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COMPARATIVE STUDIES OF EPIDERMAL APPENDAGES IN SOME WEEDS OF SARGODHA DISTRICT

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ABSTRACT

Present work has been conducted to investigate the diversity of the epidermal appendages or trichomes in some common weeds of district Sargodha. Epidermal anatomy was studied by staining and impression techniques. Wide varieties of trichomes were observed in different weed species. Trichomes play a significant role in development of adaptations to different environmental conditions for the survival in adverse situations. Most common types of the trichomes observed were Multiseriate, uniseriate, multicellular, unicellular, single, tufted, shaggy and rugose hairs. Plants growing in stressed conditions such as *Croton bonplandianum and Tribulus terrestris* showed most diverse structures of trichoms. These trichomes are type of radiating stellate trichomes and tufted. All other species have totally different trichome anatomy from it. Staining and impression techniques are proved best to study the anatomy of epidermal appendages. It was observed that the trichomes help the plants and play defensive roles against environmental stresses.

1. INTRODUCTION

Weeds are the plants that are growing where these are unwanted. Weed science focuses on moderating the negative effects of weeds. Weeds may improve soil microbial activity, improve soil physical and chemical properties, decreases soil erosion and provide pollen or nectar for parasitoids and pollinators. It is proved that epidermis of leaf and its characters are taxonomically important and provide information about the epidermal characters (Ogie-Odia *et al.,* 2010). Epidermal traits are epidermal cells, stomata and epidermal appendages. These traits are important in allocation of taxa in numerous families of plants (Metcalfe and chalk, 1950 -1989; Ditsh *et al*., 1995; Barthlott *et al*., 1998; Stenglein *et al*., 2003). It is confirmed that leaf epidermal features assist to explain taxonomic relationships at different levels Stebbins, 1956; Metcalfe, 1960; Ellis, 1979; Palmer and Tucker, 1981; Palmer *et al*., 1985; Davila and Clark, 1990; Cai and Wang, 1994; Mejia-Saules and Bisbey, 2003). These are called epidermal appendages or trichomes. They have different shapes and provide safety against the herbivores, insects and microbes. The histochemical images, morphology and distribution pattern differs on different organisms in many species (Hassan, 2009). The morphology of trichomes varies and the trichomes are showing two main types. These are the glandular and non-glandular or epi-glandular trichomes (Sinha *et al*., 2001). In many plant families, the trichome characters are important in delimitation of taxa (Krak and Patrik, 2008).Glandular trichomes have glands in them and these acts as storage of metabolites which may be biologically active and provide sites for synthesis. All these functions are performed by trichomes (Alonso *et al*., 1992; Antonious, 2001; Iijima *et al*., 2004; Siebert, 2004; Deschamps *et al*., 2006; Nagel *et al*., 2008; Wang *et al*., 2008; Biswas *et al*., 2009; Sallaud *et al*., 2009). Trichomes are found on the outermost surface of almost all plants (Wagner, 1991). These are important in taxonomy and defensive roles. The non-glandular trichomes form a dense indumentum and acts as a barrier against the pathogens, herbivores, ultraviolet radiations and extreme environmental conditions like high temperature and increased water loss (Werker, 2000; Gonzales *et al*., 2008). On the upper surface of leaves and stem in all plants in different species, epidermal hairs are present. There are two types of trichomes based on cellular level, unicellular and multicellular. The multicellular trichomes are very useful to discharge phytochemical compound such as organic acids, polysaccharides, terpenes or salt. They also help in the secretion of the secondary compounds produced in trichomes such as terpenoids, flavonoids and phenylpropanoids (Duke *et al.*, 2000). From the apical or basal part of the trichomes, it could be recognized that whether trichomes are unicellular or multicellular (Werker, 2000). Comprehensive references about secreted particles from plant trichomes are present (Callow, 2000; Wagner *et al.*, 2004).

2. OBJECTIVES

The main objectives of the present study are following.

- To inspect the variety of weeds and their taxonomic significance.

- To observe the epidermal structures of leaf and stem.
- To understand the trichomes functions.
- To compare the trichomes anatomy of various weed species.
- To see the number, size, shape, structure and location of trichomes on leaves and stems.

3. MATERIAL AND METHOD

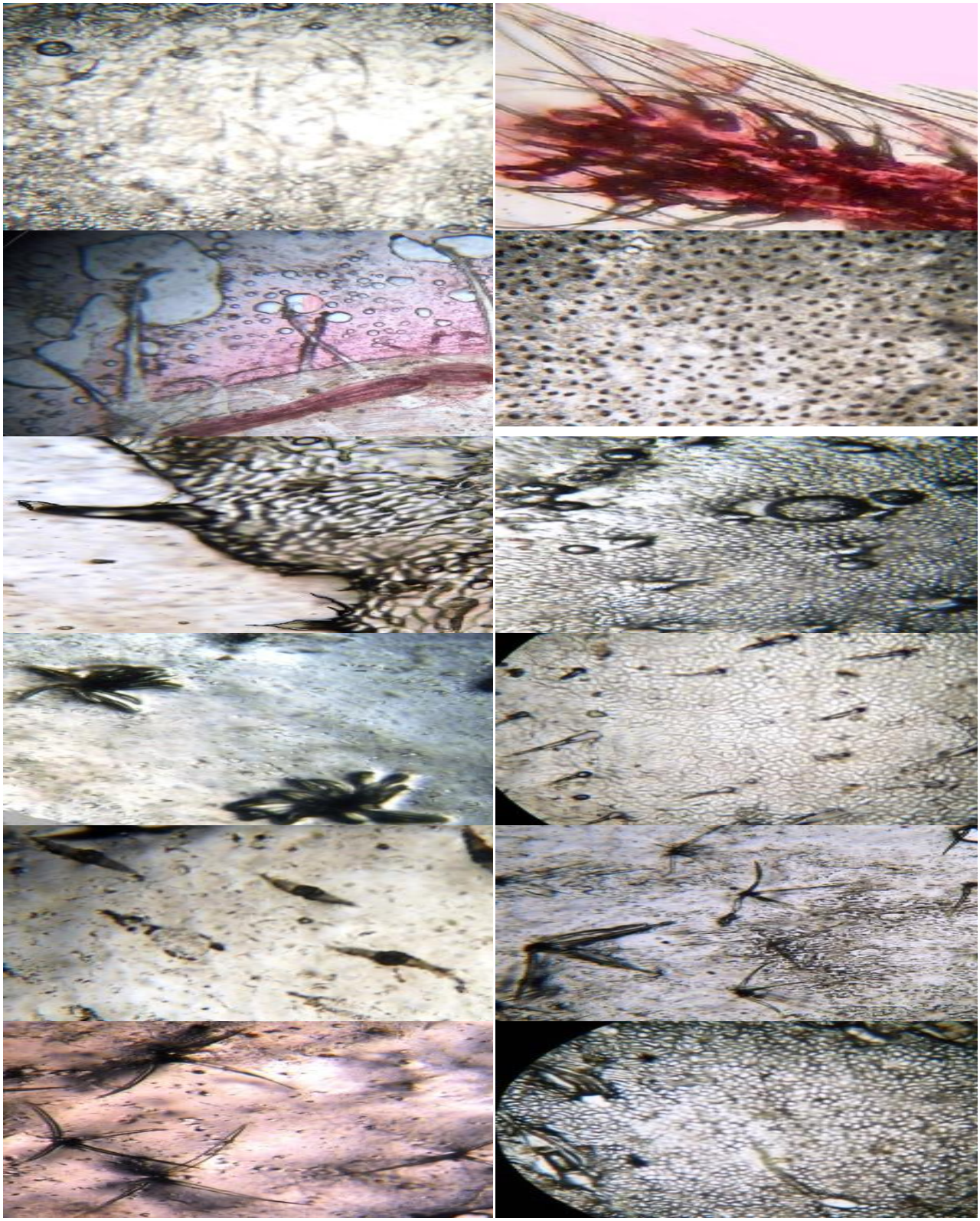
For this purpose a detailed survey was conducted in the district of Sargodha region. During the survey distribution pattern of different species were observed and plants were collected for preservation and anatomical investigation. Two different techniques were used for this purpose, one is simple free hand peeling and other is nail paint method. Comparative anatomy of leaf structure from the permanent slides and photography was taken by using electrical microscope and digital camera. Slides, beaker, petri dishes, cover slips, blades, forceps, transparent nail paint, scotch tape, potato cutter and tissues were used for the cross-section cutting and pealing of epidermis. Photographs of leaf and stem anatomy were taken by using electrical microscope and scientific digital camera. Different alcohol grades of 30% alcohol, 50% alcohol, 70% alcohol, 90% alcohol, and 100% alcohol, Safranine, Fast green, Xylene and Canda Balsam are used during slide preparation.

For the preparation of slides, first many sections were obtained by free-hand sectioning technique or by pealing of epidermis of leaf, and then placed these sections in the petri dish. Then put a best section on the slide and observed it under the microscope. The trichomes bearing section was applied by the different grades of alcohols. First of all 30% alcohol was added and it removed after 5 minutes. Then 50% alcohol was added and it also removed after 5 minutes, then 70% alcohol was added and after removing it stain (fast green or Safranine) is added. After 2 or 3 minutes it was removed and washed it by 100% pure alcohol. Then 1-2 drops of xylene solution added to give brightness to the section. Now section was fully stained and preserved it by adding some Canda Balsam and placed a cover slip on it. Now observed this slide under the electron microscope and photographs were taken by the scientific digital camera.

In impression technique first took fresh leaf samples and applies transparent nail paint on it. Let it dry then put scotch tape on it and pressed it gently to get impression of leaf. Now removed the tape carefully, the impression left on nail polish film produced an excellent detailed image of epidermis and cut 1cm piece and place it on glass slide and cover with the help of cover slip. Then these observed it under microscope. The weeds which are under anatomical investigation are *Achyranthus aspera, Alhagi maurorum, Broussonetia papyrifera, Calotropis gigantea, Chenopodium album, Citrullus vulgaris, Croton bonplandianum, Crozophora tinctoria, Datura stramonium, Euphorbia hirta, Heliotropium europium, Kunzea ambigua, Parthenium hysterophorous, Pennisetum glaucum, Trianthema portulacastrum, Tribulus terrestris, Withania coagulans and Withania somnifera.*

4. RESULTS AND DISCUSSION

Epidermal cells are sometimes extended outwards forming projections of variable shape and size. If it is short and conical it is called papillae and the epidermis is described as papillosed e.g. Coca, but if the projections are long and well protruding they form trichomes or hairs. The part of trichome embedded in the epidermis is called foot while the free part is the body. Hairs are classified into glandular and non-glandular or covering hairs. These trichomes may be unicellular or multicellular, uniseriate or multiseriate and may be single or tufted. *Withania coagulans and Calotropis gigantea* have Compositae type of trichomes. These are glandular and may be branched or unbranched. These trichomes are one celled. These are simple and cystolitic hairs. *Tribulus terrestris* have cottony unicellular trichomes. These are non-glandular and may be branched or unbranched. These trichomes are one celled. These are simple and cystolitic hairs. *Euphorbia hirta* have peltate rugose trichomes. These trichomes are with very short axis surrounded by a plate-like structure of closely joined cells. Non-glandular trichomes vary greatly in structure and distribution among different plant taxa. As determined by the dense cytoplasm present, it appears that the trichomes are still alive, and thus immature. Non-glandular trichomes are commonly dead upon maturity. A radiating stellate trichome is with unbranched processes. The individual processes merge into the basal stalk cell.A multicellular stellate trichome with bifurcated processes from the round-leaf bladder pod of the mustard family that shows siliceous warts on the surface in *Chrozophora tinctonia* and *Croton bonplandianum.* The stellate are radiating unicellular hairs. Many hairs are present in the form of cluster but are unicellular in *Achyranthus aspera* and *Heliotropium europium.* Unicellular trichomes are formed of one cell and these may be unbranched and simple or these may be branched and complex. e.g. *Broussonetia papyrifera*, *Alhagi maurorum*, *Chenopodium album*, *Citrullus vulgaris*, *Pennisetum glaucum* and*Withania somnifera.* Multicellular that is formed of more than one cell and these are uniseriate if formed of one row of cells such as in *Datura stramonium*, *Kunzea ambigua*, *Parthenium hysterophorous* and *Trianthema portulacastrum.*



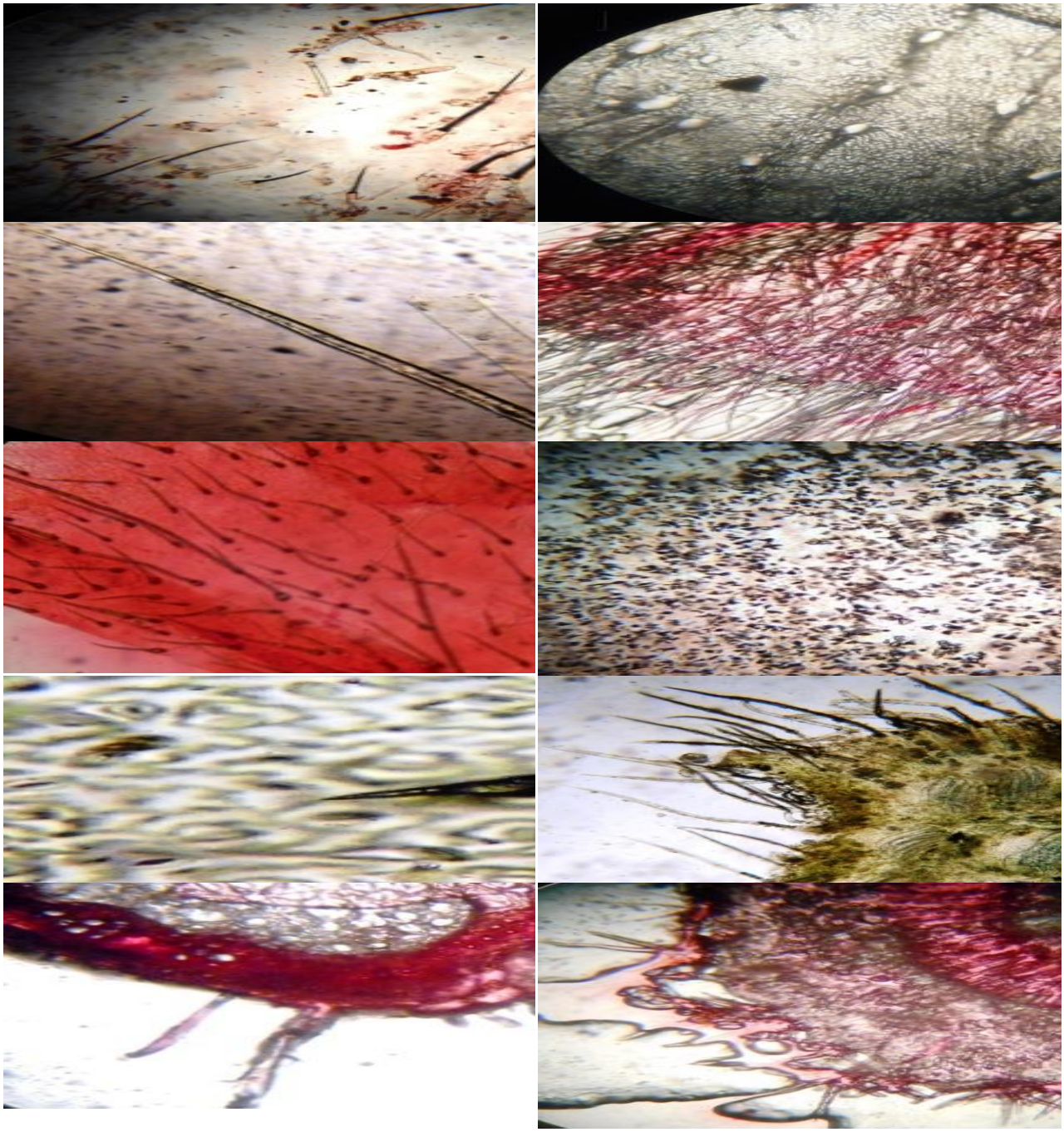


Figure 1. Comparative morphology of epidermal appendages of different weeds of Sargodha district (a) *Alhagi maurorum* (b) *Achyranthus aspera* (c) *Broussonetia papyrifera* (d) *Calotropis gigantean* (e)*Chenopodium album* (f)*Citrullus vulgaris* (g)*Croton bonplandianum* (h)*Datura stramonium* (i)*Euphorbia hirta* (j)*Heliotropium europium* (k)*Heliotropium europium* (l)*Kunzea ambigua* (m)*Parthenium hysterophorous* (n)*Parthenium hysterophorous* (o)*Pennisetum glaucum* (p)*Tribulus terrestris* (q) *Tribulus terrestris* (r)*Withania coagulans* (s)*Withania somnifera* (t)*Achyranthus aspera* (u)*Broussonetia papyrifera* (v)*Crozophora tinctoria*

Table 1. Percentage of types of trichomes of species in the study area.

Categories	Number of species	Percentage	Total number of species
Compositae	02	11.11%	18
Cottony Unicellular	01	5.55%	18
Peltate rugose	01	5.55%	18
Radiating stellate	02	11.11%	18
Stellate	02	11.11%	18
Unicellular	06	33.33%	18
Uniseriate	04	22.22%	18

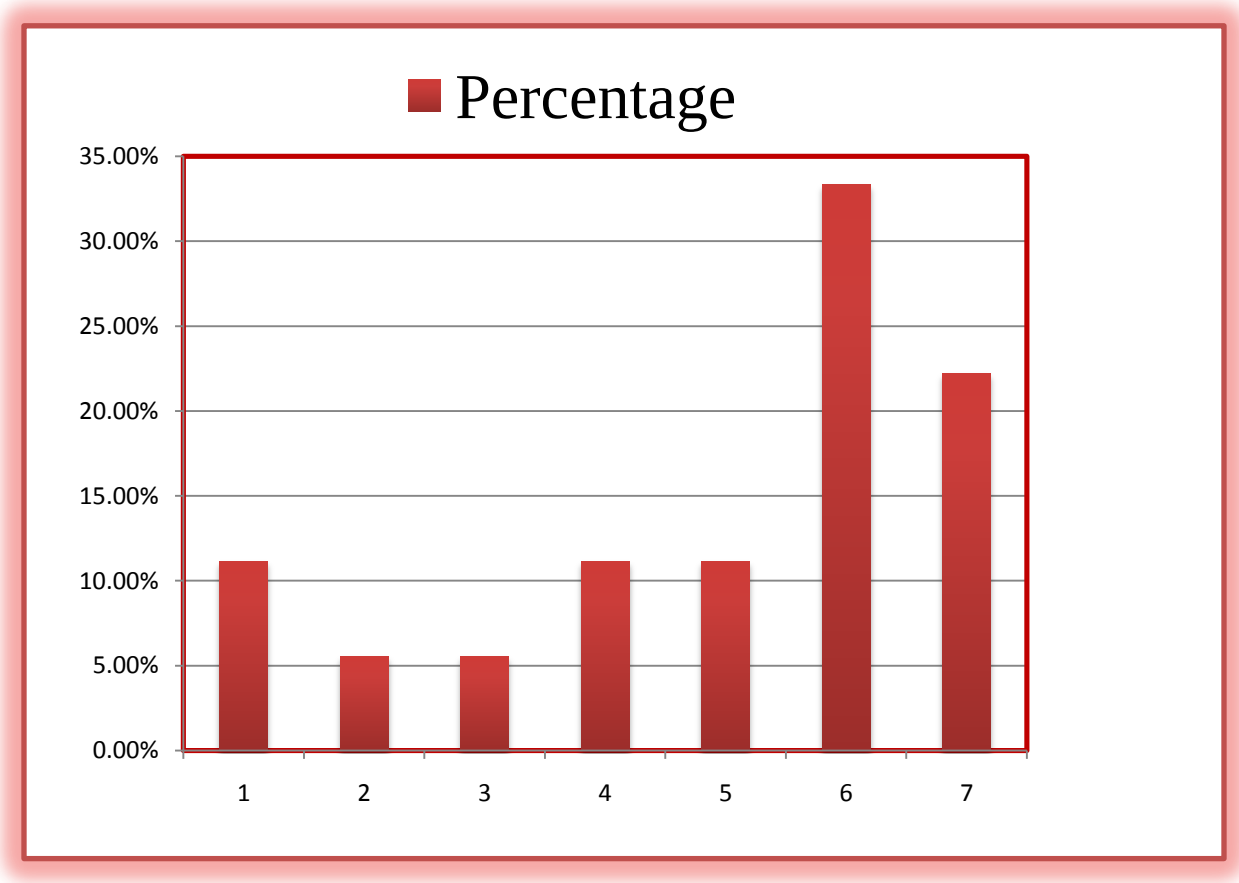


Figure 2. Graph showing percentage of Compositae, Cottony Unicellular, Peltate rugose, Radiating stellate, Stellate, Unicellular and Uniseriate trichomes

Table 2. Percentage of nature of trichomes of species in the study area.

Categories	Number of species	Percentage	Total number of species
Single	13	72.22%	18
Tufted	05	27.78%	18

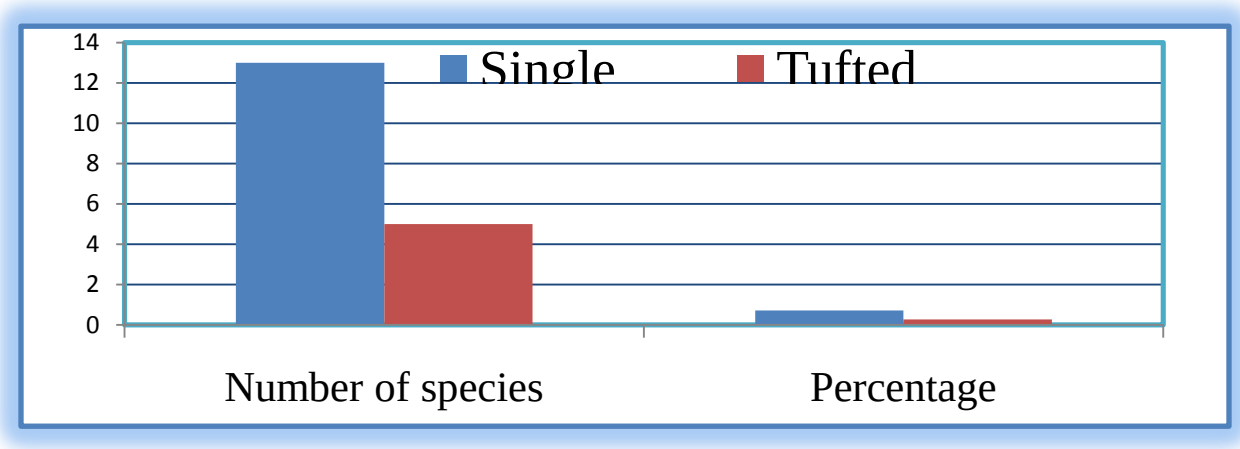
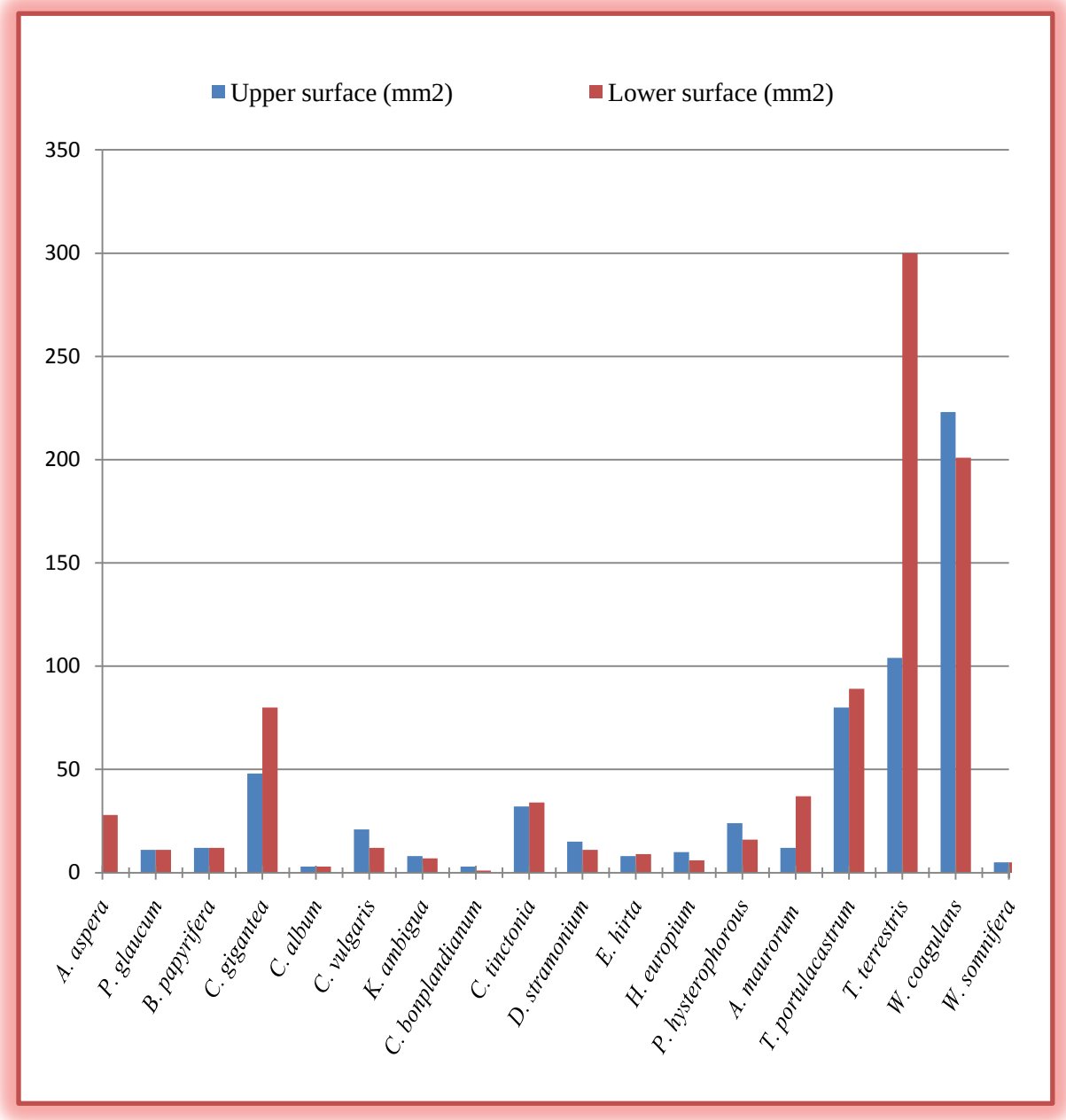


Figure 3. Graph showing nature of trichomes in all species

Table 3. Measurement of different parameters of trichomes on the upper and lower surface of leaf

No.	Name of Species	Density of trichomes on leaf (mm^2)		No. of branches	No. of cells	Length of trichomes (µm)		Width of trichomes (µm)	
		Upper	Lower			Upper	Lower	Upper	Lower
1.	*A. aspera*	34	28	2-3	0	1453.9	1564.9	65.1	68.6
2.	*P. glaucum*	11	11	0	0	3255	3259	173.6	143.6
3.	*B. papyrifera*	12	12	0	0	1548	1449	195.3	185.0
4.	*C. gigantean*	48	80	0	0	390.6	380.9	44.4	43.9
5.	*C. album*	3	3	0	0	802.9	602.9	130.2	129.3
6.	*C. vulgaris*	21	12	0	0	694.4	798.5	87.8	86.5
7.	*K. ambigua*	8	7	0	1-2	998.2	908.9	238.7	298.7
8.	*C. bonplandianum*	3	1	12-15	0	347.2	337.9	43.4	43.4
9.	*C. tinctonia*	32	34	4-5	0				
10.	*D. stramonium*	15	11	2- 6	0	781.2	989.3	86.8	76.8
11.	*E. hirta*	8	9	0	3-4	694.4	734.3	65.1	65.1
12.	*H. europium*	10	6	0	2- 3	781.2	781.2	43.4	43.4
13.	*P. hysterophorous*	24	16	0	0	737.8	737.8	65.1	65.1
14.	*A. maurorum*	12	37	0	0	824.6	824.6	43.4	43.4
15.	*T. portulacastrum*	80	89	0	2				
16.	*T. terrestris*	104	300	0	0	455.7	455.7	21.7	21.0
17.	*W. coagulans*	223	201	0	0	75.1	65.1	21.7	22.9
18.	*W. somnifera*	5	5	0	0	307.7	390.6	65.1	63.1


Upper surface (mm2)
Lower surface (mm2)
350
300
250
200
150
100
50
0
A. aspera
P. glaucum
B. papyrifera
C. gigantea
C. album
C. vulgaris
K. ambigua
C. bonplandianum
C. tinctonia
D. stramonium
E. hirta
H. europium
P. hysterophorous
A. maurorum
T. portulacastrum
T. terrestris
W. coagulans
W. somnifera

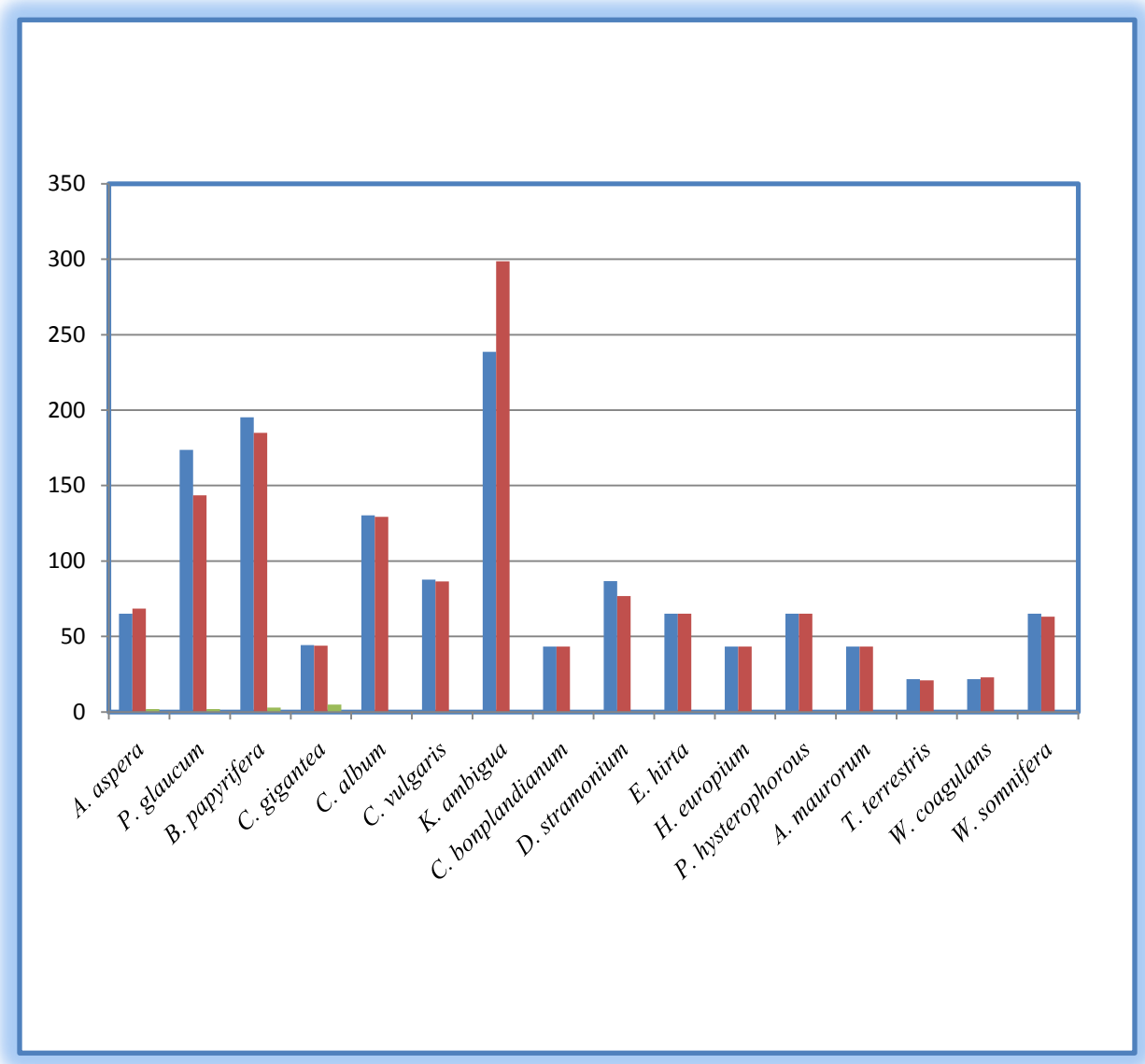


Figure 4. Graphs showing density, length and width of trichomes on leaf surfaces.

Table 4. Different parameters of trichomes on the stem surface

No.	Name of Plant	Density of trichomes on stem (mm^2)	Length of trichomes (µm)	Width of trichomes (µm)
1	*A. aspera*	31	1367.1	86.8
2	*B. papyrifera*	16	1453.9	151.9
3	*C. tinctonia*	32	477.4	43.4
4	*T.portulacastrum*	80	238.7	21.7

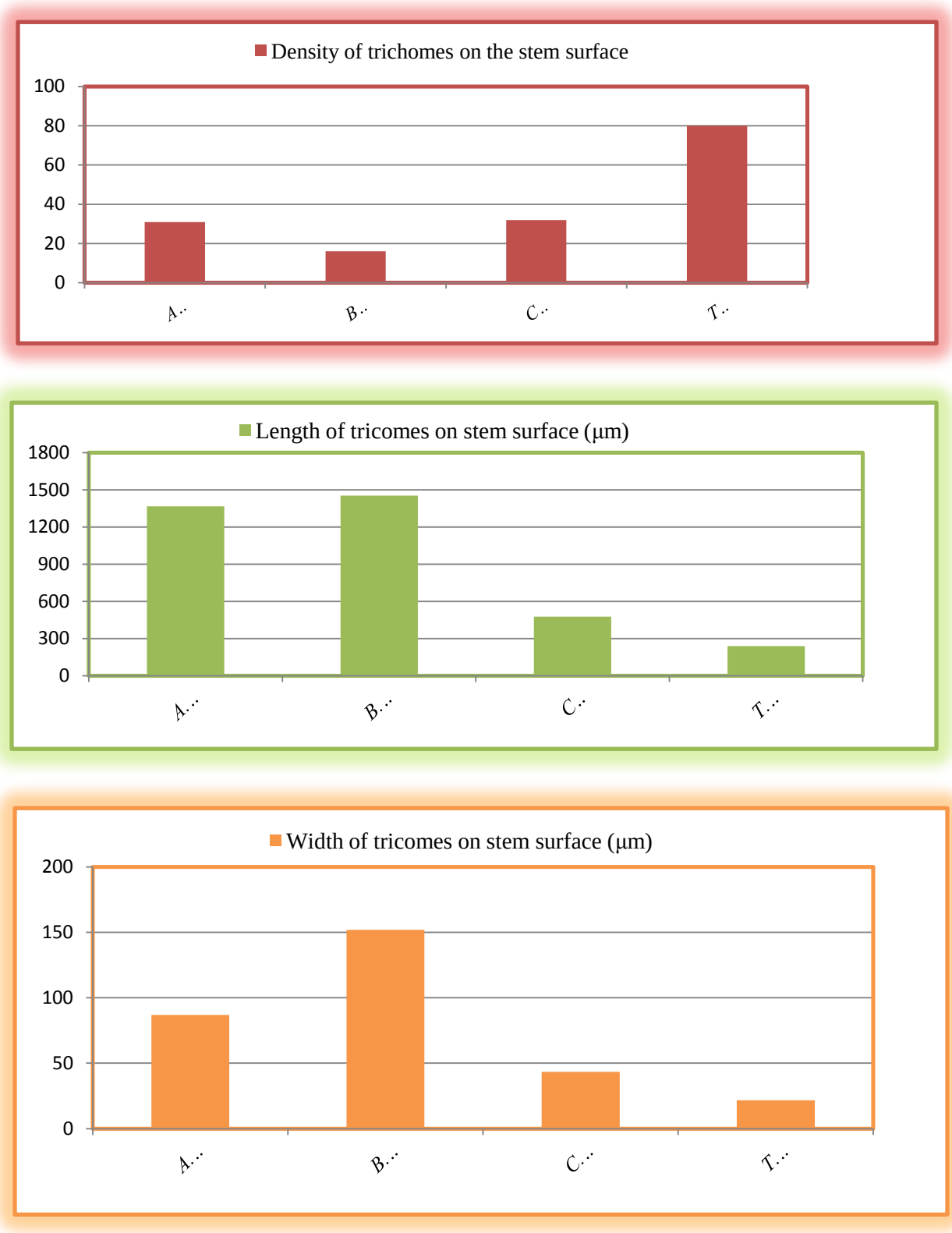


Figure 5. Graphs showing Density, length and Width of Trichomes on stem surface.

Euphorbia hirta differs from the others in having multicellular uniseriate rugose hairs (trichomes) on all parts of the plant except style and stigma, as well as on leaves and stems, with special ornamental epidermis. The hair base is surrounded by peltate arranged epidermal cells ranged between 3-4 cells around the base of each hair in *E.hirta* (Table 3). Although trichomes vary widely in structure within families and smaller groups of plants, they are sometimes remarkably uniform in a given taxon and have long been used for taxonomic purposes. The non-glandular trichome of *Achyranthus aspera*, coated by an evident papillose cuticle described in our work has corresponded to the epidermal appendages cited by Delaporte *et al.* (2002a). In our studies the non-glandular, trichomes are abundant on the lamina in *Withania species*. They are densely crowded and random in distribution. Table 3 showed that *Withania coagulans* have dense epidermal appendages on leaf surfaces than that of *Withania somnifera*. The terminal cells are pitted and the pits are not prominent. The sectional view showed with rough surface periderm accordance with studies of Okoli (1988) and Edoga and Okoli (1995). Our studies depicts that in *Parthenium hysterophorus*, trichome are of four types i.e. cylindrical hair, moniliform hair, simple multiseriate hair and shaggy hair have been recognized mostly on the basis of their structural differences. According the studies by Sahu (2008), different parameters in the trichomes of the different species of *Parthenium* as noticed from their structure are useful in distinguishing these species and we have observed these in Table 3. In *Pennisetum glaucum,* the leaf blade margin trichome length was 21.7 µm, leaf collar trichome length was approximately 65.1 µm, leaf blade edge at collar trichome was approximately 130.2 µm long for about 130.2 µm from collar, no membranous ligule present, lined with trichomes, and sheath trichomes were approximately 43.4 µm long for about 434 µm from collar (Graph 4) and these are in line with results by Hanna *et al.* (2009).

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