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FOLIAR ORNAMENTATION OF SERPENTINE SUNFLOWER (*HELIANTHUS BOLANDERI* A. GRAY; FAMILY ASTERACEAE)

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ABSTRACT

The leaves of *Helianthus bolanderi* A. Gray (family: Asteraceae) were collected from Karachi University Campus, Karachi and were studied for foliar micromorphology through epidermal impressions under compound optical microscope. Leaves were amphitrichomic. There were two types of trichomes; non-glandular were long multicellular (2 to 7-celled) with apical cell tapering in a sharp point. They were appressed with the leaf surface and directed to the apex. Glandular (presumably) trichomes were 3-celled with apical cell apically rounded. The trichome density was larger on ventral surface (49.51 ± 1.69 per mm^2) compared to that on the dorsal side (21.52 ± 1.02 per mm^2). As per Prabhakar's (2004) scheme of stomatal classification, there were several types of stomata - tetracytic, anomocytic, anisocytic, staurocytic, isotricytic and anisotricytic types. Tetracytic type was the most abundant. Stomatal density on dorsal surface averaged to 165.52 ± 4.70, varying from 88-256 stomata per mm^2.

Key words: *Helianthus bolanderi* A. Gray, Foliar micro-morphology, Trichomes and stomatal types.

INTRODUCTION

Asteraceae is a very large family consisting of 1620 genera and 23600 species (http://www.britannica.com/plant/Asteraceae). A number of studies have recently been conducted on foliar epidermal structure of many taxa of this family (Tuberosa *et al.,* 1985; Adedeji and Jewoola, 2008; Qureshi *et al.,* 2002; Milan *et al.,* 2006; Badmus and Afolayan, 2010; Hayat *et al.,* 2009, 2010; Inceer and Ozean, 2011; Kumekawa *et al.,* 2013; Paniagua-Ibáñez *et al.,* 2015; Bano *et al.,* 2015; Tahir *et al.,* 2016). Only a few studies as regards to the epidermal characteristics in Asteraceae have been conducted in Pakistan. Qureshi *et al.* (2002) carried out taxonomic studies of six species of the genus *Sonchus* (Asteraceae). Ahmad (2005) conducted morphological and anatomical studies of 23 species of the genus *Saussurea* (Asteraceae) from Pakistan. Hayat *et al*. (2009) reported that foliar trichomes of genus *Artemisia* are good taxonomic markers. Hayat *et al*. (2010) studied the stomatal variation in 24 taxa of the genus *Artemisia* which can be utilized to resolve taxonomic issues at infra-generic level. Bano *et al.* (2015) described micromorphological characters of 12 asteraceous species of alpine zone of Deosai plateau. However, the available information on the leaf epidermal anatomical characteristics of this family still appears to be quite limited.

Helianthus bolanderi A. Gray (Fam. Asteraceae) is a perennial herb and commonly called Serpentine Sunflower or Bolander's Sunflower. It is native to mountainous area of California and Oregon, USA, sometimes cultivated or grows as an escape plant in Karachi and other areas. It often grows on serpentine soils. For the last few years it is grown in UAE and Pakistan as an ornamental plant. In the current paper we focus on the surface ornamentation of its leaves. To the best of our knowledge literature on surface micromorphology of *Helianthus bolanderi* is not available and even on economically valuable species like *H. annuus* L it is rather limited particularly on stomatal frequency (Lovett and Campbell, 1973; Rawson and Craven, 1975; Dhopte and Aher, 1976). This study is pertinent in view of the importance of leaf surface structure in plant taxonomy (Stace, 1984) in general and for the family Asteraceae in particular (Adedeji and Jewoola, 2008).

MATERIALS AND METHODS

The leaves of *Helianthus bolanderi* A. Gray were collected from the Institute of Environmental studies, Karachi University Campus, Karachi. Hickey (1973) and LWG (1999) were followed for description of leaf. To study stomatal types, leaf epidermal impressions were made with clear nail polish (Wang *et al.,* 2006) and studied under compound optical microscope with various magnifications (see Results and Discussion section). Owing to the presence of trichomes, much densely over ventral surface of the leaf, some spaces of the polish imprint were blurred and consequently not utilized in this investigation. Stomatal nomenclature suggested by Prabhakar (2004) owing to its simplicity and being based upon structure of stomata and not their ontogenetic pathways, was adopted to ascertain stomatal types. The data were analyzed statistically where necessary in accordance with Zar (2010).

RESULTS AND DISCUSSION

Leaf architecture: Leaves were simple, alternate, leaf apex acute (apex angle < 90^o), leaf lamina is ovate i.e. axis of the greatest width intersecting the leaf axis basal to the midpoint of the latter axis. (L: W ratio = 1.813 ± 0.121, cf. Hickey, 1973), lamina base obtuse (base angle > 90^o), lamina margin serrate, tooth sinus rounded, tooth apex

pointed, petiole base winged. Leaf surface is velvety due to dense crop of trichomes on both the surfaces. Dried leaves are dorsally brown but ventrally gray-white. Single vein enters the lamina from petiole, pinnate single midrib and forms camptodromous-brachidodromous venation (secondary veins joined together in the form of a series of prominent arches. There are 5-6 pairs of secondary laterals. First pair of secondary veins arise from the midrib in lamina base area almost in opposite manner. Upper secondary laterals are generally alternate but occasionally nearly opposite due to irregular spacing between secondaries. Veins are more distinct dorsally (Fig. 1 and 2).

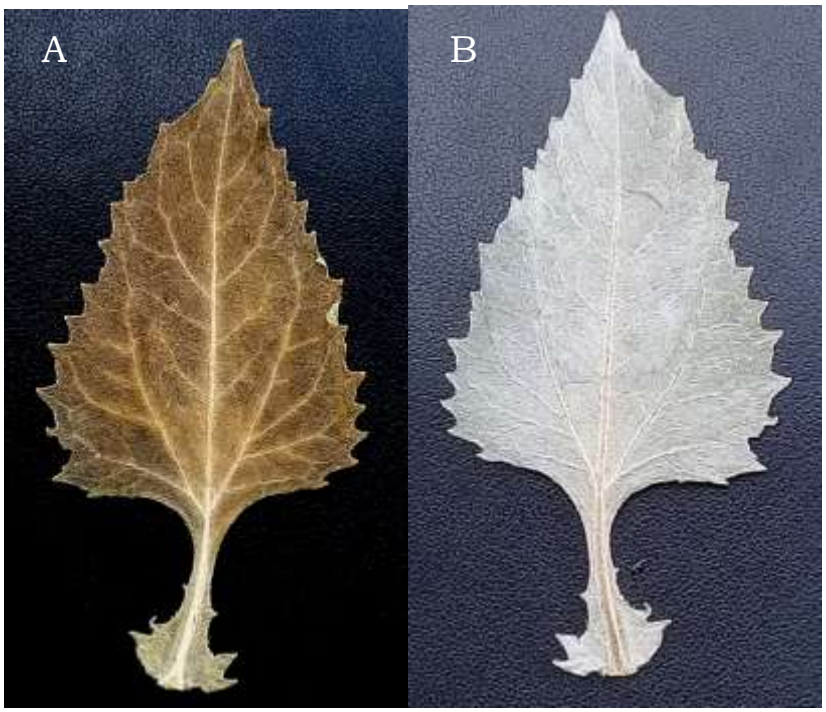


Fig. 1. Dorsal (A) and ventral (B) surface of herborized dry leaf – venation obviously discrete dorsally. Petiole is winged. Both surfaces are densely hairy but markedly differentiated in colour.

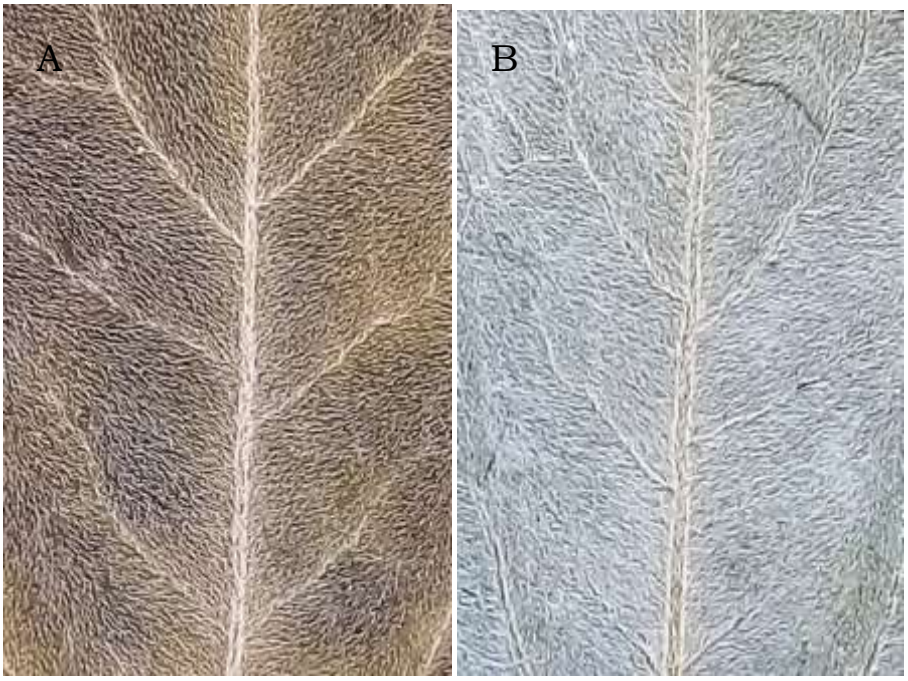


Fig. 2. Dorsal and ventral surfaces showing dense crop of trichomes (4X).

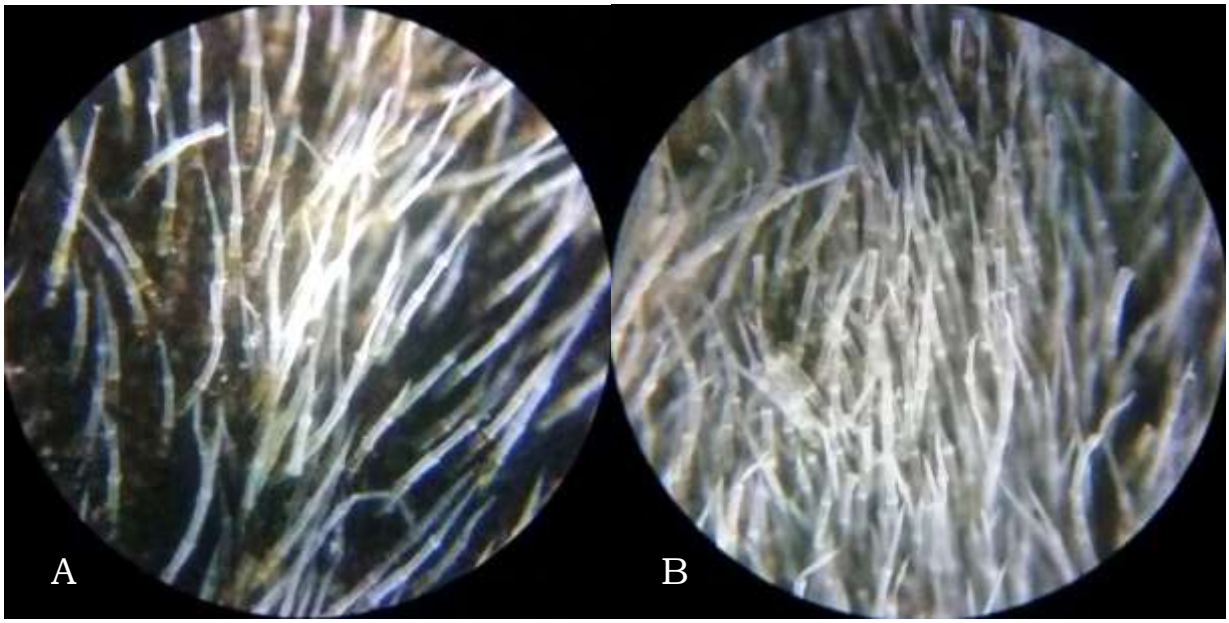


Fig. 3. Trichome crop on the dorsal (A) and ventral (B) surface of leaf of *H, bolanderi* (10 x 10 X). It is much denser on ventral surface compared to the dorsal one.

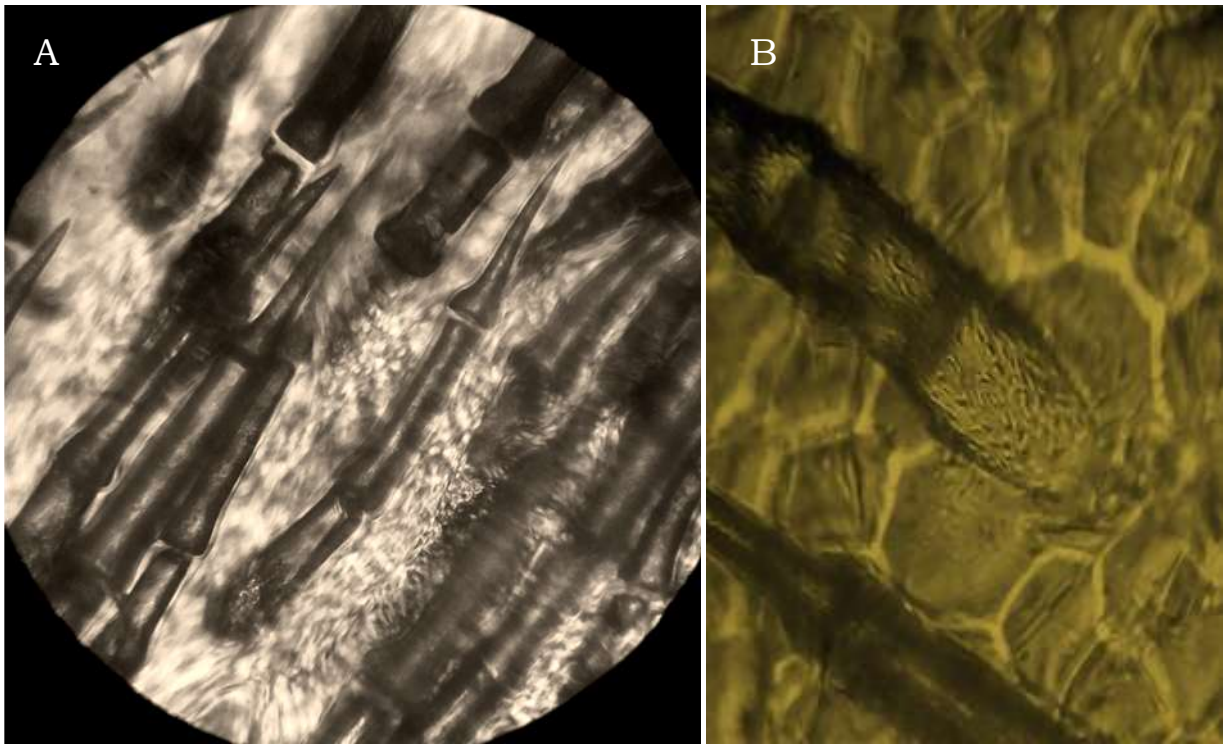


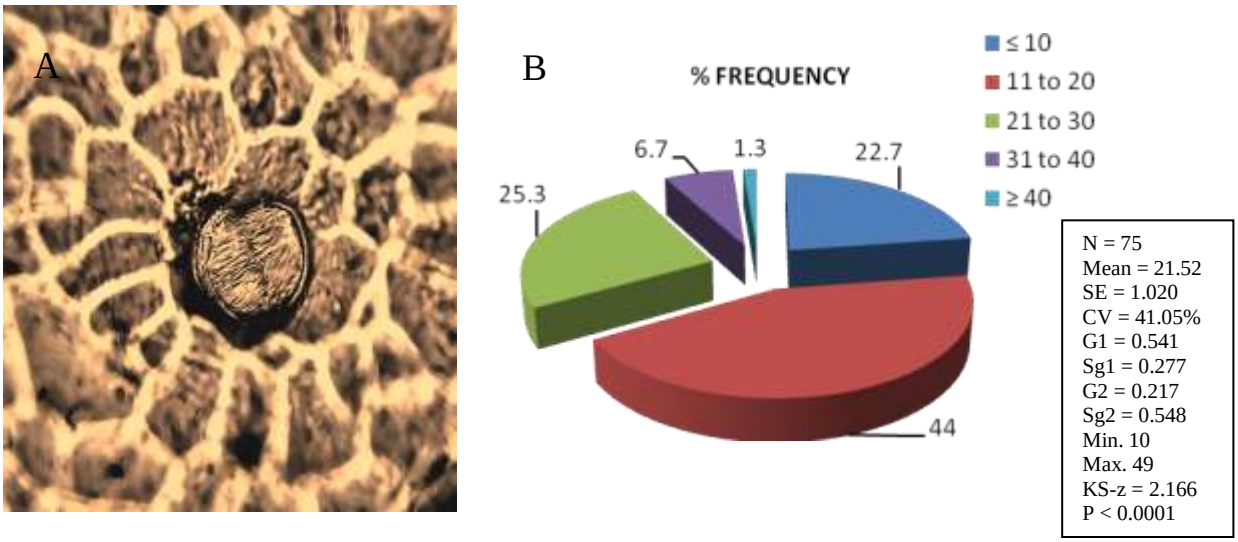
Fig. 4. A, Nail polish imprint of ventral surface of leaf – enlarged at 45 x 10 X. Trichomes are 2 to 6 celled with sharply- pointed apical cell. B, Trichomes of *H. bolanderi* also appear to be cuticularized (45 x 10 X, zoomed at 4X).

Trichomes

The leaves of *H. bolanderi* are amphitrichomic and somewhat velvety due to the presence of a dense crop of trichomes on both of the surfaces (Fig. 3). Trichomes are present on stem, leaves, inflorescence axes and involucral bracts. Several asteraceous taxa bear amphitrichomic leaves e.g. *Conyza japonicus* and *Aster himalaicus* that are also found to be amphitrichomic (Bano *et al.*, 2015).

Trichomes in *H. bolanderi* are long, cylindrical and tapering to a sharp point, directed towards the apex and appressed with the surface as also reported in *Costus bipinnatus* (Paniagua-Ibáñez *et al., 2015*) and *Felicia muricata* (Ashafa *et al., 2008*). Trichomes appeared to be rigid and sometimes broken at the base leaving a scar often filled with waxy cuticular substances (Fig. 5A).Trichomes appeared to be of two types: 1) Non-glandular Trichomes (NGT) and 2) Linear glandular Trichomes (LGT). NGTs are long, linear and multicellular with long tapering apical cell. They are ornamented with short moderately dense cuticular striae which is more prominent on basal cells (Fig. 4B). LGT's are relatively short with basal cell striae but apical cell is round (*Fig. 6A*). There appears thick encrustation of cuticle and compact wax flakes on leaf surfaces, thicker on the ventral surface (Fig. 4A). Aschenbrenner *et al.* (2013) have investigated trichome types in 38 *Helianthus* species and several other Helianthinae taxa and reported three types of trichomes – Linear glandular trichomes (LGT, 6-11 celled) and Non-glandular trichomes (NGT) from leaf and capitate glandular trichrome (CGT) from anther and some leaves. We found no CGT in *H. bolanderi*.

The trichome crop was much denser on the ventral surface of the leaves compared to that on the dorsal surface. Based on the observation of 80 microscopic frames of vision, the trichome density on ventral surface averaged to 49.51 ± 1.69 trichomes per mm^2 (varying from 19.66 to 88.46; CV= 30.53%). The trichome density on dorsal surface averaged to 21.52 ± 1.02 trichomes per mm^2 (N = 65) varying from 10 to 49; CV = 41.054%). The parameter of trichome density distributed asymmetrically (KS-z: 2.166, $p < 0.0001$) on dorsal surface but normally on the ventral surface (KS-z: 1.313, $p < 0.064$) (Fig. 5B and 6B). Thus, the trichrome density was significantly higher on ventral surface compared to that on dorsal surface ($t = 14.82$, $p < 0.001$).



Fig,5. **A**) A scar of the broken trichome filled presumably with waxy cuticular substances. **B**) Pie-chart showing the Frequency distribution of trichome density per mm^2 (for five density classes) on the dorsal surface of *H. bolanderi* leaf. *Acronyms:* SE, standard error of mean; CV, coefficient of variation; g1, skewness, Sg1, standard error of skewness; g2, kurtosis; Sg2, standard error of kurtosis; KS-z, Kolmogorov-Smirnoff z.

The trichome size on dorsal and ventral surface is presented in Figs. 7 and 8. The trichome length averaged to 348.06 ± 10.347 µm on dorsal surface (CV = 24.83%; varying from 112 to 602 µm) and 383.11 ± 15.508 µm (CV = 32.64%; varying from 150.4 to 768 µm). The value of $t = 1.85$ between the means (383.14 and 348.617) tended to be significant at $p < 0.05$ i.e. trichomes tended to be somewhat longer on ventral surface than dorsal surface. On both surfaces the parameter of trichome size tended to follow normal distribution.

The unbroken trichomes on the dorsal surface were on an average composed of 3.78 ± 0.083 cells per trichome (N = 110, varying from 2 to 6; CV = 23.04%) where as on ventral surface trichomes had 4.20 ± 0.073 cells per trichome (N = 150; varying from 3 to 7, CV = 21.31%). Around 94% of the trichomes on dorsal surface and 91% on the ventral surface were composed of 3-5 cells. The mean number of cells per trichome was statistically higher on ventral surface of leaf ($t = 4.09$, $p < 0.001$) as compared to that on dorsal surface.

Epidermal cells

The epidermal cells are polygonal shape and have straight to curved anticlinal walls.

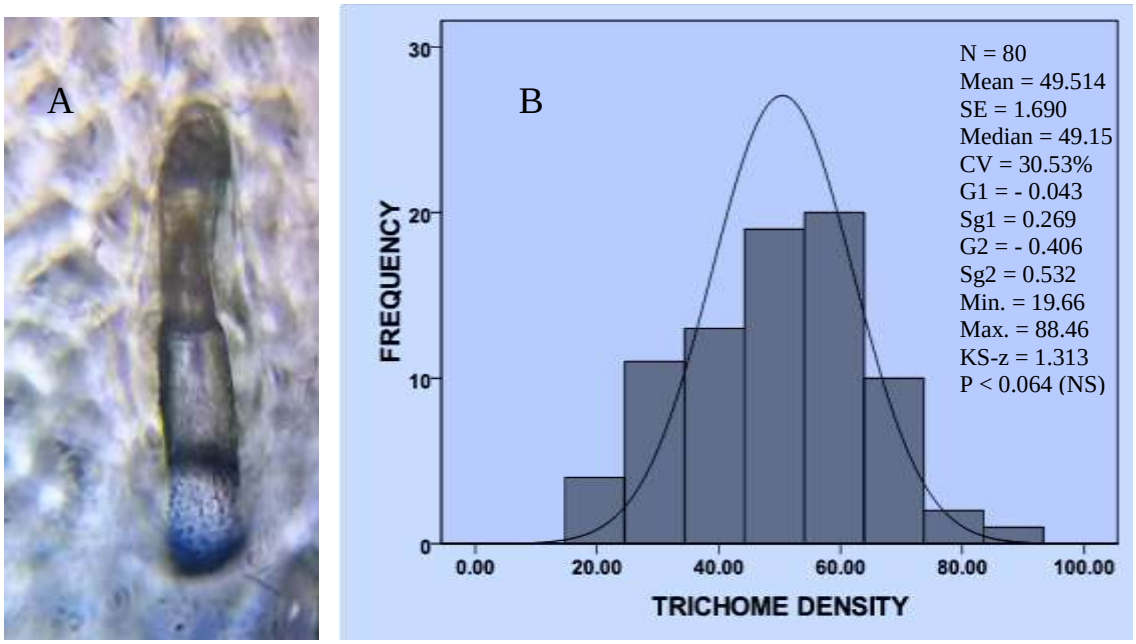


Fig.6. **A**) Linear glandular trichome interspersed rarely among non-glandular hairs. **B)** Frequency distribution of trichome density over ventral surface of leaf. Acronyms are the same as given in Fig. 5B.

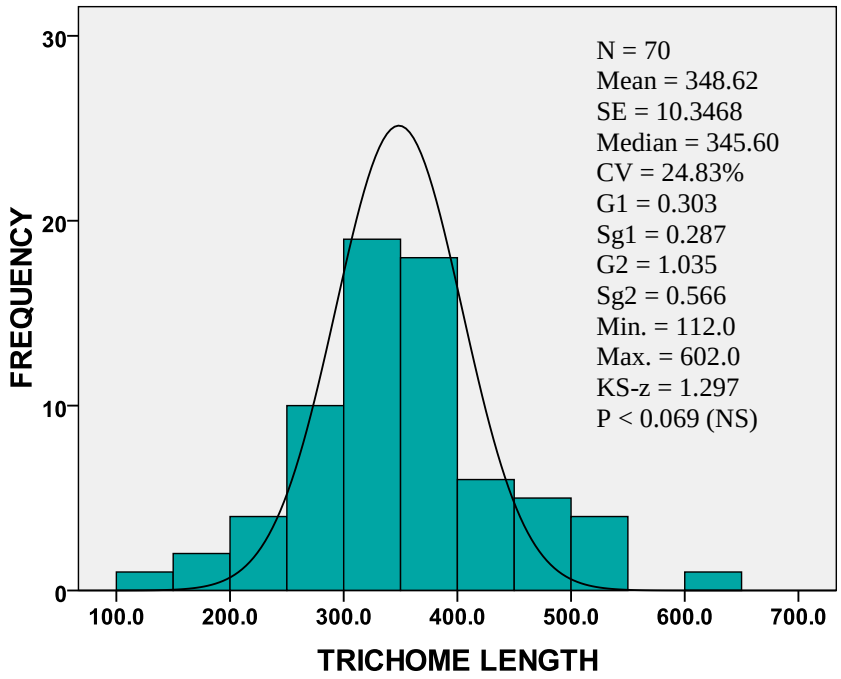


Fig. 7. Frequency distribution of trichome lengths (µm) on dorsal surface of leaf measured at magnification 45 x10 X Acronyms as in Table 2; Fig. 5B).

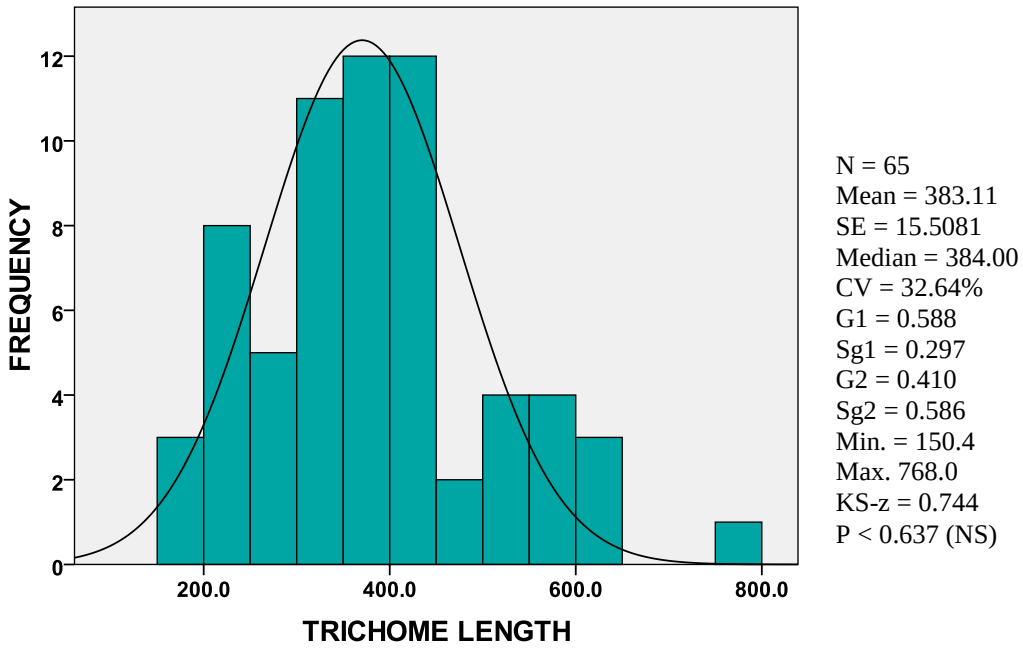


Fig. 8. Frequency distribution of trichome lengths (µm) on ventral surface of leaf (magnification: 45 x10 X). Acronyms as in Fig. 5B.

Stomata

The leaves of *H. bolanderi* are amphistomatic and asper classification scheme of Prabhakar (2004) bear a variety of stomatal types (*sensu* Prabhakar, 2004) which included tetracytic, anomocytic, anisocytic, staurocytic, isotricytic and anisotricytic types of 'subsidiaries arrangement' around the stomatal apparatus (Fig. 9 – 15). Tetracytic type of stomata was the most abundant (65.07%) and isotricytic, anisiotricytic types were substantially of much lesser occurrence (Fig. 16). Stomata surrounded by three subsidiaries were 13.2%, surrounded by four cells were 73.34% and those with five subsidiaries were 13.46 %. Stomata were generally observed in close vicinity with common subsidiaries among them (Fig. 10, 12, 15). There were a few abnormal stomata also e.g. contiguous stomatal pores (Fig. 12). The stomata were oriented in various directions. They are wide elliptical in shape.

There appears a diversity of stomata types in family Asteraceae. Anisocytic and anomocytic stomata have been reported in *C. bipinnatus* (Paniagua-Ibáñez *et al. (*2015) and in *Gillardia pulchella, Helianthus annuus* and *Wedelia trilobata* (Kaur and Nagpal, 2016). Bano *et al.* (2015) have found three types of stomata in asteraceous taxa studied from Deosai, Pakistan - anisocytic in *Artemisia persica*, actinocytic in *Circium falconeri* and *Erigeron multiradiatus* and anomocytic in nine other asteraceous species studied from Alpine zone of Deosai. Inceer and Ozean (2011) reported anomocytic stomata in 18 taxa of Asteraceae (*Matricaria* and *Tripleurospermum* from Turkey). Ahmad (2005) reported it from many species of *Saussurea* (Ahmad, 2005). Anomocytic stomata have also been reported in *Mikania glomerata*, *Porophyllum ruderalre* and *Vernonia caudensata* (Milan et al., 2006) and *Tithonia divrersifolia* from Paraná, Brazil (Duarte and Empinoti, 2012). All asteraceous taxa studied by Perveen *et al.* (2007) within the flora of Karachi (*Launaea resedifolia, Eclipta prostrata, Pluchea arguta, Pullicaria angustifolia, Sonchus asper, Tridax procumbens Vernonia cinera, Iphionia grantioides and Lactuca remotifera*)are reported to be anomocytic. Stomata are slightly striated in *Tithonia diversifolia* (Duarte and Empinoti, 2012).

Hayat *et al.* (2010) reported anisocytic stomata in *Artemisia* and Badmus and Afolayan (2010) in *Arctotis arctotoides*. Metcalfe and Chalk (1979) have included three stomatal types in family Asteraceae – Anomocytic, anisocytic and helicocytic. *Bidens pilosa* from Ibom State, Nigeria, has, however, been reported to be more diverse in having a variety of foliar stomatal types – diacytic, anisocytic, staurocytic and anomocytic stomatal on both the surfaces of leaf in addition to brachyparacytic type only on adaxial surface (Essiett and Archibong, 2014).

Tetracytic stomata have been reported in several asteraceous species e.g. *Bidens bipinnata, Lactuca sativa, Zinnia elegans, Tagetes erecta, Galinsoga parviflora* (Tahir *et al.,* 2016), several *Senecio* spp. (Joshi and Bajacharya (2015) and *Lactuca serriola, Launaea procumbens* and *Zinnia elegans* (Stace, 1965).

Tricytic stomata have been found in *Artemisia vulgaris*, *chrysanthemum indicum* and *Cosmos sulphureus* in association with abundantly present anomocytic, anisocytic and tetracytic stomata) (Sri Lakshmi and Naidu, 2014) and some *Senecio* spp. (Joshi and Bajacharya, 2015*).*

Dicytic stomata have been reported in *Bidens pilosa* besides anisocytic, staurocytic, anomocytic and brachyparacytic –anisocytic being the most abundant (Essiett and Archibong, 2014).

In a congeneric species, *Helianthus annuus*, only anomocytic stomata on both surfaces of leaf has been reported by Tahir *et al.* (2016) in a specimen collected from Azad Jammu & Kashmir. However, MahbuburRahman (2013) reported two types of stomata in this species from Northern areas of Bangladesh – anisocytic stomata on dorsal surface and anomocytic stomata on ventral surface.

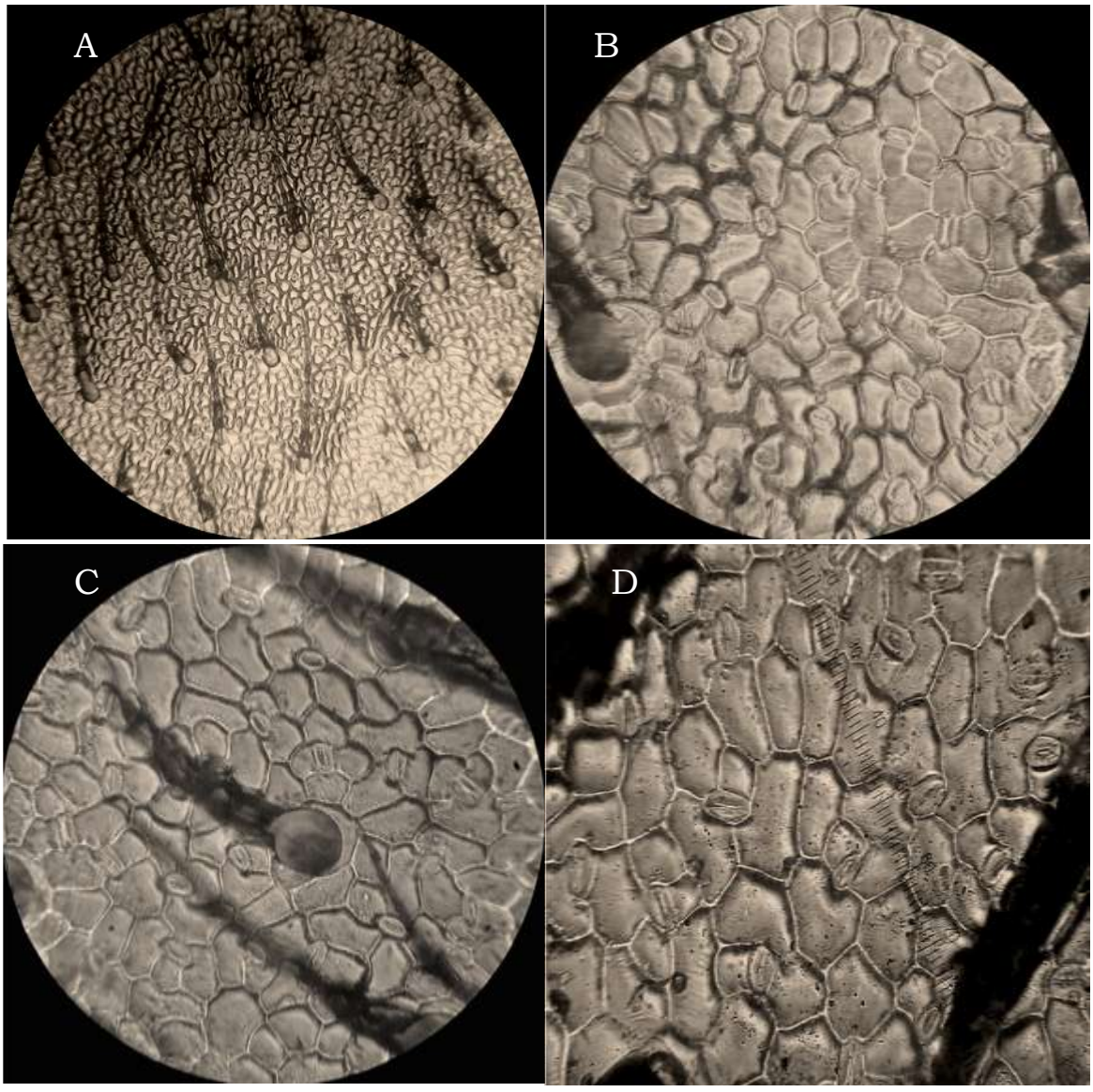


Fig. 9 Nail polish imprint of dorsal surface of leaf showing trichomes and Stomata- A, 10 x10X. B and C 45 x 10 X and D, 45 x 15 X. A number of stomatal types in these images are visible

The stomatal density on the dorsal surface of leaf averaged to 165.52 ± 4.70 varying from 8 to 256 stomata per mm^2 (CV = 24.60%). They appeared to be distributed normally on the leaf surface (Fig. 17). A comparative account of stomatal density of *Helianthus annuus* and *H. bolanderi* is presented in Table 1. The stomatal density of *H.*

bolanderi is similar to that reported for *H. annuus* by several workers such as Eckerson (1908), Tuberosa *et al.,* (1985) and Tahir *et al.* (2016).

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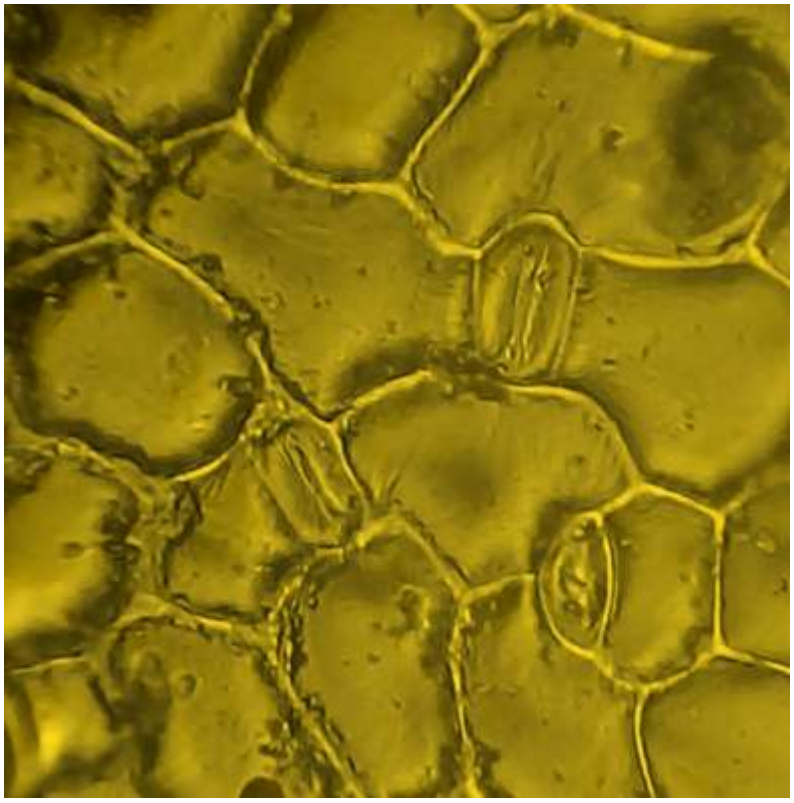

Fig. 10. Nail polish imprint of dorsal surface of leaf showing three stomatal types (Tetracytic, anomocytic and anisocytic) with subsidiaries common amongst them. Subsidiaries are indistinct.

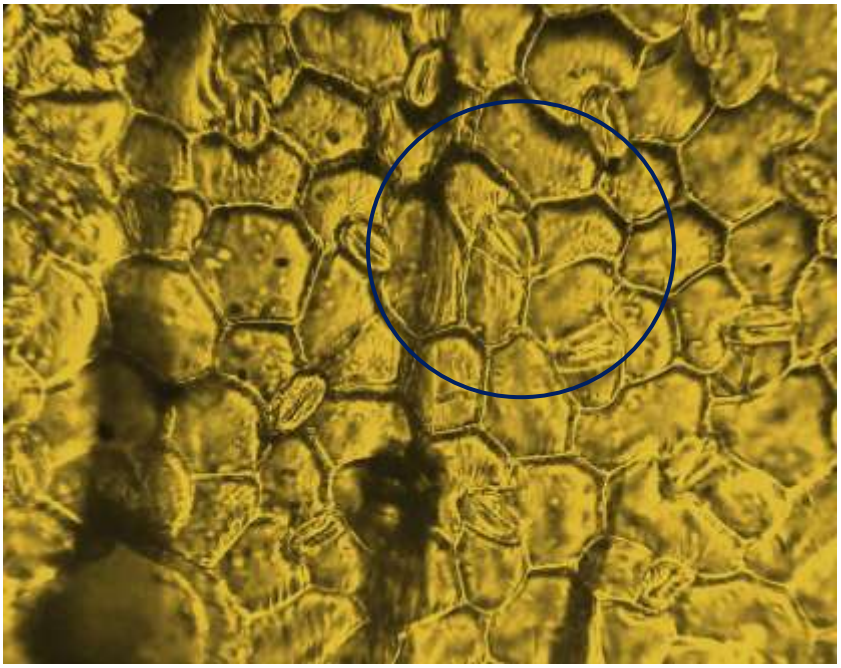

Fig. 11. Nail polish imprint of dorsal surface of leaf showing isotricytic stoma (in circle) besides other types.

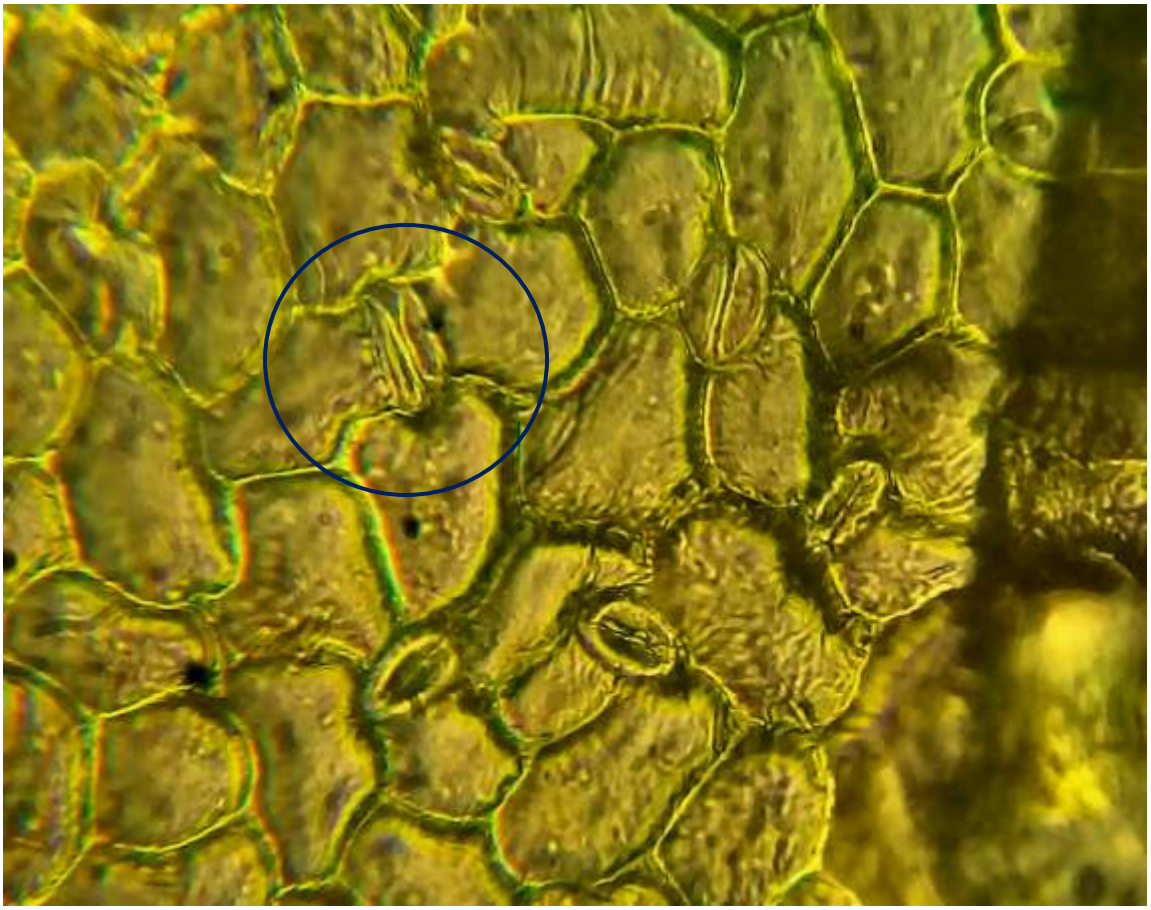

Fig. 12. Nail polish imprint of dorsal surface of leaf showing various stomatal types [Tetracytic, staurocytic, anomocytic and abnormal stomata (inside circle)]. There are several common subsidiaries amongst stomata. The subsidiaries are indistinct.

Table 1. Comparative account of stomatal density per mm^2 of *Helianthus annuus* and *H. bolanderi.*

Locality	*Helianthus annuus*		*H. bolanderi*	Reference
	Abaxial (ventral)	Adaxial (dorsal)	Adaxial (dorsal)	
Ganzolle, Italy	125.67 ± 6.07 * CV= 8.90%	100.33 ± 7.31 * CV = 12.621%	-	Tuberosa *et al.* (1985)
Momseille, Italy	144.33 ± 4.98 * CV = 4.48%	114.87 ± 5.04 * CV = 7.60	-	Tuberosa *et al.* (1985)
Green House, USA	156 (96-268)	85 (52-118)	-	Eckerson (1908)
India	276.17	-	-	Kaur and Nagpal (2016)
Azad Jammu & Kashmir	208.23 ± 8.92	178.82 ± 14.17	-	Tahir *et al.* (2016)
Karachi (Pakistan)	-	-	165.52 ± 4.70 (88 – 256) CV = 24.60	Present study

*, irrespective of the canopy sector (Lower, central or upper).

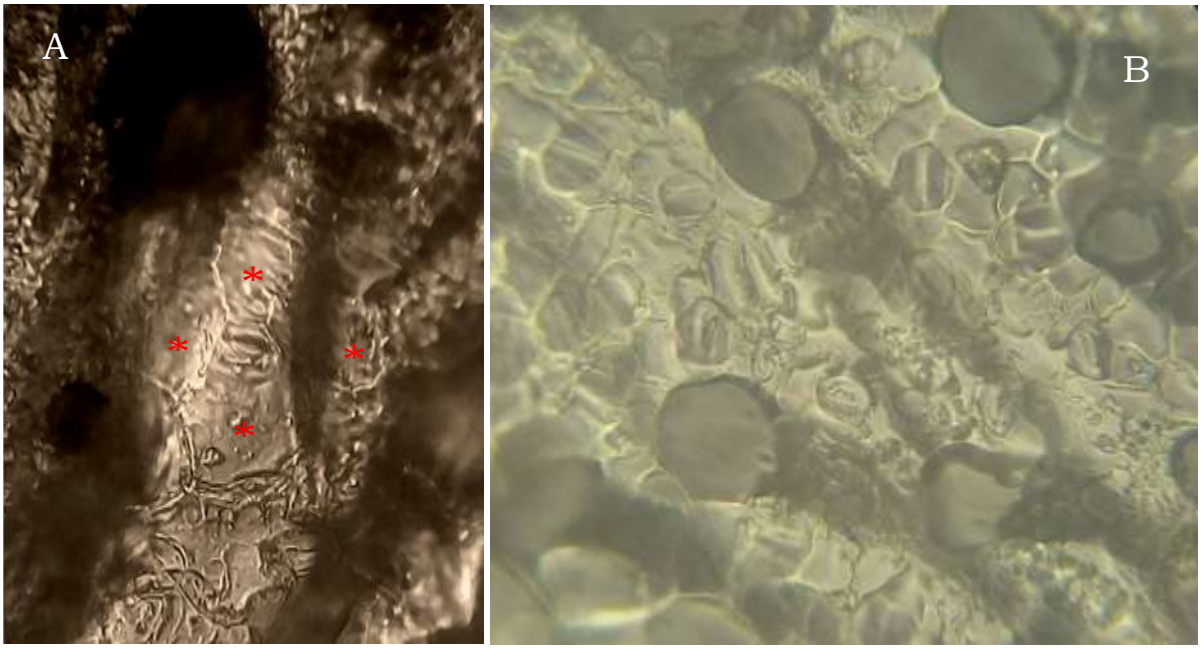

Fig. 13. Nail polish imprint of ventral surface of leaf. A tetracytic stoma (A) and some stomata peeping amongst the scars of the trichomes removed mechanically with the help of a scalpel (B). A, 45 x 10 X, zoom 1.2X and B, 45 x 10 X

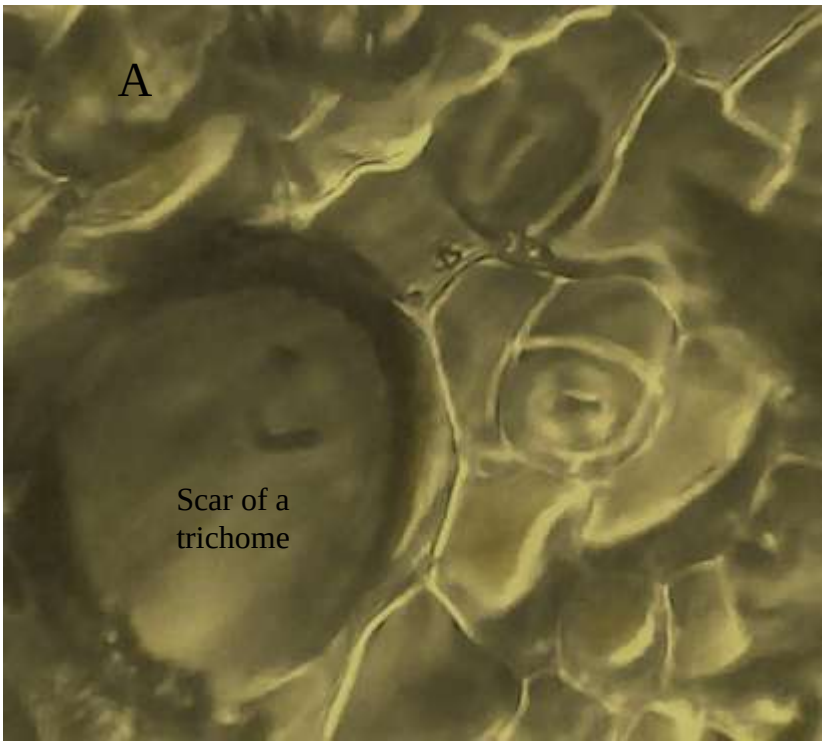

Fig. 14. The ventral surface of leaf showing a scar of a trichome and tetracytic stomata. The trichomes were gently removed from the leaf mechanically with the help of a scalpel and a brush.

The stomatal size of *H. bolanderi* in terms of length and width is presented in Table 2. The stomatal length averaged to 24.06 ± 0.03179 and stomatal width averaged to 13.37 ± 0.372 µm. Paniagua-Ibáñez *et al. (*2015) reported stomatal size of 30.16 ± 0.25 um in length and 20.64 um in width in *Cosmos bipinnatus*.

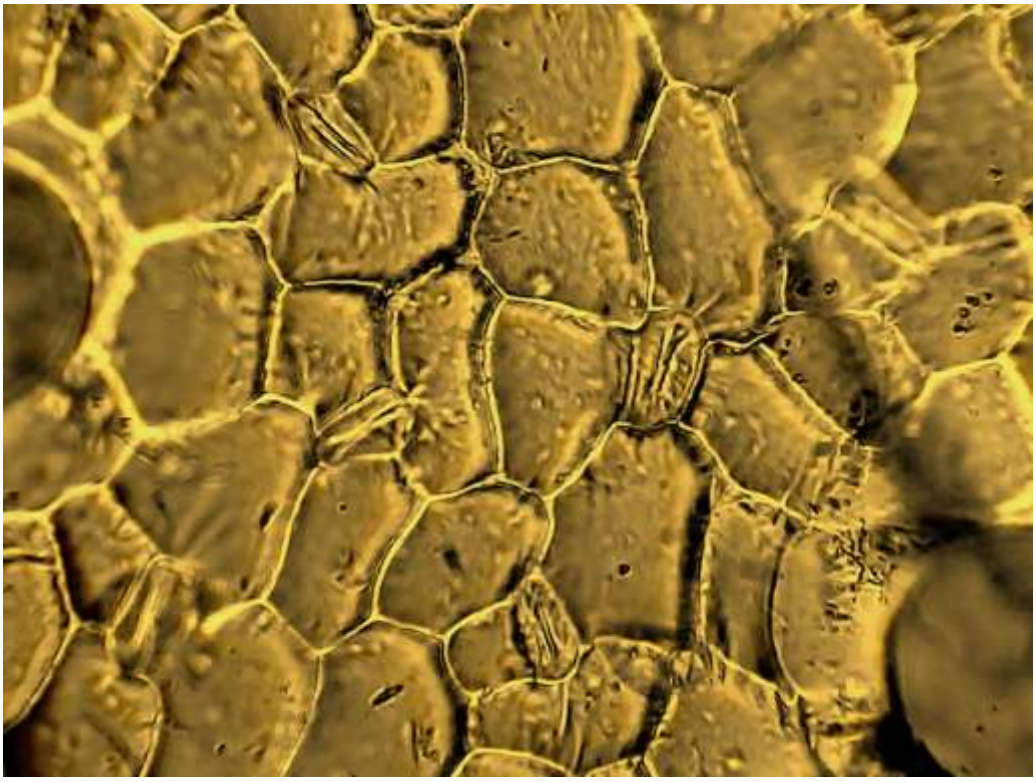

Fig.15.The ventral surface of leaf showing scar of a trichome and staurocytic, tetracytic and anomocytic stomata. Some cuticular striae and waxy flakes are visible. Trichomes were gently removed with the help of scalpel and a brush.

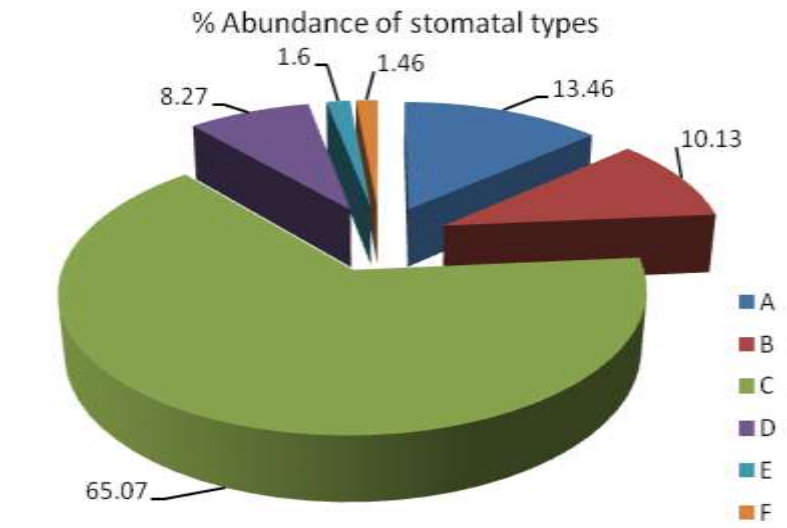

Fig.16. *Percent* abundance of various stomatal types on foliar dorsal surface of *H. bolanderi* grown in Karachi, Pakistan. Key to the acronyms; A, Anomocytic; B, Anisocytic; C, Tetracytic; D, staurocytic; E, isotricytic; F, Anisotricytic. The statistics is based on the inspection of 750 stomata in 45 fields of vision at magnification: 45 x 10 X.

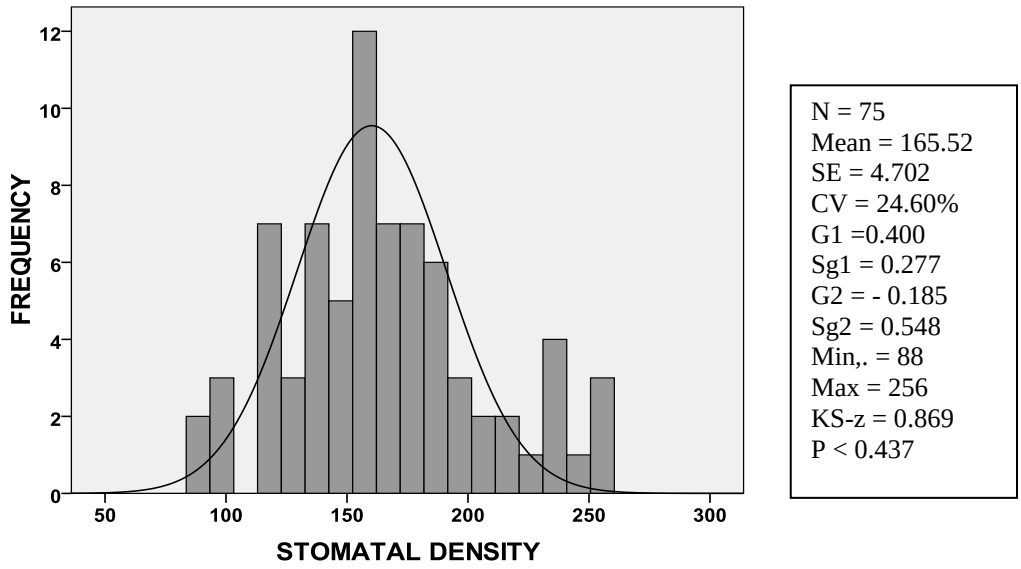

Fig. 17. Distribution of stomatal density per mm^2 on foliar dorsal surface of *H. bolanderi*. Acronyms as in Table 2

Table. 2. Length and width (µm) of stomata (Pore + guard cells) of *H. bolanderi*.

Statistical Parameter	Size of stomata	
	Length (µm)	Width (µm)
N (sample size)	50	50
Mean	24.016	13.373
SE of mean	0.31794	0.37234
CV (%)	9.36	19.69
G1(Skewness)	-0.286	0.213
Sg1 (SE of skewness)	0.337	0.337
G2 (kurtosis)	0.083	- 0.682
Sg2 (SE of kurtosis)	0.662	0.662
Minimum	19.20	9.60
Maximum	28.80	19.20
KS-z*	1.552	1.457
P	0.016	0.029

*, Kolmogorov-Smirnoff z. CV = Coefficient of variability (%).

The distribution of trichomes in Asteraceae is amazingly complex due to a great deal of diversity of their types and marked differences in their density. They may range from unicellular, bicellular to multicellular (Bano *et al.*, 2015). They may be eglandular or glandular and sessile or stalked. There are no trichomes in *Launaea taraxacifolia* (Adedegi and Jewoola, 2008) and also in *Circium reniforme, C. falconerii, Saussurea nepalensis, and Cremanthodium ellisii* Bano *et al.*, 2015). There is only one type of trichrome in (long, multicellular non-glandular, cylindrical, tapering to a sharp point and running parallel to the leaf surface in the direction of the apices) in *Felicia muricata* (Ashafa *et al.*, 2008). They may also be K-shaped, stellate, and tetraradiate. *Vernonia* has T-shaped trichomes. They may be bulbous at the base, capitate or non-capitate in *Tithonia diversifolia* (Duarte and Empinoti, 2010). They may vary in length (60-99.5 µm in *Erigeron multiradiatus* and 215-275 µm in *Aster himaliacus* (Bano *et al.*, 2015). The trichomal characteristics may be useful taxonomic parameter in Asteraceae which is a very large family and only some of its taxa have been studied for foliar micro-morphology.

Several types of stomata have been reported in various taxa of the family Asteraceae. Their comparative study is, however, cumbersome as various workers have either employed different stomatal classifications in their studies or in many cases they have failed to mention the classificatory system employed. This not only necessitates the comparative studies of various schemes of stomatal classification to adopt a unanimously accepted system but also uniform application of a particular system to facilitate comparison throughout the family.

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