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MORPHO-PALYNOLOGICAL AND LEAF EPIDERMAL ANATOMY OF WEEDS OF DISTRICT TANK, N.W.F.P, PAKISTAN

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ABSTRACT

An account was made to investigate morphology, palynology and leaf epidermal anatomy of ten weed species i.e. Asphodelus tenuifolius Carvan, Chenopodium album L., Chenopodium murale L., Convolvulus arvensis L., Cyprus defformis L. Amoen, Euphorbia helioscopia L., Lathyrus aphaca L., Plantago major L., Tribulus teristris L. and Vicia faba L. from District Tank. Pollen characters i.e. Type, shape and diameter of pollen grain and P/E ratio were important characters; while in leaf epidermal anatomy the type, shape and diameter of epidermal cell, type and diameter of stomata, length of stomatal complex, type of trichome (glandular / non glandular) etc were used as taxonomic characters of these weeds. The average polar diameter was 28.14, the smallest was 14.12 in Chenopodium album L. and C murale L. while the largest (52.5) was in Convolvulus arvensis L. Five types of stomata were found while trichomes were present only in two species. It is concluded that such type of study of weeds clarify its taxonomic position which is helpful for proper weeds management.

Key words: Morphology, palynology, leaf epidermal anatomy, weeds, District Tank.

INTRODUCTION

District Tank is located at 32.00°–32.30° N latitude and 70.05°–70.40° E longitude, with an altitude of 320.04 m ASL. Tank is a semi-desert, dry-area and only small chunk is under irrigation. Farming depends on rain-fall. The total area of the district is 1679 km square kilometers with a population of 238216. (Anonymous, 1998). Plant growing at an undesirable place is called weed. Weeds cause enormous reduction in crop yield, wastage of resources and human energy and are also health hazards to human beings (Man, 2006). Plants growing in a limited resources area are in competition for resources essential for their growth like water, light, space and nutrients etc. Weeds are often excellent competitors due to extensive

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root system and having high root-to-shoot ratio. Weeds produce an extensive amount of seeds often reduces the food value of cereal crops and interfere in harvesting. Their seeds may also go through dormancy to ensure its survival in the next growing season. It also decreases the aesthetic aspect of the crop fields.

Knowing the taxonomic status of weeds makes their management easier. The study of weeds morphology, palynology and leaf epidermis anatomy explain its exact taxonomic position. The scientists believe that the palynological studies can provide more accurate basis for identification of plant species or variation in plant species (Diaz and Lifante, 1991). Leaf epidermal anatomical features such as stomata, trichomes and other characters are useful anatomical tools. Anatomical features are of particular value to scientists who need to identify small scraps of plant material (Stace, 1980).

MATERIALS AND METHODS

For morphological studies, one to five specimens per species were used for assessment of morphological characters. Observations and measurements were made 3-6 times to ensure the readings. Fresh polleniferous material was used for pollen studies (Ronald 2000). Anthers were dissected from flower under binocular light microscope and treated with acetic acid. Pollen were stained with glycerine jelly (Erdtman, 1952).

Leaf samples for epidermal anatomical studies were prepared according to the modified method of Clark's (1960) technique. The leaves were treated with 88% lactic acid, in water bath (model memert GmbH+Co.KG D-91\26, Schwabach FRG, Germany) at 100 C for 30 minutes. Slides of both abaxial and adaxial sides of leaves were prepared and observed under light microscope. Microphotographs of pollen and leaf epidermal characteristics were taken by using CCD digital camera (Model: DK 5000,) fitted on Leica light Microscope (model DM 1000). These micrographs were useful for identification and differentiation of taxa on the basis of microscopic features.

RESULTS

***Asphodelus tenuifolius* Carvan. Liliaceae**

Local name: Pyozikai

An annual herb occurs mostly as weeds in chickpea and grams, up to 45cm tall, leaves sessile, up to 42cm long and 3.5mm wide initially forming a rosettes, leaf margins entire, tip acuminate, base truncate, shape ensiform and having parallel venation. Two to many floral stalks appear from the rosette up to 45cm in height. Inflorescence recemose, flowers many having 5mm long pedicle. Perianth 04, white membranous, up to 5mm long and 1.5mm wide

having midrib at the back up to its apex, tip acute, base truncate, shape lanceolate. Stamens 06, 3 long and 3 short. Longer ones 4mm and shorter ones are 3mm long, anther 1mm long and 0.5mm wide. Carpel 01, 4mm long, ovary superior, basal portion swollen of 1mm long and wide, style and stigma 01, 3mm long, respectively. Fruit capsule, rounded, 4mm in diameter. Bear flowers from March to April.

***Chenopodium album* L. Chenopodiaceae**

Local name: Spina surma

Erect annual herb up to 45cm tall, variously branched. Stem yellowish green in colour, striated. Leaves opposite, abaxially dark green and green adoxially, 3-5cm long and 1-2cm wide, tip acute, base obtuse, shape ovate-lanceolate, margins mostly entire and occasionally serrate. Upper most leaves lanceolate and entire, teeth mostly obtuse. Inflorescence terminal, cymosely panicle, each panicle is of 3-florates in the axial of a leaf which is 3mm long and 0.5mm wide having cuspidate tip, attenuate base and lanceolate to oblanceolate shape. Perianth segments 05, persistent, each one is 1mm long and 0.5mm wide, tip obtuse, base rounded, shape ovate, dorsally keeled. Stamens 05. Carpel 01 and 0.5mm long. Seeds 1-1.6mm in diameter, black, horizontal, margins obtuse, nearly smooth. Flowering season is March-May.

***Chenopodium murale* L. Chenopodiaceae**

Local name: Thura surma

Annual herb up to 60cm tall, stem yellowish to light green in colour, erect, glabrous, much branched at the base. Leaves usually opposite, dark green in colour, petiolate having 1-1.5cm long petiole, 4cm long and 2cm wide, tip acute, base obtuse, shape rombate, margins coarsely dentate. Inflorescence axillary and terminal divaricately branched, leafy. Flowers bisexual. Perianth segments 05, green, persistent, 1mm long and 0.5mm wide, tip rounded, base truncate, shape elliptic having a prominent keel at the mid. Stamens 05 and 0.5mm long. Carpel 01, 0.6mm long, ovary superior, stigmas 2. Seeds black in colour, 1-1.5mm in diameter, apparently rounded, horizontal, margins acute, obtusely keeled. Flowering season is March-May.

***Convolvulus arvensis* L. Convolvulaceae**

Local name: Perwatha

Annual trailing or climbing herb, length up to 80cm, stem smooth, striated, weak and green. Leaves simple and alternate. Petiolate having petiole up to 1.5cm long. Leaf length up to 4cm, width up to 1.2cm, tip rounded, base truncate, shape sagitate, margins entire. Flower solitary, pedicle length up to 2.5cm, stipules two at the base of flower stalk having up to 3mm length and 1mm width, tip rounded, base truncate, shape ovate. Sepals 05, free, 5mm

long and 4mm broad, tip retuse, base truncate, shape ovate. Petals 05, whitish to pinkish white in colour, united and form a campanulate tube having truncate base and 3-lobed tip and 1.7mm length. Stamens 05, 5mm long, anther 2mm long, sagitate. Carpel 01, 7.5mm long, ovary superior having 2mm length and 1mm wide, style and stigma one. Bear flowering from March-April.

***Cyperus difformis* L. Cyperaceae**

Local name: Dela

Annual up to 45cm tall. Leaves somewhat shorter than stem. Stem trigonous up to 4mm in diameter. Leaves are sheathing at the base, sheathed portion is 5cm, free portion is 30cm long, leaf width is up to 4mm, flaccid, tip acute, base truncate, venation parallel, ligule absent. Inflorescence panicle up to 4cm long, glubose, peduncle of 0.8-1.5cm long, individual floret length is 1.2cm, bracts 02, one larger of 8mm and 2nd one shorter of 4mm length. Stamens 03, 5mm long, filament length 0.8mm and anther length is 4.2mm and 2-celled, attachment basal. Glumes 02, both same, 6.5mm long and 4mm wide, tip bidentate, base obtuse, shape ovate, concave and keeled. Carpel 01, 5.5mm long, stigma and style 03 and 2.5mm in length each. Flowering season is March-April.

***Euphorbia helioscopia* L. Euphorbiaceae**

Local name: Perparai

Annual erect herb, 25-45 cm tall, its branches arise at the base. Stem cylindrical with sparse hirsute hairs. Leaves simple, alternate, sessile to sub-sessile, leaves whorled at the tip having inflorescence. Leaf length 2-2.7 cm and width 1-1.7cm, tip complete rounded, base cuneate, shape obovate, margins entire but dentate only at the tip, venation pinnate-netted. Inflorescence is cythaium, terminal, peduncle length is 1-2cm. The disc formed of yellow petals is 3mm long and 2mm wide. The middle longer flower is 6mm long, its base which is like style is 4mm long, then a swollen portion of 0.8mm long and wide occur, then above three stigma are found, each is 1.2mm long and the tip of each stigma is bifurcated. The stamens in disc are 15, the anther seems as like two are attached with one filament. The stamens length varies. The longer stamen is 2.5mm having 2mm long filament, and 0.5mm long anther. The length of short stamen is 1.2mm only. Flowering season is March-April.

***Lathyrus aphaca* L. Papilionaceae**

Local name: Jangli mater

Annual climbing herb and weak stemmed. Stem not winged. Stipules foliaceous, opposite, sessile, 3cm long and 1.5cm wide, tip obtuse, base ariculate, shape sagitate, margins entire, venation parallel. With the two opposite stipules arise a tendril-which is a

modified leaf of varying length up to 15cm long, its tip become thin and used for climbing. Flower solitary, yellow in colour, pedicelate having a pedicle of 4mm length. The pedicle has one minute stipule of 1.5mm long. Sepals 05, 9mm long, united at the base, free portion of sepals are 5mm long, tip narrowly acute, base truncate, shape ovate. Petals 05, papilionaceous, 1st petal large of 1cm long and 5mm wide, tip notched at the mid up to 1mm, then the 2 portions are rounded, base truncate, shape spatulate, 2nd and 3rd petals are same having 8mm length and 2mm wide, tip complete rounded. The 4th and 5th combine at apices to form a cap like structure having 8mm long and 4mm wide, all petals have long and narrow limb. The bases and shapes of all are same. Flowering season is March-April.

***Plantago major* L. Plantaginaceae**

Local name:

Perennial acaulescent herb with short stout root stock and adventitious roots. Leaves in rosettes, few in number, spirally arranged, long petioled of 5cm but no clear distinction occur between petiole and lamina. Leaves up to 25cm long and 3cm wide, tip acute, base attenuate, shape oblanceolate, margins entire, venation parallel. Scapes many, erect, 12-42cm long, sparsely covered by pilose hairs. Inflorescence spikes, dense, slender, each 1.5-4cm long, somewhat broad at base and narrow at apex. Bracts 02, glabrous, 5mm long and 1.5mm wide, tip acuminate, base obtuse, shape subulate. Sepals 04, glabrous and membranous, 3mm long and 1.5mm wide having black keel at the back up to the apex, tip acute, base obtuse, shape ovate. Petals 04, fused to form a tube of 3mm length, next nearly same as sepals. Stamens exerted out of corolla tube. Carpal 01, 5mm long, ovary superior and dilated of 1mm length, style length is 4mm. Seeds few, minute, not smooth. Flowering season is March-April.

***Tribulus terrestris* L. Zygophyllaceae**

Local name:

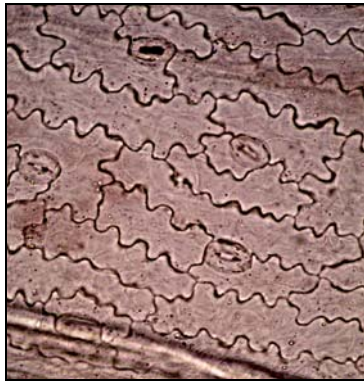
Annual prostate herb, branches not exceeding then two feet. The whole plant is covered by tomentoluse hairs. Leaves opposite, petiolate of 7mm long petiole, peripinnate compound, leaflets 4-8 pairs, 2.5cm long and 4mm wide, tip obtuse, base oblique, shape ovate, margins entire. Stipule lanceolate to falcate. Flower solitary, axillary, pedicelate of 7cm long pedicle. Sepals 05, green, free, 7mm long and 1.5mm wide, tip acute, base truncate, shape ovate, sides membranous. Petals 05, yellow in colour, free, 4mm long, 3mm wide, tip complete rounded, base truncate having long and narrow limb, shape ovate, having tomentoluse hairs on inner side. Stamens 05, 3.5mm long, filament 3mm and anther 0.5mm long-basifixed, 2-celled. Carpel 01, 3.5mm long, ovary superior of 1mm long and wide, style and

stigma 1, stigma dilated and seems like an anther. The base of carpel is densely covered by 2.5-3mm long hirsute hairs. Fruit mericarp with two long and two short spines. Flowering season is March-April.

***Vicia faba* L. Papilionaceae**

Local name: Jangli mater

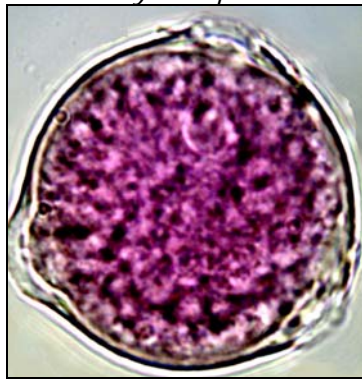
Annual herb having length up to 40cm, stem weak. Leaves simple. Sessile, opposite, up to 6cm long and 6.8mm wide, tip obtuse, base truncate, shape lance-ovate, margins entire, venation parallel. Flower solitary, axillary, pedicle up to 7mm long. Sepals 05, green, attached at the bases, up to 8mm long and 2mm wide, tip acute, base truncate, shape lanceolate. Petals 05, blue in colour, papilionaceous. Membranous staminal tube of 6mm long and 2.3mm wide of which stamens are protruded out up to 6mm, stamens 08, anther 1mm long and 0.5mm wide. Carpel 01, 1.2cm long, ovary superior, style and stigma 01. Flowering season is March-April.



Lathyrus aphaca



Tribulus terrestris



Vicia faba



Cyperus difformis

Fig. 1. Some Photographs from Slides of Pollen and Leaf Epidermal Anatomy

TABLE: 1 QUALITATIVE AND QUANTITATIVE ANALYSIS OF POLLEN MORPHOLOGY

Taxa	Shape in Polar view	Polar diameter (µm)	Shape in equatorial view	Equatorial diameter (µm)	Type	PVE Ratio	Exine thickness (µm)
<i>Chenopodium album</i> L.	Circular	14.12 (12.5-17.5)	Irregular but mostly near to crescent shaped	10.87 (10-12.5)	Pentaporate	1.3	1.2
<i>Chenopodium murale</i> L.	Circular	14.12 (12.5-17.5)	Prolate to apple-shaped to oblate-spheroidal	17 (15-21.25)	Pentaporate	0.83	0.5 (0.2-1.2)
<i>Convolvulus arvensis</i> L.	Circular	52.5 (45-50)	Spheroidal to subprolate to prolate	46 (30-50)	Tricolpate	1.14	3.375
<i>Cyperus difformis</i> L.	—	—	Irregular	24.25 (22.5-30)	—	—	1.25
<i>Euphorbia helioscopia</i> L.	Circular	25.6 (17.5-30)	Subprolate to prolate to perprolate	26.25 (20-30)	Tricolpate	0.97	2.43 (2-2.5)
<i>Asphodelus tenuifolius</i> Carvan.	—	—	Spheroidal to prolate to perprolate	39.5 (30-50)	—	—	1.41 (1-2.25)
<i>Lathyrus aphaca</i> L.	Probate	23 (17.5-25)	Irregular	17.7 (15-22.5)	Tricolpate	1.29	1.2
<i>Vicia faba</i> L.	Circular	30.75 (28.75-35)	Cup shaped to perprolate	26.09 (22.5-30)	Tricolpate	1.17	1.25
<i>Plantago major</i> L.	Circular	24.25 (20-27.5)	Variable i.e. having no specific shape	18.25 (17.5-22.5)	Tricolpate	1.32	1.25
<i>Tribulus terrestris</i> L.	Circular	40.87 (37.5-45)	—	—	Pentaporate	—	3.6 (21.5-5)

TABLE-2. QUANTITATIVE ANYLYSIS OF LEAF EPIDERMAL CHARACTERS

Taxon	Length x Width of epidermal cells (μm)	Diameter of stomata (μm)	Length of Stomatal complex (μm)
<i>Chenopodium album</i> L.	70 (55.84-84.17) x 29.6 (29.16-30)	17.91 (17.5-18.75) x 9.2 (8.75-10)	63 (30-87.5)
<i>Chenopodium murale</i> L.	51.6 (40-63.325) x 20.7 (16-25.33)	18.125 (17.5-20) x 7.18 (5-8.75)	58.33 (55-65)
<i>Convolvulus arvensis</i> L.	95.5 (62.5-129.17) x 43.75 (41.76-45.8)	Adaxially 22.5 (20-25) x 7.08 (6.25-7.5) Abaxially 25 x 8.7 (7.5-10)	28.5
<i>Cyprus difformis</i> L.	62.084 (27.17-100) X 22.92 (22.5-23.34)	31.7 (30-32.5) x 1.67 (1.25-2.5)	29.3 (27.5-31.2)
<i>Euphorbia helioscopia</i> L.	22 (9.167-35.84) x 18.34 (16.67-20)	15 (12.5-17.5) x 7.5 (5-10)	30 (27.5-32.5)
<i>Asphodelus tenuifolius</i> Carvan.	51.3 (38.35-65) x 27.3 (23.35-31.25)	17.5(15-20) x 5	23.5 (20-25)
<i>Lathyrus aphaca</i> L.	60.62 (37.5-83.75)	14.16 (12.5-15) x 4.16 (2.5-5)	35 (30-40)
<i>Vicia faba</i> L.	43.3 x 51.7 (Abaxial) 31.6 x 41.6 (Adaxial)	14.16 (12.5-15) x 4.16 (2.5-5)	35 (30-40)
<i>Plantago major</i> L.	47.5 (35.48-58.3) x 30 (25-4)	15 x 8 (6-10)	53.34 (45-65)
<i>Tribulus terrestris</i> L.	27.91(20-35.8) x 18.3 (15-21.67)	9.4 (7.5-12.5) x 3.7 (2.5-5)	38 (30-42.5)

TABLE-3. QUALITATIVE ANALYSIS OF LEAF EPIDERMAL CHARACTERS

Taxon	Type and shape of epidermal cells	Type of stomata	Type of trichomes	Difference b\^t the two leaf surfaces
<i>Convolvulus arvensis</i> L.	On adaxial side are polygonal & are polygonal to nearly undulating abaxially	Axillocytic and staurocytic	Absent	The diameter of stomata is larger on abaxial side
<i>Chenopodium album</i> L.	Polygonal	Staurocytic	Absent	Both sides of the leaf are same
<i>Chenopodium murale</i> L.	Polygonal to nearly undulating	Staurocytic	Absent	Both sides of the leaf are same
<i>Cyperus difformis</i> L.	Mostly tetragonal but penta and hexagonal cells are also found with wavy walls	Paratetracytic	Absent	Adaxial side has less number of stomata
<i>Euphorbia helioscopia</i> L.	Tetra to penta and hexagonal on adaxial side and its walls are wavy on abaxial side	Staurocytic	Absent	No stomata found on adaxial side.
<i>Asphodelus tenuifolius</i> Carvan.	Long, narrow and rectangular with smooth walls	Anomocytic	Unicellular and non-glandular	Both sides of the leaf are Same
<i>Lathyrus aphaca</i> L.	Wavy	Diacytic	Absent	Both sides of the leaf are same
<i>Vicia faba</i> L.	On Abaxial side are smooth, longitudinal and linear but wavy on Adaxial side	Amphianisocytic	Absent	The epidermal walls are different on both sides of the leaf
<i>Plantago major</i> L.	Tetragonal to polygonal	Diacytic and amphianisocytic	Absent	The number of stomata were more on adaxial side
<i>Tribulus terrestris</i> L.	Polygonal and isodiametric	Actinocytic	Unicellular & non-glandular	Both sides of the leaf are same

DISCUSSION

According to this study the species were distinguished morphologically as: *Lathyrus aphaca* by its climbing habit, foliaceous stipules and leaves modified into tendrils, *Vicia faba* by its simple and sessile leaves which are lance-ovate in shape, flower solitary axillary. *Plantago major* by perennial acaulescent herbaceous nature, oblanceolate leaves, bracts subulate in shape, sepals 04, ovate. *Tribulus terrestris* by annual prostrate herbaceous plant covered by tomentulose hairs, leaves compound peripinnate, petals covered by tomentulose hairs on inner sides, stamens 5, carpel densely covered by hirsute hairs, fruit mericarp with 02 long and 02 short spines. Similarly the species with in the same genus *Chenopodium* were differentiated as: *Chenopodium album* by entire leaf blades, terminal cymosely panicle inflorescence and seeds nearly smooth, margins obtuse and *Chenopodium murale* by coarsely dentate leaf blades, axillary and divaricately branched inflorescence, and seeds apparently rounded, margins acute, obtusely keeled. And all the above findings of individual species distinguishable characters were comparable to the Flora of Pakistan by Nasir and Ali. Morphological studies for taxonomic purposes are a very old and useful technique and majority of the world floras are based on morphological description. It also solves the taxonomic positioning of problematic taxa for their proper ranking. A lot of new species have been introduced to the world of taxonomy on the basis of morphological findings (Ahmad et al, 2008).

The palynological findings were as: *Lathyrus aphaca* and *Vicia faba* have tricolpate pollens and psilate in sculpturing, prolate and circular in polar view, irregular and cup shaped to perprolate in equatorial view respectively. The pollen morphology of 157 species, representing 37 genera, of the subfamily Papilionoideae from Pakistan have been examined by (Perveen and Qaiser 1997), using light and scanning electron microscopy. According to them Papilionoideae is a eurypalynous subfamily. The pollen grains are generally free, radially symmetrical, isopolar, mostly tricolporate, rarely colpate or porate. The shape is commonly prolate to sub-prolate, or prolate-spheroidal, less commonly to oblate-spheroidal or sub-oblate, often perprolate.

In *Tribulus terrestris* pollen diameter was much larger i.e. 40.87 μm (37.5-45 μm) with exine thickness 2.15 μm , periporate pollen type and echinate sculpturing. According to the findings of Perveen et al. (2006), the *Tribulus terrestris* has a prolate Oblate-spheroidal shape, 32.3 (35.9 +0.71) 34.5 lengths in μm , 1.52 μm Pore diameter and 6.82 (7.14 +0.06)7.18 exine thickness in μm .

The detail of 02 species i.e. *Chenopodium album* and *C. murale* were as: Both have the same polar shapes and diameters i.e. circular

and 14.12 μm respectively and same pollen types- periporate and psilate sculpturing. *Chenopodium album* have irregular but mostly near to crescent shaped pollens in equatorial view while *C. murale* have prolate to apple-shaped to oblate-spheroidal shapes. According to the findings of Pinar and Inceoglu (1998), the *Chenopodium murale* L. pollen grains have radial symmetrical, isopolar, pentoporate, spheroidal.

The equatorial diameter, P/E ratio and exine thickness in both *Chenopodium album* and *C. murale* were 10.87 μm and 17 μm , 1.298 and 0.830, 1.2 μm and 0.5 μm respectively.

Pollen grains of 12 species of the genus *Chenopodium* L. (*Chenopodiaceae*), of which morphological separation is problematical, have been examined in detail by Pinar and Inceoglu (1998). According to them pollen grains of *Chenopodium* L. species examined are radially symmetrical, isopolar, pantaporate and spheroidal. Their exine structure is similar. In the genus, five pollen types have been defined, mainly on the basis of pollen size. *Chenopodium murale* L. and *Chenopodium album* L. both have just the same characters except its diameter.

In this study the species were also differentiated on the basis of some diagnostic anatomical characters. As in case of *Lathyrus aphaca* the epidermal cells were wavy on both sides of the leaf. In *Vicia faba* on abaxial side are smooth, longitudinal and linear and on adaxial side it's wavy. The stomata types were diacytic in *Lathyrus aphaca* and in *Vicia faba* it was amphianisocytic. MacDonald *et al.* (2000), described the epidermal morphology and the structure and development of stomata in 10 species of *Fabaceae*. According to them the epidermal cells varied from irregular to straight-walled and in some taxa sinuous patterns were observed. The leaves are hypostomatic. Anomocytic stomata follow an aogenous ontogenetic pathway, while paracytic stomata are eumesogenously derived. These stomatal features are considered to be of systematic value in delimiting hardwood species in this family. The *Tribulus terrestris* was as: polygonal and isodiametric walls, unicellular and non-glandular trichomes. The species of the same genus were also distinguishable on the basis of leaf epidermal characters e.g. The *Chenopodium album* has polygonal walls while *C. murale* have polygonal to nearly undulating walls.

CONCLUSION

Hence it is concluded that the detail taxonomic studies are the basic tools by which we not only differentiate the various weed species but can also placed them at their proper positions.

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