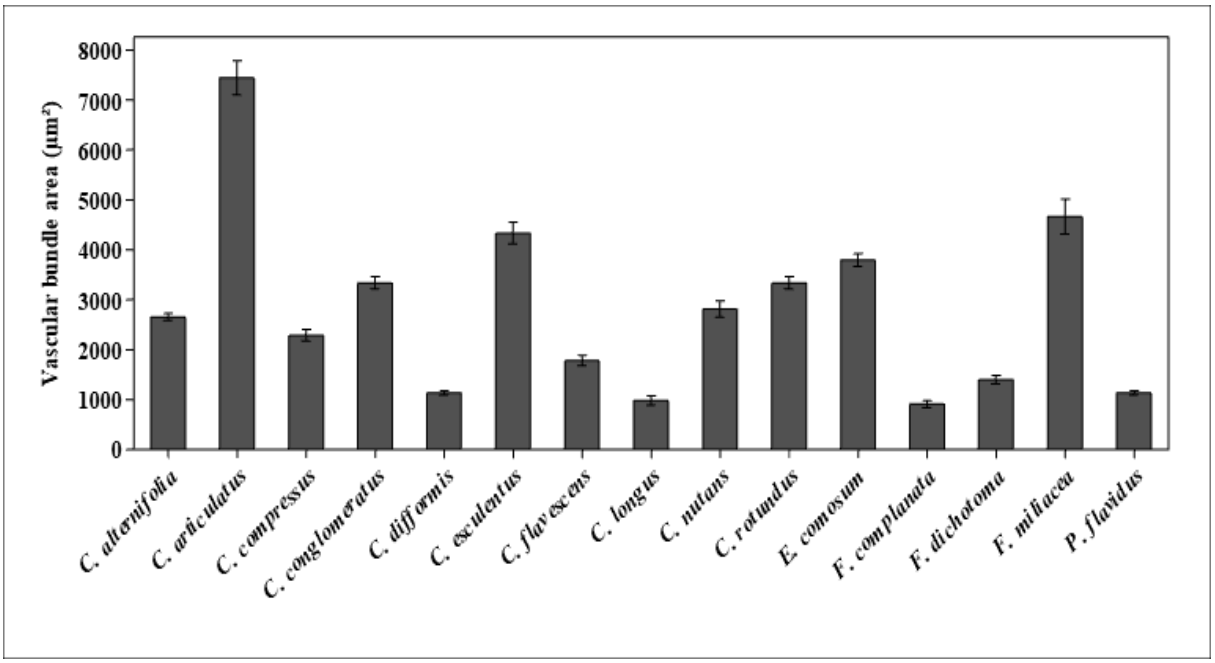



**Fig.9. Leaf anatomical characteristics (Vascular bundle area) of some Cyperaceae species collected from Rasool Headworks, District Mandi Bahauddin.**

**Table 10. Analysis of variance (ANOVA) for leaf bulliform cell thickness (µm) of some Cyperaceae species**

| SOV | df | SS | MS | F-Value | P-Value |
|---|---|---|---|---|---|
| **Species** | 14 | 15041 | 1074.34 | 13.93 | 0.000 |
| **Error** | 30 | 2314 | 77.13 | | |
| **Total** | 44 | 17355 | | | |

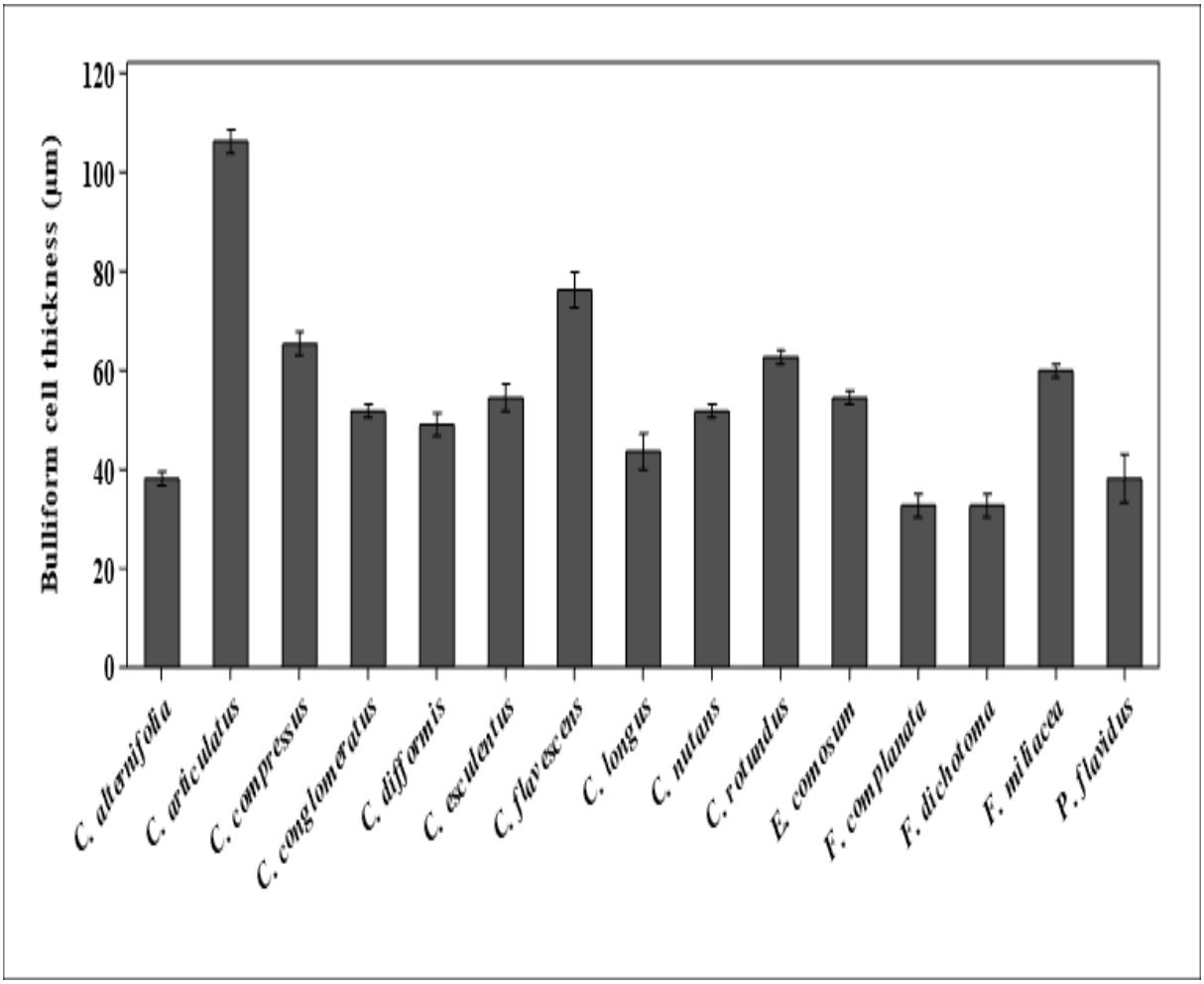



**Fig.10. Leaf anatomical characteristics (Bulliform cell thickness) of some Cyperaceae species collected from Rasool Headworks, District Mandi Bahauddin.**

**Table 11.leaf Analysis of variance (ANOVA) for bulliform cell area (µm²) of some Cyperaceae species**

| SOV | df | SS | MS | F-Value | P-Value |
|---|---|---|---|---|---|
| **Species** | 14 | 48321071 | 3451505 | 8.94 | 0.000 |
| **Error** | 30 | 11583429 | 386114 | | |
| **Total** | 44 | 59904500 | | | |

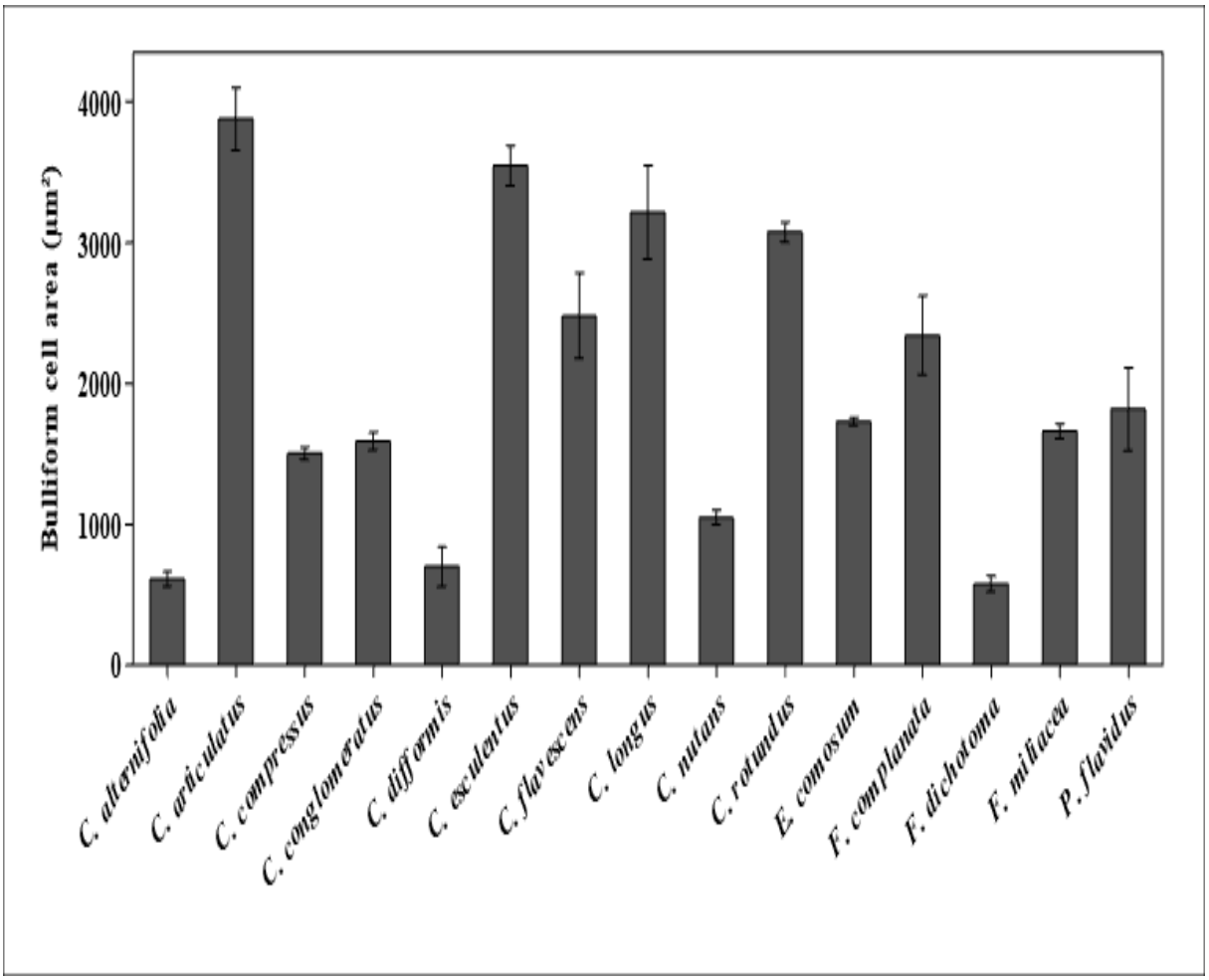



**Fig.11. Leaf anatomical characteristics (Bulliform cell area) of some Cyperaceae species collected from Rasool Headworks, District Mandi Bahauddin.**

**Discussion**

A survey was conducted at Rasool Headworks, District Mandi Bahauddin to explore flora of Cyperaceae family. In flora of District Mandi Bahauddin three genera Cyperus, Eriophorum and Fimbristylis with 16 species had been reported. Ever before flora of District mandi Bahauddin had been explored and one species of Cyperaceae Cyperus rotundus reported as ethnomedicinal plant (Nisar *et al*., 2011). Within extreme habitats certain plants adapt one of common strategy of anatomical modifications (Hlwatika and Bhat 2002). Even though in stressed habitats certain characters are same for plant growth but for same environment certain species exhibit variant survival strategies (Piazza et al. 2015).

Leaf cortical cells refers to living cortex and remain after aerenchyma formation (Jaramillo *et al*., 2013). Studies revealed that leaf cortical cells in maize are an indicator of root metabolic cost, a portent root respiration determinant as compared with root cortical cells. Under stressed or well-watered conditions in roots, leaf cortical cells were associated with reduction of specific root respiration. Hence cortical cell area of leaf in *Pycreus flavidus* is clear indication of this adaptation under stressed conditions.

Sclerenchyma form continuous sheath in leaf of sugarcane under epidermis of midrib portion (Joarder *et al*., 2010). These cells also provide compression or tensile strength to vascular bundles of leaf (Wang *et al*., 2013). Leaves of *Cyperus articulatus* showing maximum sclerenchyma thickness than rest of recorded species therefore having more capability of survival in harsh environments. Sclerenchyma cells provide strength to cells playing vital role by preventing collapse of tissues in terrestrial or aquatic ecosystems (Cholewa and Griffith, 2004). Larger sclerenchyma cells in stem of *Cyperus articulatus*, *Cyperus conglomeratus* and *Fimbristylis miliacea* clearly shows their better adaptation in stressed conditions. Vascular system includes xylem and phloem interconnecting and distributing shoot and root system. Within leaf vascular system play crucial role for mechanical support and for two way and long interval transport of water (Brodersen and McElrone, 2013) photosynthetic consequences (Lalonde et al., 2003) and inorganic ions (Miller *et al*., 2001). Increased vascular bundle area was recorded in less tolerant species (Awasthi *et al*., 1999). Vascular bundle areas in leaf of *Cyperus articulatus*, stem of *Cyperus compressus* and within root of *Cyperus cuspidatus* surpassed all remaining species showing their efficient water and nutrient uptake in highly salt tolerant habitats. Large phloem and metaxylem area of halophytic species play crucial role in photosynthesis and conduction of water (Awasthi and Pathak, 1999). Large metaxylem area of *Cyperus conglomeratus, Fimbristylis miliacea* and phloem area of *Fimbristylis dichotoma* and *Fimbristylis complanata* showed their survival efficiency in harsh conditions with wide distributional range.

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