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SURFACE MICROMORPHOLOGY OF *MILLINGTONIA HORTENSIS* LINN. f. CULTIVATED IN DUBAI, UAE

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ABSTRACT

Various morphological components of *Millingtonia hortensis* Linn. f. (cultivated in Dubai as road-side ornament) were studied for their surface micromorphology. The surface of this plant presented three important structures – much distributed trichomes on the surface of all organs of this plant, stomata present on leaf, pedicel, petals and fruit and tracheoidal system making the wing surface. The leaf was characterized with peculiar cuticular striation running parallel to each other and occasionally twined as the rope. Striations were generally confined within the perimeter of an epidermal cell but sometimes passing over several cells. Thirteen types of glandular and non-glandular trichomes are described *in toto* in this study – many of which were already described from this species but some of the trichomes were probably not observed in earlier studies e.g., 1) Branched moniliform trichomes on petals imparting velvet touch to the petal, 2) Multicellular uniseriate conical and stiff NGT with curved apical cell on peduncle, 3) Unicellular soft non-glandular trichome on petal, 4) Glandular trichomes present in pit and attached laterally with epidermis of the leaf and 5) Club shaped glandular trichomes on pedicel. Stomata were anomocytic type, raised above epidermis and with well-defined rim around. Guard cells outer ledges were very prominent. Contiguous stomata were frequent. Occasionally, triplet stomata were also present. Seed wing was thin and papery and composed of tracheoids running all over the seed surface in a fan like manner. Seed wing was porous with air spaces of varying sizes, well-suited for dispersal of seeds. The large air spaces showed tracheoidal ingrowths forming knot-like structures of various shapes and sizes, possibly for mechanical support to the wing. The findings are discussed in the light of available literature.

Key Words: *Millingtonia hortensis* Linn. f., surface micromorphology, trichomes, stomatal type, seed wing tracheoids.

INTRODUCTION

Millingtonia hortensis Linn. f (Family Bignoniaceae with 120 genera and c 800 spp.), Synonymy- *Bignonia hortensis* (L. f.) Oken., *B. suberosa* Roxb., *B. azedarachta* König & Sims, *B. cicutaria* K.D. Koenig ex Mart., *Nevrilis suberosa* Raf. Nom. Ileg.), known as Cork tree, Akash Neem, Neem Chameli and Tree Jasmine, is a useful as a garden ornament and medicinal plant. Its generic name comes from Thomas Millington, an English Botanist while hortensis means "grown in gardens" (Kiewchaum *et al.,* 2008). Tree is never naked of leaves and fruits. It is native to Southeast Asia. It is reported to have high air-pollution tolerance (Girish *et al.,* 201, Uka *et al.,* 2017). *M. hortensis* has anthelmintic activity (Chumbhale *et al.,* 2016). Aq. alcoholic extract of leaf showed anti-microbial activity against gram negative *Escherichia coli* and *Salmonella typhimurium* (MlC: 25 µg / mL.) (Jetty and Iyengar, 2000). Glycosidal alkaloid, millingtonine, has been reported from *M. hortensis* (Hase *et al.,* 1996). The plant has broad spectrum antimicrobial activity (Sittiwet, 2009). It plant is rated as antifungal, antibacterial, larvicidal (against *Aedes aegypti* L.), antioxidant, antiproliferative, antimutagenic, antihelmintic and hepatoprotective (Kumari and Sharma, 2013; Thongpoon and Poolrasert, 2015). Leaves sometimes used as substitute of tobacco in cigarettes. Leaves are low arrester of dust.

It grows in several types of soils. It is cultivated in Sindh and Punjab provinces of Pakistan (Stewart, 1972). In Pakistan (Karachi) (www.eflora.org) and in some parts of India (Agra, Delhi, Indo-gangetic plain and Pune) (Natesh, ND), it is reported to produce no or very few fruits and seeds. In Dubai, several plants of Cork tree have been cultivated along the roadside at Oud Metha - near Pakistan Education Academy. Flowering phenology in *M. hortensis* is annual (*Cornucopia* type; Gentry, 1974) occurring for several weeks and producing large number of flowers each day. Dubai trees were seen flowering and fruiting copiously in winter months. Normal fruiting and seed-setting is reported to take place, in some locations of India like Bengaluru, Chennai, Hyderabad, Jammu, Kolkata and Panaji. Such location-specific characteristics of certain Bignoniaceae are considered to be environmentally related (Chauhan *et al.,* 1987; Heslop-Harrison and Shivanna, 1977). Pollination is by a variety of insects and sunbirds (in India). Temperature and relative humidity somehow mediate molecular, physiological and morphological changes in plants. The availability of vegetative and reproductive materials of the plant in Dubai during recent visit led us to study them in botanical interest. The results obtained on surface micromorphology structures (trichomes, stomata and seed wing tracheoids) on various components of the plant are reported here which are based on the material collected from the trees of Oud Metha, Dubai (Fig. 1).

Climatic features of Dubai

UAE is located in Middle East, situated on Arabia Peninsula between Oman and Saudi Arabia bordering the Gulf of Oman and the Persian Gulf. It covers an area of 83,600 Sq. km. Its largest city is Dubai, landscape of which is sandy – extreme hot. Days are sunny all the year around. Humidity is discomfortingly high in coastal region. According to Köppen classification, its climate is of Bwh type (Tropical desert climate) (Köppen and Geiger, 1954)

and bioclimate as given by Holdridge (1947) falls into the category of Tropical Desert Bush formation. Brief description of climate of Dubai is given in Khan and Ismail (2019).

Fig.1. *Millingtonia hortensis* (in bloom) growing near Pakistan Education Academy, Oud Metha, Dubai. Image: November 2016.

MATERIALS AND METHODS

The plant material (leaves, flowers, fruits, seeds etc.) of *Millingtonia hortensis* was collected from the trees growing near Pakistan Education Academy, Oud Metha, Dubai in November 2016 and again in December 2018. To study surface micromorphology of leaf, fruit and seeds epidermal impressions were made with clear nail polish (Wang *et al.,* 2006) and studied under compound optical microscope. Stomatal nomenclature suggested by Prabhakar (2004) being simple and based upon structure of stomata and not their ontogenetic pathways was adopted to ascertain stomatal types. This nomenclature does not recognize actinocytic and stephanocytic stomata and categorize them as anomocytic type. As a basic criterion, all the cells abutting the guard cells are considered distinct by Prabhakar (2004) from the other epidermal cells by virtue of their position (i.e. abutting nature to the guard cells) hence he prefers to call them subsidiaries. Length and width of stomatal (including peristomatal rim) was measured in μm with calibrated micrometer. For scanning electron microscopy (SEM), un-imbibed (air-dried) plant material was mounted on brass stubs and coated with a 250 A gold layer with JFC-1500 gold coater. SE micrographs were made at 15kV with JEOL JSM-6380A electron microscope at various magnifications. The data was analyzed statistically (Zar, 2010).

RESULTS AND DISCUSSION

Phytography: *M. hortensis* is an ornanetal evergreen tree with brownish pyramidal stem which may break under strong wind gusts. Branches drooping. Leaves are large, opposite, exstipulate, imparipinnate, bipinnate, dorsiventral and ornamental. Leaves are green dorsally but less green ventrally. Petiole and rachis with longitudinal groove on dorsal surface. Young leaves shiny with raised midrib on ventral side (Fig. 2A). Leaf area measures around 0.0052112 sq. m. per leaf (Naik *et al*., 2006). Long leaf bears widely spaced pinnae, each with 3-5 (-7) leaflets. Terminal leaflets are larger than the laterals. Leaflets oval, pointed, slightly toothed and large. The leaves are slightly bitter in taste and odourless. Petiole's upper part somewhat flat and the lower part semicircular. Midrib prominent - slightly concave on the upper side and semi-circular on the lower side. The leaflet venation is brachidodromous (Fig. 2C). Flowers pendulous in panicles. Flowers are infundibuliform, showy, bisexual, hypogynous, zygomorphic, bell-like, opening in night and are pleasantly fragrant. Calyx campanulate, 5-lobed. Corolla tube: 6.5 – 8.0 cm and width: 3.2-4.6 cm. Five-lobed corolla in two lips (upper lip with 2 petals, lower lip with 3 petals), gamopetalous, white and petals are waxy which ensure their freshness for quite some time. Stamens are four in number, epipetalous and each anther is dithecous. Ovary bicarpellary, syncarpous, superior with axile placentation. Fruiting November to February. Fruit is a capsule - long, c. 40 cm at maturity - tapering at both ends. It is smooth flat septicidal capsule and partitioned lengthwise. Seeds broad, winged and viability of seeds short-lived. Seeds should be sown immediately as fruits ripen. The plant is, therefore, propagated through cuttings. *M. hortensis* flowers from November to February in Dubai. In Bangkok it is reported to flower in December to March (April) (Kiewchaum *et al.*, 2008). Fruit is long, pendulous and green in colour when young and brown when mature. The young fruits are much moisture-laden. Seeds are broad, flat, thin, laterally winged and arranged in partially

overlapping manner on both sides of septum in the fruit. Seeds viability is short. The plant is propagated through cuttings.

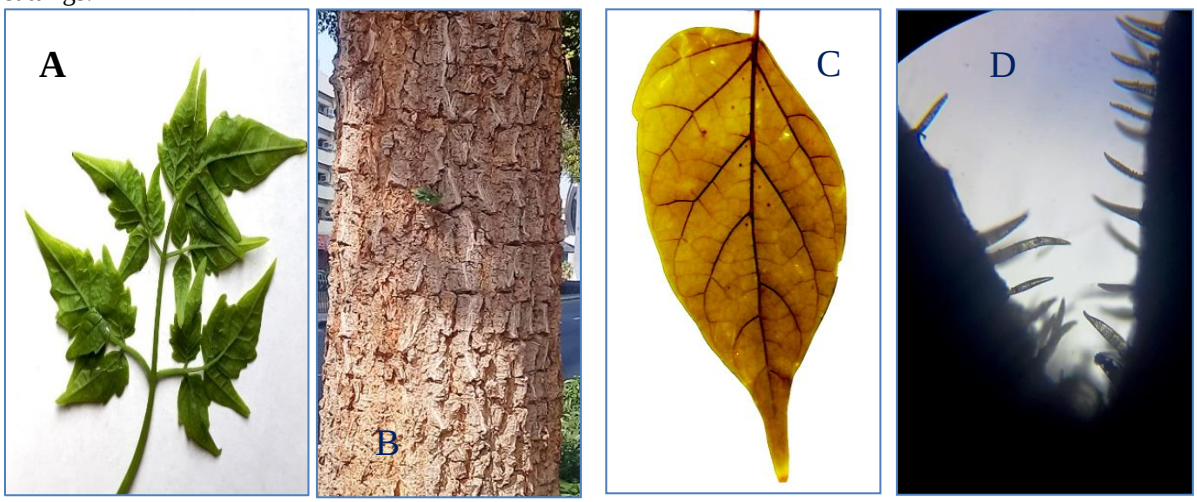


Fig.2. (A) a part of the young leaf (Ventral surface) – veins are raised above the ground surface. Bark of stem (B) - image taken from 100 cm away from the trunk). C) Venation in a NaOH treated leaflet – Brachidodromous type. D) Conical trichomes on the surface of rachis and rachilla.

Stem and Bark: The stem of *M. hortensis* is woody, columnar reaching to around 10m. The bark of *M. hortensis* stem is yellowish brown in colour, thick and corky longitudinally fissured and transversely cracked giving an impression of broken horizontal ridges (Fig. 2B). It has characteristic odour. This is used as a substitute for cork (Sharma, 1993) but inferior to true cork (http://www.terraforma.ae). The cork of *M. hortensis* is also rated of inferior quality (Natesh, ND). Stem DBH influences the bark crop and structure. Bark shows poor larvicidal activity against mosquitoes (Kumar and Karimani, 2014). Aqueous extract of the bark is anti-inflammatory (Kumar *et al.*, 2013).

Surface micromorphology

Various morphological components of foliage of *M. hortensis* (leaf, peduncle, pedicel, flowers, young and mature fruits, and seeds) were studied for their surface micromorphology. Leaves appeared to be superficially smooth to necked eyes but they were found studded with microscopic trichomes. Three important structures observed on the surface of this plant were much distributed trichomes on the surface of the plant, stomata present on leaf, pedicel, petals and fruit and tracheoids of wing surface. The leaf was characterized with peculiar cuticular striations running parallel to each other. Striations were generally confined within the perimeter of an epidermal cell but sometimes passing over several cells (Fig. 3) particularly around large stomata (Fig. 9A). Striate sculpturing was sometimes twined as in rope (Fig. 11C). Cuticular striations of *M. hortensis* resembled to that in *Melastoma sanguineum* (Haron *et al.*, 2015). Striae were also present in other Bignoniaceae species e.g., on adaxial surface of *Oroxylum indicum* and on abaxial surface of *Spathodea campanulata* (Ugbabe and Ayodale, 2008). The epidermal cells in *M. hortensis* were small in size but varying considerably being sometimes more or less isodiameteric (27.2 x 25.6 um) and sometimes roughly rectangular (c 42 x 22 µm) and irregular in shape, lobed and somewhat papillose. The cells were wavy in contour with U-shaped undulations (Fig. 3B). Avery (1933) related such undulations to the developmental stresses of the leaf differentiation. Watson (1942) reiterated that waviness may be due hardening of the differentiated cuticle. The wavy contours in epidermal pavement cells are considered to be of biomechanical benefits (Jacques *et al.*, 2014; Sapala *et al.*, 2018) and their formation to be regulated by sub cellular cytoskeleton organization of microtubules, cellulose micro fibrils and actin (Panteris *et al.*, 1994; Jacques *et al.*, 2014; Sapala *et al.*, 2018). They are affected by environmental conditions during development. In case of petals the anticlinal walls of epidermal cells, were, however, straight (Fig. 23 A and B). Epidermal cells in Bignoniaceae species in Nigeria are reported to be polygonal irregular on both surfaces of leaf. Anticlinal wall may be straight, curved or undulate (Ugbabe and Ayodale, 2008).

Trichomes: Both glandular trichomes (GTs) and non-glandular trichomes (NGTs) were observed. GTs had two parts - the basal stalk cell and head. Head consists of several thin-walled cells. GTs maximally admeasured around 50µm in diameter; the glands are embedded on both dorsal and ventral surfaces of the leaf in shallow pits in epidermis - a position slightly lower than the level of the epidermis. Eglandular trichomes were unicellular conical (broad at the base and pointed terminal cell) trichomes present – more on midrib and the veinlets than on the interveinal islands (Fig. 4) and leaf margin (Fig. 5A). Such trichomes were present on rachis and rachilla as well

(Fig. 2D). These non-glandular trichomes on rachis and rachilla averaged to 107.43 ± 11.67 µm (N = 15, CV: 42.05%). The basal cell of the trichomes was broad and more or less rectangular (Fig. 4B). A brief description of various types of trichomes observed in *M. hortensis* is presented as follows.

1. Disk-shaped, peltate, shortly stalked trichomes (8-celled and 12-celled head) were abundant on leaf (Fig. 10 A, B, and C), peduncle (Fig. 13, 17), filament (Fig. 14), floral bud (Fig. 21C, 22), petal (Fig. 19), and fruit (Fig. 25B, 26A).
2. Large peltate glands with head composed of numerous cells were found on fruit (Fig. 26B, 27B, 28).
3. Peltate gland present on leaf connected with epidermis through lateral stalk (Fig. 12).
4. Capitate glands with few-celled head present on leaf (Fig. 11 B and C) and petal (Fig. 19).
5. Conical NGTs stiff and bristly trichomes on leaf midrib, margins and less frequently on lamina (Fig. 4 A and B; 5) and young rachis and rachilla (Fig. 2D) - Sometimes leaving scar on epidermis on breakage (Fig. 9B), on peduncle (Fig. 13, 17), pedicel (Fig. 14B), corolla margins (Fig. 19).
6. Shortly stalked GTs with multicellular head were present on seed wing and the embryo region (Fig. 31).
7. Two celled NGTs with narrow and bent apical cell present on peduncle (Fig. 18A).
8. Club-shaped NGT on pedicel (Fig. 14B).
9. Multicellular, uniseriate conical and tough trichome with curved apical cell on peduncle (Fig. 17B).
10. Unicellular soft NGT on petal (Fig. 20 E).
11. Branched but soft moniliform NGT on petal's inner surface and margins (Fig. 20 B, F, and D).
12. GTs with 2-celled apex and broad and short stalk were observed on calyx (Fig. 21 A).
13. A club-shaped GT with multicellular stalk on calyx (Fig. 21B).

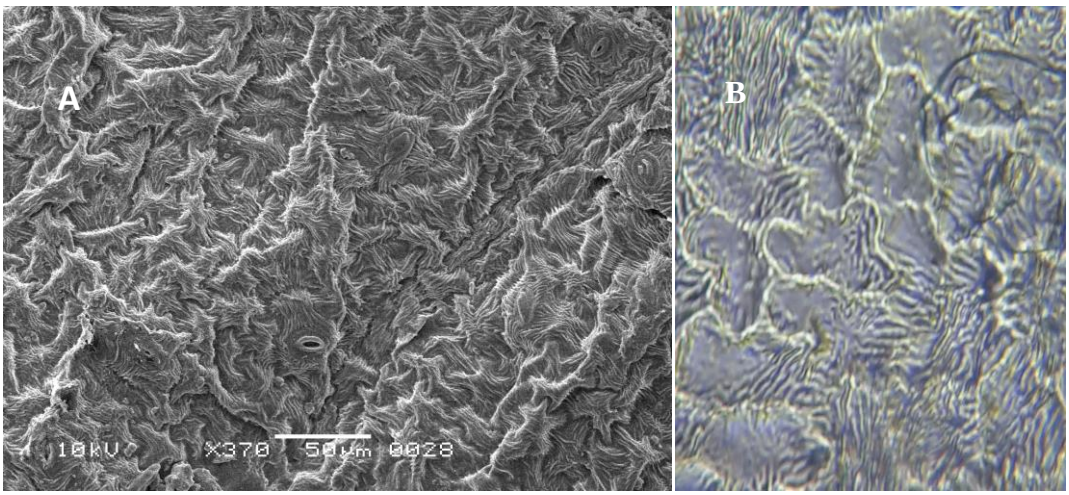


Fig. 3. A) Foliar dorsal surface showing some stomata and the peculiar cuticular striations. The striae remain generally restricted to a cell but at times striae from one cell move over adjacent cell. B) Dorsal surface of a leaf treated with NaOH and lactic acid. The epidermal cells are irregular in shape and wavy on the contour (U-shaped) (B). Mag. 40 x 10X, zoom 4X).

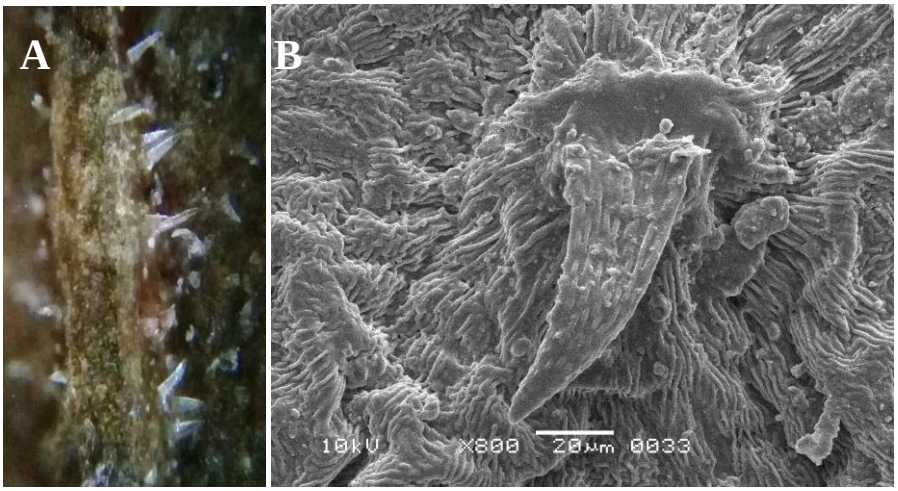


Fig. 4. Foliar trichomes: A, Trichomes on midrib of the ventral surface of leaf (B) as viewed in nail polish imprint at 45 x 10 X.; B, Conical trichome (c 80 um in length) with cuticular striations similar to those present on the epidermal cells (SEM Magnification: 800 X).

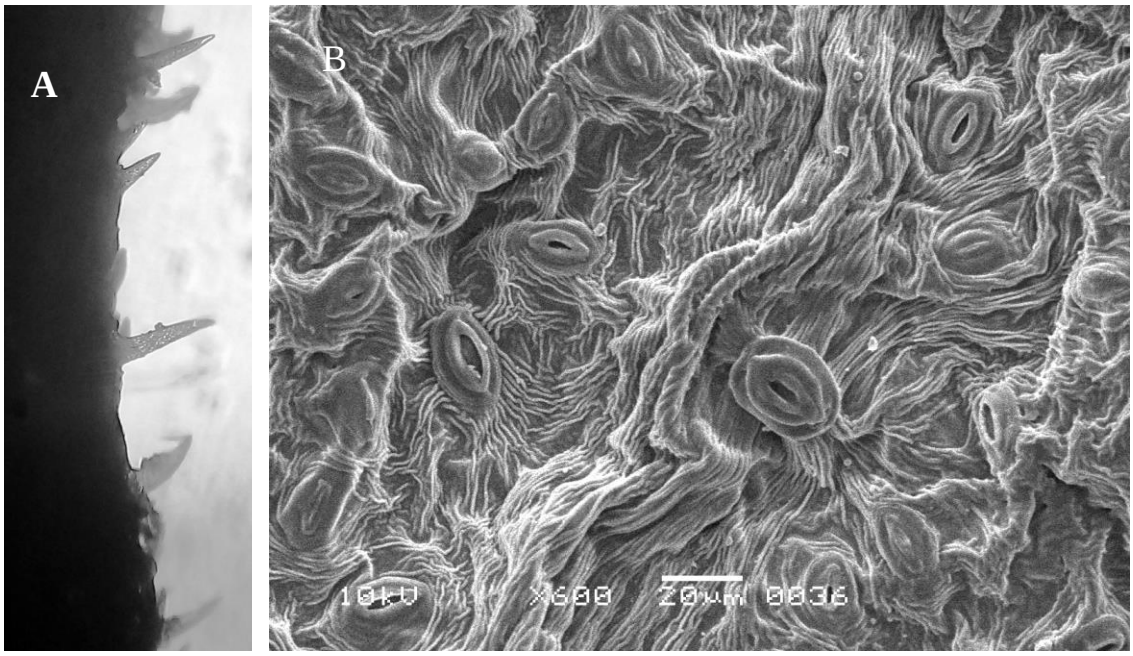


Fig. 5. Trichomes on the leaf margin (A) and Ventral surface of leaf showing several stomata with characteristic pattern of cuticular striations (B). The arrangement of subsidiaries appears to be anomocytic.

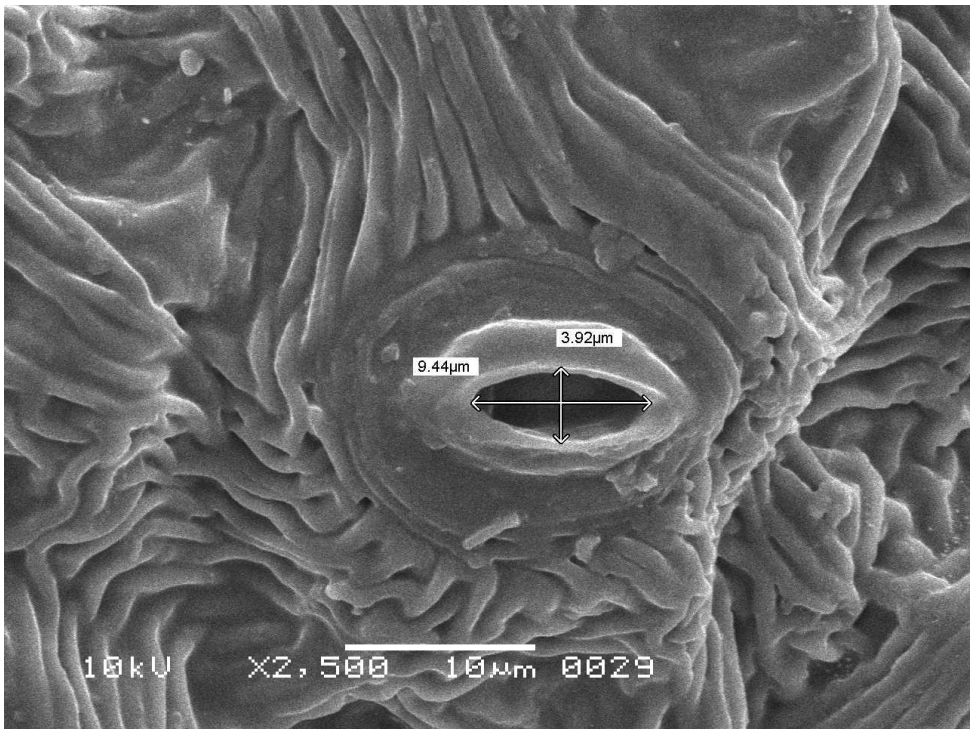


Fig. 6. Stoma on the ventral surface of leaf. Peristomal pavement is mostly devoid of cuticular striations which radiate away and run parallel to each other. The outer ledges of guard cells are raised above the pavement and stomatal pore is small (9.44 x 3.92 um in size). Stoma anomocytic.

According to Muravnik *et al.* (2019), the surface of leaves and flowers of *M. hortensis* are covered with the glandular trichomes (GTs) of three types. Most common amongst them are peltate trichomes as also observed in the present study. They are situated on the peduncles, calyx, petal, ovary and leaves. GTs were of three kinds. Type 1) – They are short stalked and have head composed of 12-16 cells. Type 2) – Capitate trichomes are short but wide stalked. They are 2-4 celled. They were reported from on corolla tube. Type 3) –These trichomes are immersed in pits. They are present on petals. (We found many trichomes situated in pits on leaf dorsal and ventral surfaces (Fig. 11 D, E, F & 12). A trichome with 2-celled apex with short stalk was observed on fresh calyx (Fig. 21A).

NGTs seen on the epidermis are stiff and unicellular; the terminal cell of the trichome was conically pointed whereas the basal cells broad and rectangular. On leaves glands are more in number on the edges of the veins. Advanced form of glands is yellowish brown owing to the secretion by them.

Peltate glands on mature leaf were more in number on the dorsal surface (9.24 ± 0.97 per mm^2) than ventral surface (5.41± 0.69 per mm^2). The size of peltate gland was, however, comparable on the two surfaces (40.05 ±0.97 and 39.96 ±0.87 µm, respectively (Table 1). They were maximally 48.6 µm wide. Glandular trichomes with a single basal stalk cells and 16-celled head are reported by Mageswari *et al.* (2017) to be up to 50µm wide.

Both ovary and style were studded with GTs. The peltate glands on fully mature brown fruits of 40 x 2.2 x 0.8 cm in size averaged to 24.57 ± 1.77 (N = 60; varying from zero to 58.97 per mm^2). Glandular density on fruit, however, varied with various developmental stages of the fruit. Glandular density varied with various developmental stages of the fruit. Larger green fruit (39 x1.4 x 0.4 cm in size) had lesser number of glands (26.21 per mm^2) as compared to the young fruit of 17.4 x 0.9 x 0.3 cm in size (49.64 ± 2.81 per mm^2) (Table 2). Young fruit had density of glands much higher comparatively (162.99 ± 6.46 glands per mm^2). This pattern of glands density with age may presumably be attributed to the expansion of growing fruit with time. However, several factors (light and temperature regimes) moisture availability and soil conditions affect the development of trichomes and their expression (Shanower, 2008).

Glandular trichomes are known to morphologically characterize the Fam. Bignoniaceae. They represent a diversity of forms. Fröes *et al*. (2015) have reported peltate and patelliform / cupular trichomes in Marinnella obovata, *Amphilophium magnoliifolium* and *Stizophyllum riparium* and capitate and stipitate trichomes from *M. obovata*. Higher the density of trichomes in these species, higher was the number of ants attracted to the plants due to substances secreted by the trichomes. Machado *et al*. (2006) have reported peltate trichomes from the ovary of *Zeyheria montana* (Bignoniaceae) from Brazil. They are continuously present and active from early budding through flowering and fruiting set. The peltate disk-shaped, shortly stalked and phenol-secreting trichomes are also reported from young fruits and their wings of *Pterocarya rhoifolia* (Juglandaceae) grown in Russia. The polygonal heads of these trichomes are c 70 µm in diameter and 15 µm in height composed of 16-20 radially arranged cells (Muravnik and Shavarda, 2011). Peltate trichomes are characteristic on reproductive parts for several families such as Lamiaceae (*Calamintha menthifolia*) (Hanlidou *et al.*, 1991), Verbenaceae (*Lippia scaberima*) (Combrinck *et al.*, 2007), and Asteraceae - *Artemisia umbelliformis*; (Cappelletti *et al.*, 1986) and *Vernonia galamensis* (Favi *et al*., 2008).

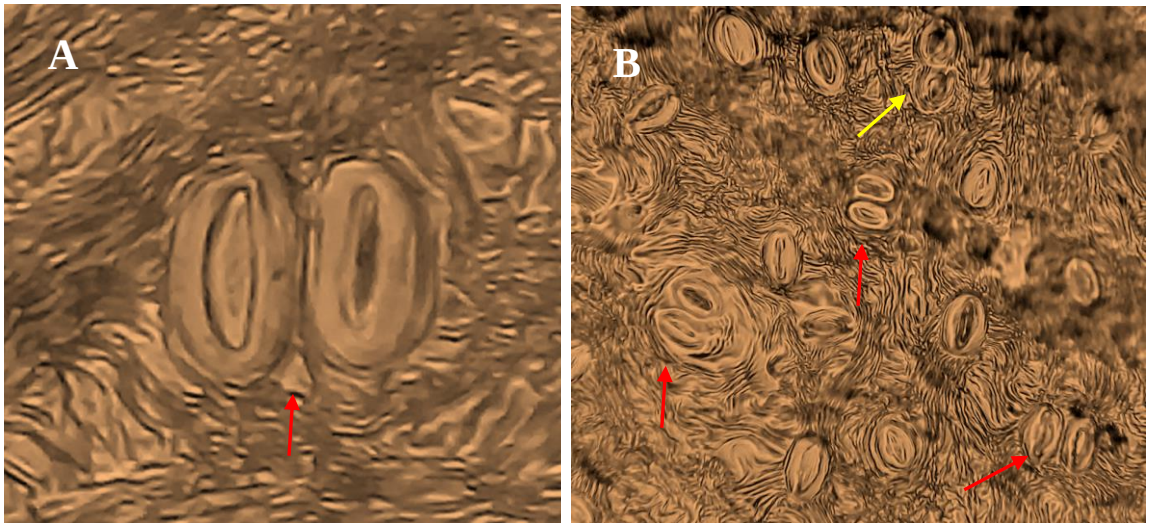


Fig. 7. Contiguous stomata in form of paired stomata without subsidiaries between them. One such juxtaposed pair on dorsal surface of leaf, 45 x15 X (A) and Four such pairs on ventral surface of leaf (B) (45 x10 X) as viewed in nail polish imprint. The contiguous stomata are generally juxtaposed (red arrows) and rarely superimposed type (yellow arrow).

Glandular trichomes are known directly or indirectly related with the protection of plant from UV radiation, drought, high salinity, heavy metals, herbivores and pathogens (Levin, 1973; Shanower, 2008; Muravnik and Shavarda, 2011; Machado *et al.*, 2006; Kim *et al.*, 2012). Advanced form of glands is yellowish brown owing to the secretion by them. They secret a variety of chemicals which are olfactory and gustatory repellent (Levin, 1973). Phenolic substances are predominant in foliar GTs where as terpenoids are present in larger degree in GTs of petal,

corolla tube and ovule. Monoterpenoids compounds viz. linalool, trans-nerolidol, alpha-farnescene and cineole are major volatile compounds that are emitted from floral tissue. (http://congresskazan2019.ofr.su).

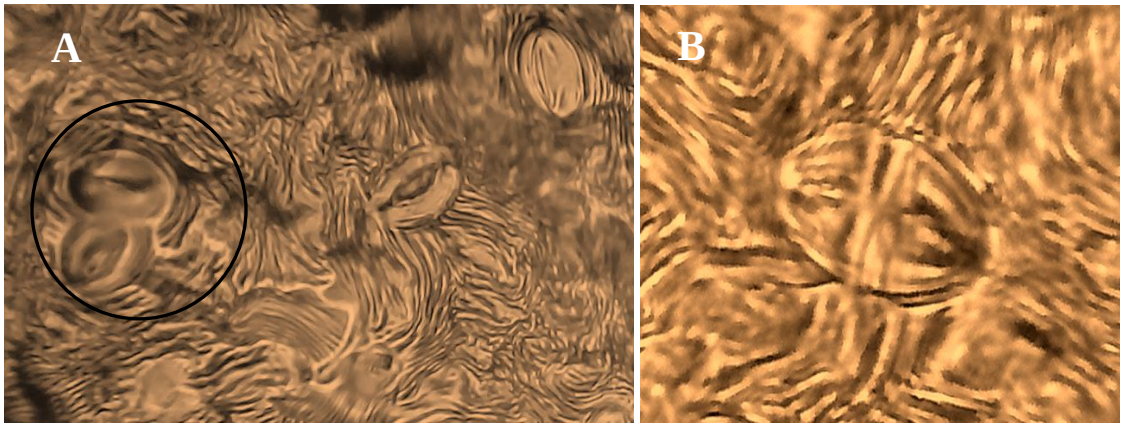


Fig. 8. Ventral surface of leaf showing contiguous stomata – One stoma lying more or less at right angle to the other (A) - such stomata are seldom unequal in size. An abnormal stoma showing its division into two stomatal segments (B).

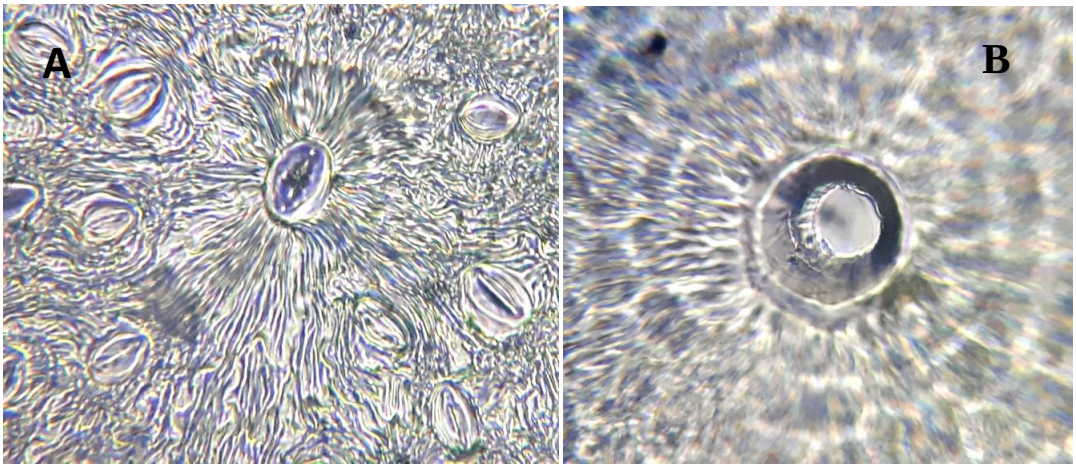


Fig. 9. A stoma with radiating striae (A) and a scar due to breaking of a trichome from the base (B) on leaf surface.

Stomata

Stomata in varying number were observed on both surfaces of leaf (Fig. 3, 5-10), pedicel (Fig. 18), petal (Fig. 23) and fruit (25B, 26 A, 27A, 28) The leaves were amphistomtatous with lesser number of stomata on dorsal surface (30.17 ± 2.12 stomata per mm^2 varying from zero to 98.29 stomata per mm^2) but numerous stomata on the ventral surface (368.96 ± 4.74 stomata per mm^2; varying from 294.87 to 491.45 stomata per mm^2) (Fig. 15 and 16). Stomatal density on dorsal surface significantly deviated from normal distribution being positively skewed and platykurtic and significant value of Shapiro-Wilk test (0.923, $p < 0.0001$). On ventral surface, the stomatal density tended to follow normal distribution (Shapiro-Wilk: 0.971, $p < 0.070$, NS). Stomatal density across eight Bignoniaceous species is 1000 – 10000 stomata per cm^2 (Wazir *et al.*, 2016). The lower surface was, however, reported to consist of only 67.5 stomata per sq.mm (Mageswari *et al.*, 2017) which is a very low quantum.

On mature fruit (40 x 2.2 x 0.8 cm) surface the stomatal density averaged to 9.99 ± 1.80 per mm^2 (N = 60, varying from zero to 39.32 per mm^2). The density of mature stomata on fruit varied with fruit size. Green but larger fruit (39 x1.4 x 0.4 cm in size) had 21.62 ± 1.598 stomata per mm^2 and smaller fruit (17.4 x 0.9 x 0.3 cm) had relatively larger stomatal density (33.42 ± 1.88 stomata per mm^2). On very young fruit of 10.6 x 0.6 x 0.2 cm in size, the stomatal density of mature stomata, due to some unknown reasons, was only slightly larger (36.22 ± 1.42 per mm^2) with quite high degree of variation around 67.72% (Table 2). They were oriented in various directions. Contiguous stomata of a pair were sometimes unequal size.

Stomata in *M. hortensis* are anomocytic- oval to wide elliptical in shape a*s also reported by* Kaushik and Saini (2008). Stomata were oriented in various directions. The outer ledges are well-developed by guard cells. Ugbabe and Ayodale (2008) investigated stomata in 11 species of Bignoniaceae. Leaflets were generally hypostomatic.

Mirkhania lutea was hypoamphistomatic. Stomata were predominantly anomocytic except *Kigella africana* which had diacytic stomata. Other types of stomata were paracytic and cyclocytic. In *Crescentia cujete*, stomata on adaxial surface were paracytic and abaxially cyclocytic. Various species of Fam. Bignoniaceae have been reported to show anomocytic stomata and glandular (patelliform / cupular) trichomes as in *Tecoma grandiflora, Campsis radicans, Catalpa speciosa, C, bignonioides. Anemopaegma album, A. scabriusculum and Bignonia aequinoctialis* (Gama *et al.,* 2013; Nogueira *et al*., 2013; Ugbabe and Ayodale, 2008; Ugbabe *et al.,* 2014; Abhimanyu *et al.,* 2016). Metcalfe and Chalk (1979) have reported two types of stomata in Bignoniaceae – anomocytic and anisocytic. Stomata are diacytic in *Kigella* (Watson and Dallwitz, 1992). Most of the Bignoniaceae species are hypostomatic. *Tecomella undulata* is, however, amphistomatic (Wazir *et al.,* 2016). The contiguous stomata (in group 2-5) are present in several families – Bignoniaceae, Fabaceae and Ranunculaceae. Also, the stomata of *M. sanguineum* had a well-defined rim around the stoma (Haron *et al.,* 2015) as found in *Millingtonia hortensis*.

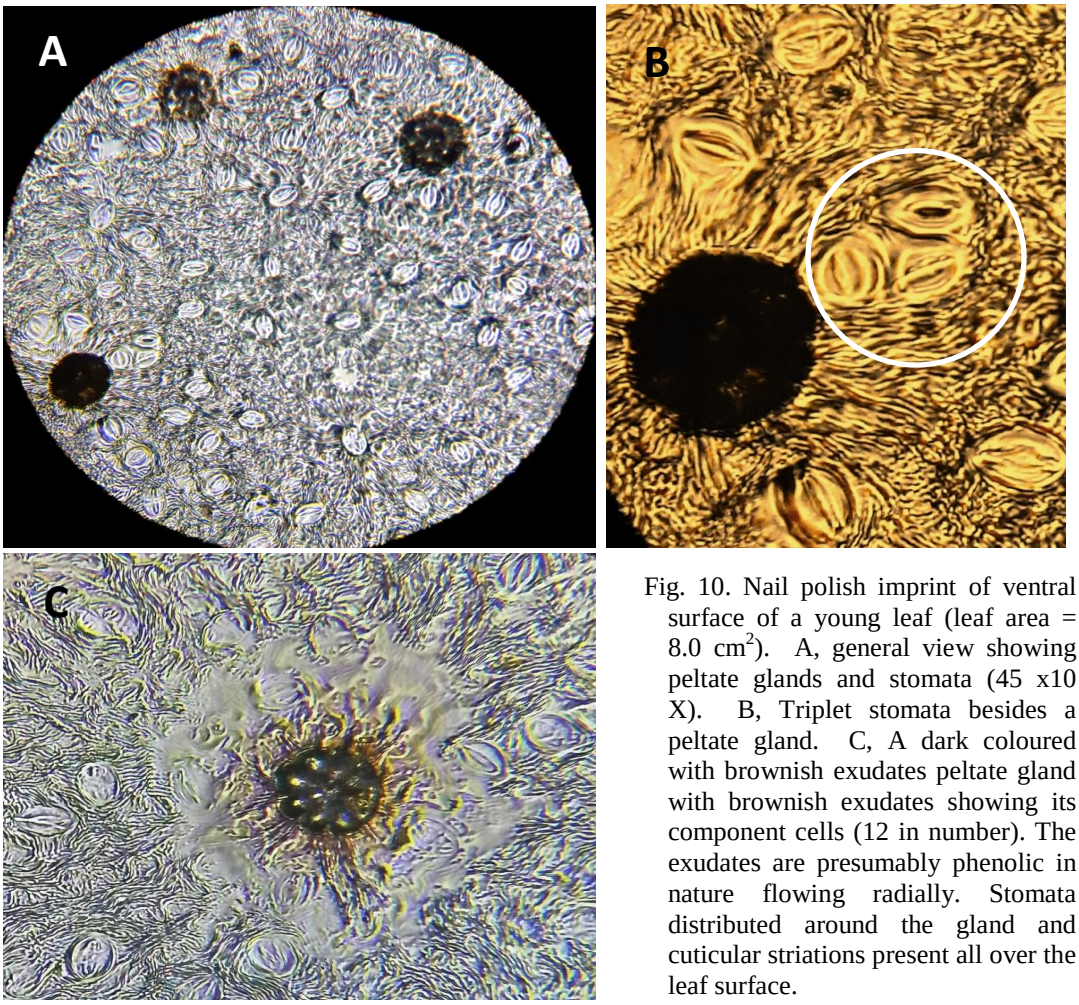


Fig. 10. Nail polish imprint of ventral surface of a young leaf (leaf area = 8.0 cm^2). A, general view showing peltate glands and stomata (45 x10 X). B, Triplet stomata besides a peltate gland. C, A dark coloured with brownish exudates peltate gland with brownish exudates showing its component cells (12 in number). The exudates are presumably phenolic in nature flowing radially. Stomata distributed around the gland and cuticular striations present all over the leaf surface.

Stomata showed sometimes its abnormal division into two stomatal segments (Fig. 8B). Contiguous stomata (Fig. 7 and 8) were frequently observed and triplet stomata (Fig. 10B) also occurred but infrequently. In a sample of 415 microscopic fields of vision, contiguous stomata occurred in around 8% of the microscopic fields of vision at 45 x 10 X magnification – the fields of vision containing contiguous stomata had in most cases one pair of contiguous stomata in a field but sometimes two or very rarely three pairs of contiguous stomata also occurred in a field of vision. Contiguous stomata of a pair were sometimes unequal size. There also occurred triplet stomata in *M. hortensis* (Fig. 10B). Besides, normal stomata in this species were seen sometimes to be arranged in groups of 3-4 (-5) stomata. Stomata in groups may also be seen in *Nigella damascana, Paeonia anomata* and *Pisum sativum* (Paliwal, 1969). Stomata may sometimes be found arranged in groups of 5 as in *Pulsatilla alabana* and sometimes in a row of 5-6 stomata (Zimmermann and Bachmann-Schwegler, 1962). Paliwal (1969) considers such occurrence of stomata in groups against the Bünning's (1952) idea of an "inhibition zone" around the meristemoids to promote regular distribution of stomata. The area of inhibition around the stomatal initials appears to be not borne out by

frequently occurring contiguous stomata in *M. hortensis* similar to the observation of Mukherji *et al.,* 2000). The frequent occurrence of contiguous stomata may presumably be attributed to the genotoxic effects of very high transport density in the Oud Metha area as suspected in *Albizia lebbeck* growing in this locality (Khan, 2020).

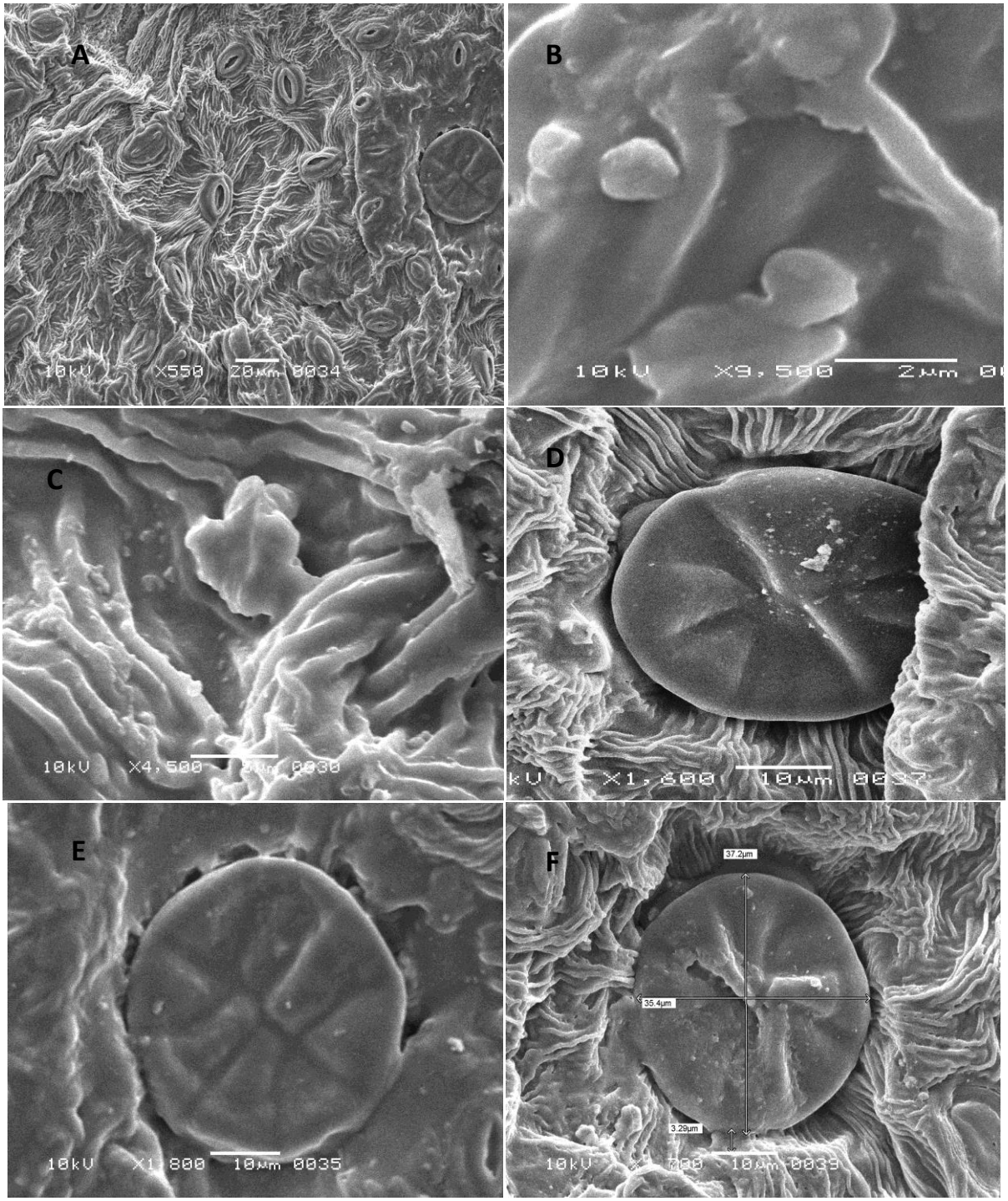


Fig. 11. A, General surface view of leaf showing stomata and the cuticular striations; B, Capitate trichomes (glands) on the leaf surface C, Some cuticular striae are twisted like cord. and stalked capitate trichome (4-celled) may also be seen besides little granular crystalloids (waxy ?) distributed over surface; D, Discoidal 8-celled peltate trichomal head placed inside pit; and stalk connected basally with the epidermis. (E) Glandular peltate trichome head connected laterally with epidermis (see Fig. 12 also). F, The peltate trichome admeasuring 35.4 and 37.2 µm in diameters at right angles.

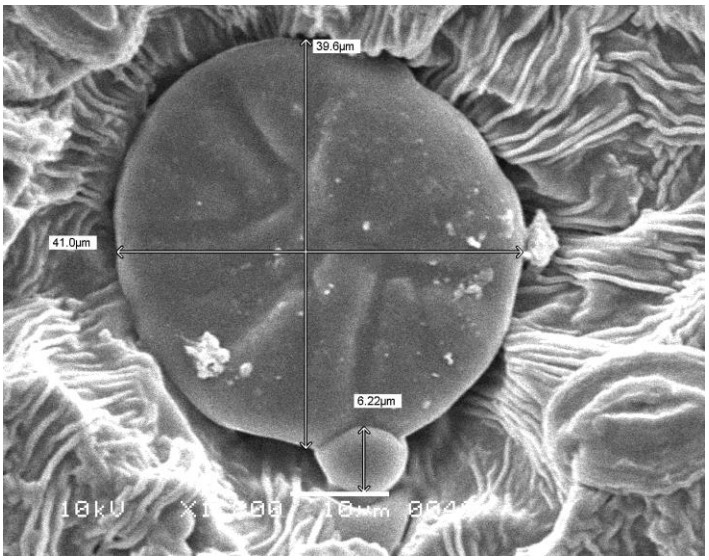


Fig. 12. Gland or glandular trichome situated in the pit or depression on the ventral surface of leaf. One-celled button-like stalk attached from lateral edge of the trichomal head to the anticlinal surface of the epidermal cell. The head of the discoidal trichome admeasured 41.0 and 39.6 um in diameter at right angle and the button like one-celled stalk 6.22 um. More or less parallel-running cuticular striations are abundantly present

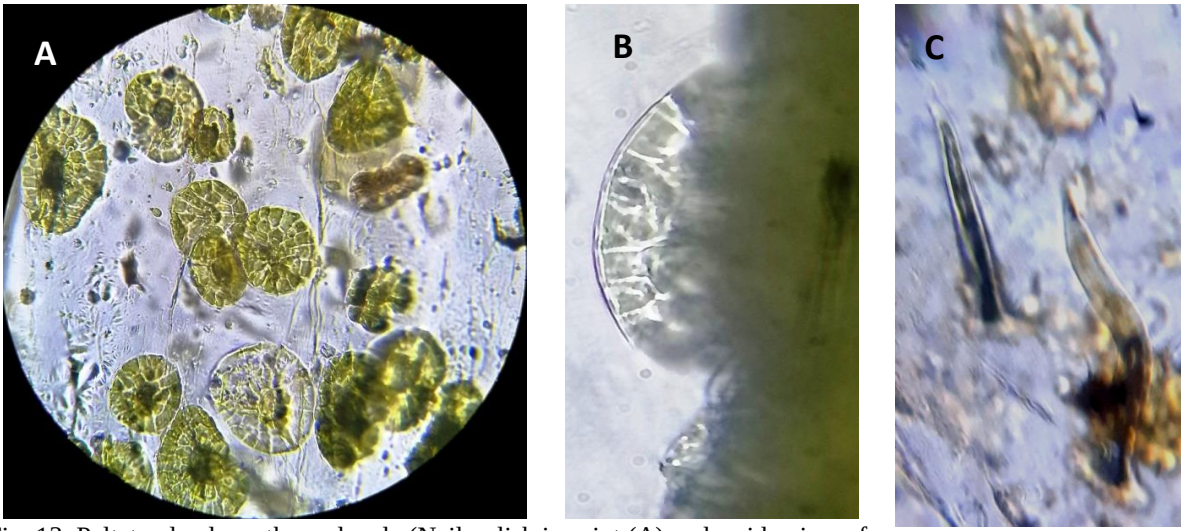


Fig. 13. Peltate glands on the peduncle (Nail polish imprint (A) and a side view of a gland (B). Conical trichomes on the peduncle (C).

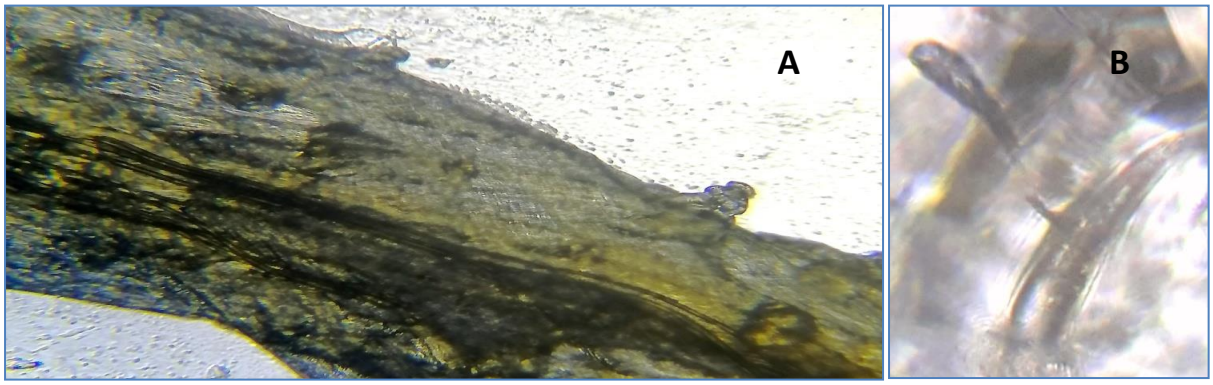


Fig. 14. Peltate Glands on the surface of filament (A) and a club shaped glandular trichome on the Pedicel (B) besides a conical non-glandular trichome.

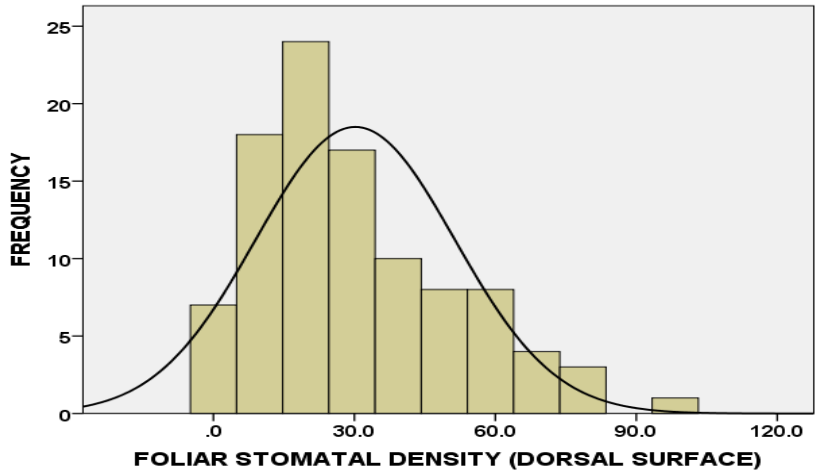


Fig.15. Distribution of stomatal density per mm^2 on dorsal surface of large mature leaflet.

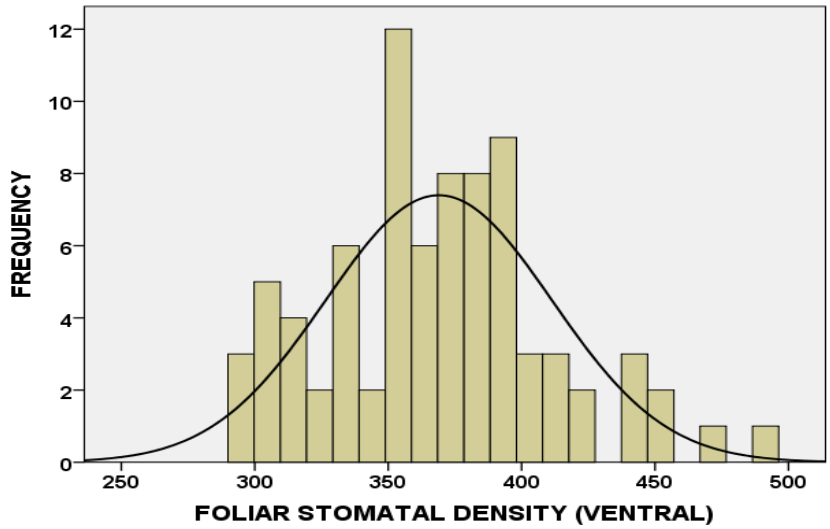


Fig. 16. Foliar stomatal density on ventral surface of large mature leaflet.

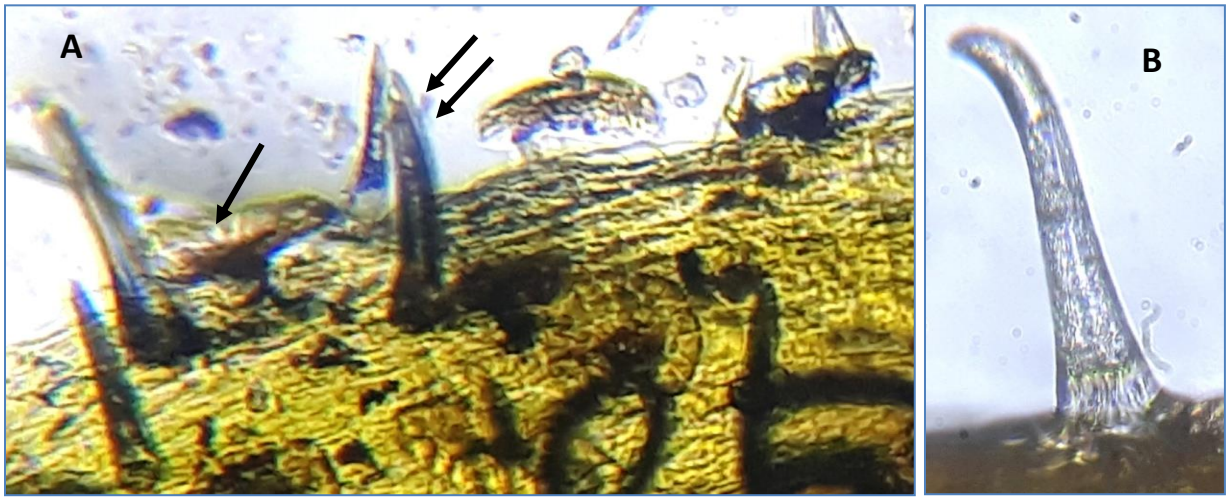


Fig. 17. A) Surface of young peduncle showing trichomes – glandular peltate gland with broad but short multicellular stalk (arrow) and conical trichomes (double arrow). The multicellular, uniseriate, conical and tough NGT having apical cell elongated and curved (B).

Stomata are generally raised above epidermis in Bignoniaceae but they are sunken in *Kigella africana*. Anticlinal walls are generally sinuous = wavy. Striation may be present or absent. It is present in *Jacranda mimosifolia. Taberbuia rosea, Tecoma stans and T. capensis* but absent in some species like *Kigella africana, Markhamia tomentosa, Stereospermum kunthianum and S. acuminatissimum* (Ugbabe *et al.,* 2014).

Stomatal size on dorsal and ventral surfaces of mature leaf were almost comparable as they varied insignificantly (mean stomatal length 24.44 and 22.17 µm, respectively. The mean stomatal width (inclusive peristomatal pavement) was 14.98 and 14.39 µm, on dorsal and ventral surface, respectively (Table 3). Mean stomatal length in some Bignonoids varied from minimum in *Markhamia lutea* (9.6 ± 1.6 µm) and maximally 18.0 ± 1.4 µm in *Kigella africana* and comparably so (18.0 µm) in *Tabebuia rosea* (Ugbabe and Ayodale, 2008) – somewhat smaller than that in *M. hortensis*. With respect to their size, however, stomata may be divided in two – larger and smaller. The larger stomata distributed randomly and were surrounded by several smaller stomata. The largest stoma recorded on ventral surface admeasured 36.8um in length and 22.4 um in width. In general, larger stomata were on an average 25.59 ± 0.42 µm in length (N = 36, CV: 10.74%) and 18.12 ± 0.64 um in width (N=36, CV: 21.06%). The smaller stomata averaged to 21.9 ± 0.38 µm in length (N= 34, CV: 6.9%) and 14.30 ± 0.40 µm (N=34, CV: 11.17%).

Table 1. Density per mm^2 and size (diameter, µm) of glands on dorsal and ventral surfaces of mature leaf.

Parameters	Gland density per mm^2		Gland diameter (µm)	
	Dorsal surface	Ventral surface	Dorsal surface	Ventral surface
N	100	80	30	30
Mean	9.239	5.4059	40.05	39.96
SE	0.9661	0.6972	0.96854	0.86957
Minimum	Zero	Zero	35.67	32.140
Maximum	39.32	19.96	48.60	48.6
CV (%)	104.56	115.26	9.12	11.19

Table 2. Trichome (glandular) and stomatal density per mm^2 in relation to fruit size.

Statistics	Fruit A (Green) Size: 39 x 1.4 x 0.4 cm	Fruit B (Green) Size: 17.4 x 0.9 x 0.3 cm	Fruit C (Green) Size: 10.60 x 0.6 x 0.2 cm
Trichome density per mm2			
Mean ± SE	26.21 ± 1.878	49.64 ± 2.814	162.99 ± 6.459
CV (%)	55.52	45.16	30.69
Mature stomatal density per mm^2			
Mean ±	21.62 ± 1.598	33.42 ± 1.884	36.218 ± 1.418*
CV (%)	57.25	43.73	67.72

Table 3. Stomatal size on dorsal and ventral surfaces of mature leaflet.

Parameters	Stomatal size (µm)			
	Dorsal surface		Ventral surface	
	Length	Width	Length	Width
N	70	70	80	80
Mean	24.948	14.987	22.166	14.965
SE	0.4793	0.2542	0.4276	0.26607
Minimum	17.82	11.34	14.58	9.72
Maximum	38.88	22.68	32.40	22.68
CV (%)	16.07	14.19	17.25	15.90

Ugbabe and Ayodale (2008) investigated stomata in 11 species of Bignoniaceae. Leaflets were generally hypostomatic. *Mirkhania lutea* was hypoamphistomatic. Stomata were predominantly anomocytic except *Kigella africana* which had diacytic stomata. Other types of stomata were paracytic and cyclocytic. In *Crescentia cujete*, stomata on adaxial surface were paracytic and abaxially cyclocytic. Striae were present but on adaxial surface of *Oroxylum indicum* and on abaxial surface of *Spathodea campanulata*.

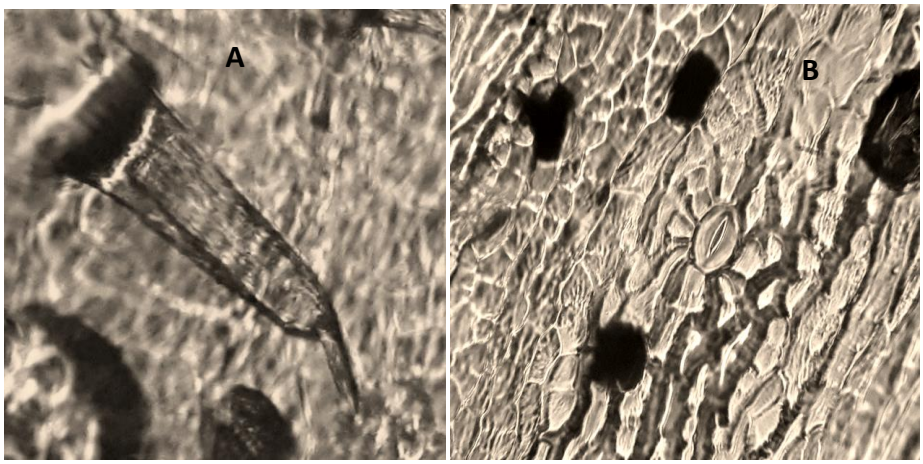


Fig.18. Trichomes on the surface of Pedicel (A)- a two-celled trichome with narrow and bent apical cell resembling stiff trichome (type i) of Mehra and Kulkarni (1989, see Fig. 32). Anomocytic stoma on pedicel (B).

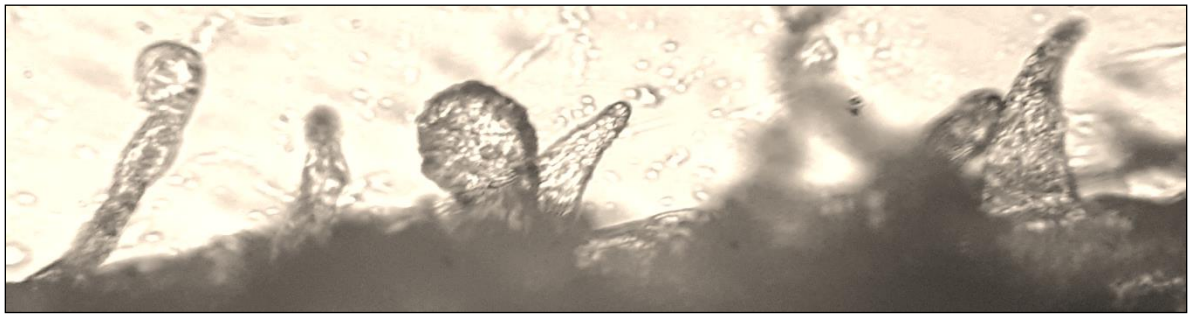


Fig. 19. Three types of trichomes on the marginal surface of petal- Conical non-glandular trichomes, soft flexible trichome and capitate stalked trichome, near margins.

Annulus tissue below ovary in *M. hortensis* contains nectaries which are reported to be anatomically similar to *Kigella pinnata* (Subramanian and Inamdar, 1985). Such discoidal nectary surrounding the base of the ovary in *M. hortensis* has been reported by Kher *et al.* (2010) to have raised type of stomata on its top rim, as is also seen in *Stenolobium stans*, another member of the Bignoniaceae.

Fruit surface micromorphology

The fruits are elongated capsules reaching to c 40 cm in length (Fig. 24A). They are green when young but brown on maturity. In Dubai, they dehisce in February/March or somewhat later by splitting longitudinally and releasing many seeds which are winged (Fig. 24B). The chocolate- brown septum dividing the fruit into two longitudinal halves is brittle. The surface cells of the septum were thick-walled, closely-fitting, polygonal and variable in shape (Fig. 25A). The anticlinal walls of these cells were straight.

The fruit surface micromorphology was more or less similar to that of leaf. Fruit surface had 8-celled and occasionally giant multicellular glands on the surface but stomata were less frequent (Fig. 25B, 26, 27, and 28) as already described in preceding pages.

Micromorphological characteristics of seed and its associated wing

Seeds in *M. hortensis* present an interesting structure. They are flat and thin and encased in thin papery wing that is derived from the seed coat as indicated by Clobert (2012) in Fam. Bignoniaceae. Seeds are released from the capsule due to dehiscing longitudinally into two halves. The wings of *M. hortensis* have numerous air- spaces of varying sizes and bounded by tracheids (a term used to describe the tracheids-like cells that may exhibit diverse ornamentations on their secondary walls). Tracheoids have variously been named by various workers as reservoir vesiformes, vascular cells, spiral cells, lignified idioblasts, mechanical cells, storage tracheids or tracheoidal idioblasts (see Rao and Das, 1979). Tracheoids of *M. hortensis* seed wings were observed to distribute over wings in fan-like manner extending from centrally-located embryo region to the wing endings on either side as also reported in Pithecocniinae, a subtribe of Bignoniaceae, by Burelo-Ramos *et al.*, 2011). The tracheoids in *M. hortensis* seeds have no ornamentation of any kind at some places and helical thickenings at the other places (Fig. 29). The tracheoids in *Campsis radicans* are also reported to radiate from the mid region of the seed to the extremities (Lersten *et al.*, 2002). The larger air spaces in wing of *M. hortensis* seeds were seen to bear tracheoidal knots of variable shape (Fig. 30) presumably they provided mechanical support to the wing. No stomata were seen on seeds.

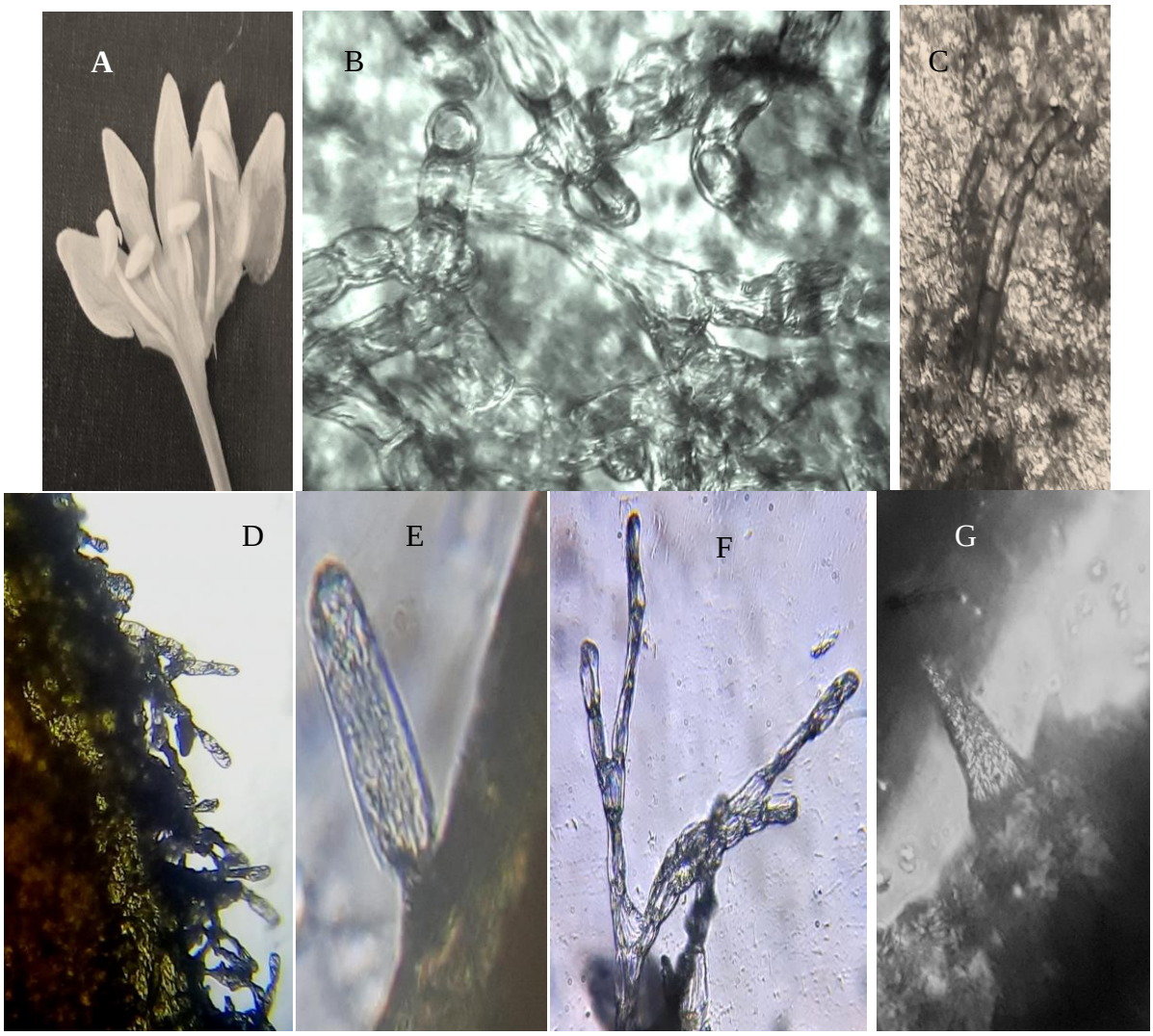


Fig. 20. A, a dissected flower – flower is white but it changes colour to pale and then to brown as it dries; B, soft flexible multicellular, soft and branched trichomes on inner side of petal; C, Unbranched multicellular trichome; D, Soft branched trichomes on the margins of petal; E, Unicellular round-headed trichome on margin of petals; F, multicellular branched trichome on margins of the petals and G, a conical trichome on the surface of a sepal (F).

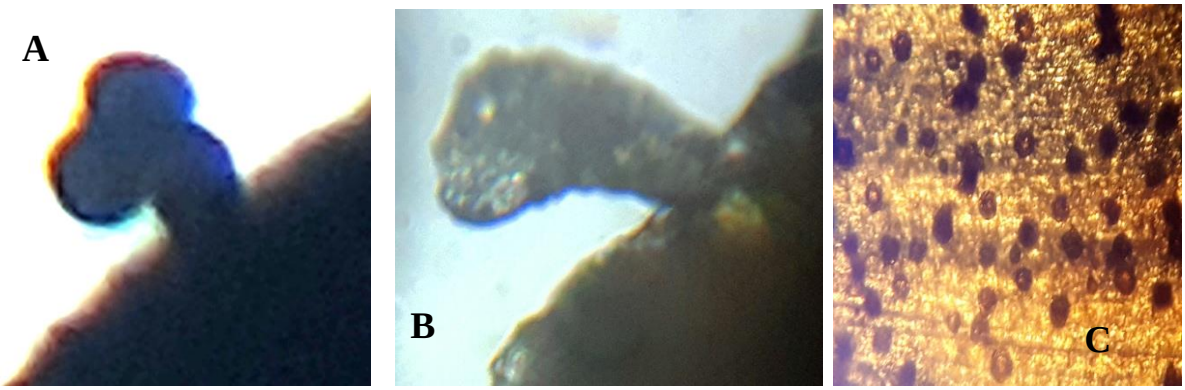


Fig. 21. Two-celled capitate GT with short and wide stalk on calyx (A) - earlier described on corolla by Muravnik *et al*. (2019). . A club-shaped GT on the calyx with multicellular stalk and multi-celled head (B) – probably type E or F GT (Fig. 32) described by Mehra and Kulkarni (1989). Dense crop of glandular peltate trichomes on the surface of floral bud (C).

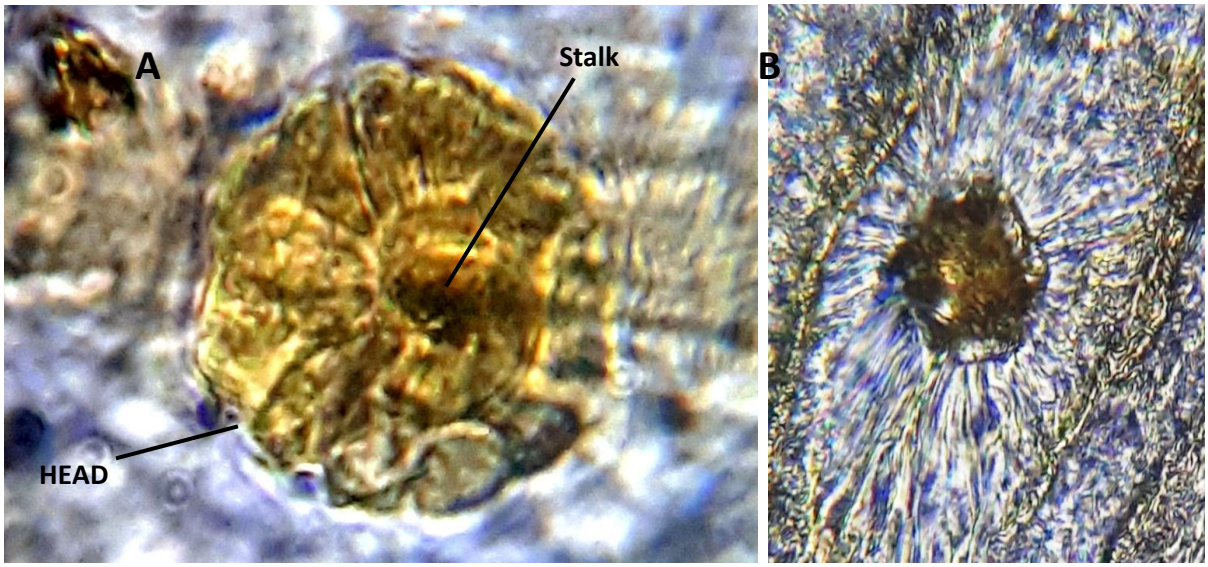


Fig. 22 A broken peltate trichome on petal inner surface showing underside - basal stalk and a multicellular head and (A) and a peltate gland in interveinal region of petal surface (B).

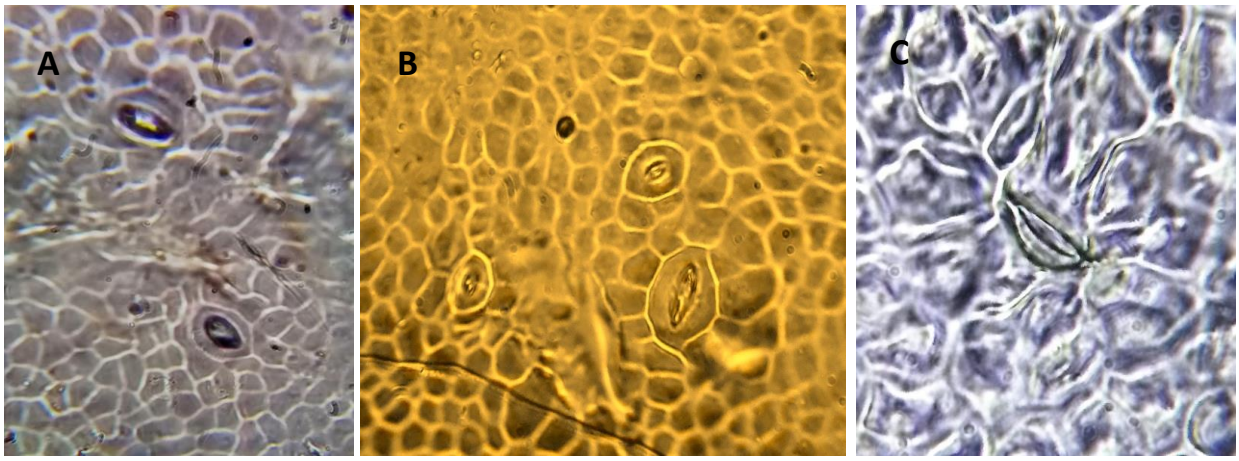


Fig. 23. Anomocytic stoma on the outer surface of petal (A and B) and inner surface of petal (C). Stomata are quite infrequent. The walls of the epidermal cells are straight.

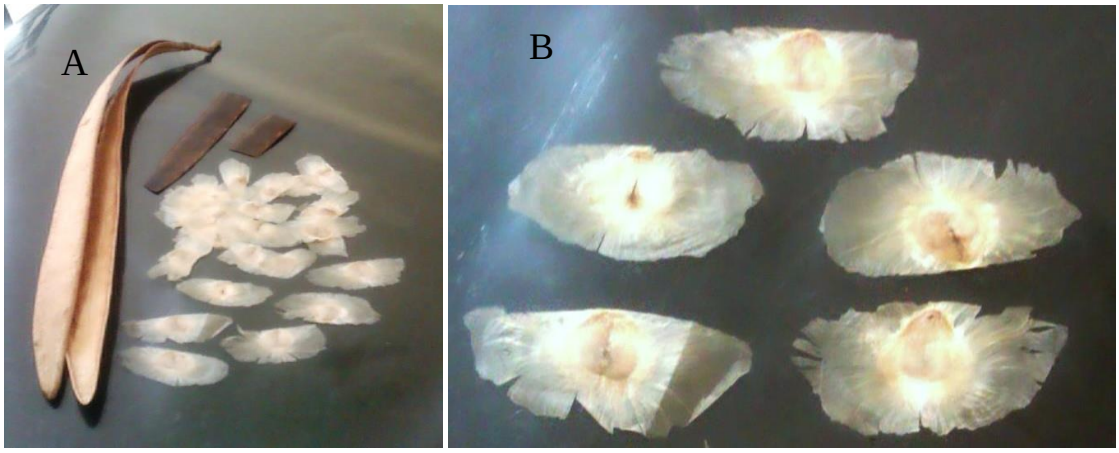


Fig. 24. Fruit opened to show chocolate brown septum and seeds (A).Close up view of the winged seeds (B). Fruits dehisce in February/ March or somewhat later by splitting longitudinally and releasing winged seeds. The fruit surface micromorphology was more or less similar to that of leaf. Fruit surface had 8-celled and occasionally giant multicellular gland on the surface but stomata less frequently.

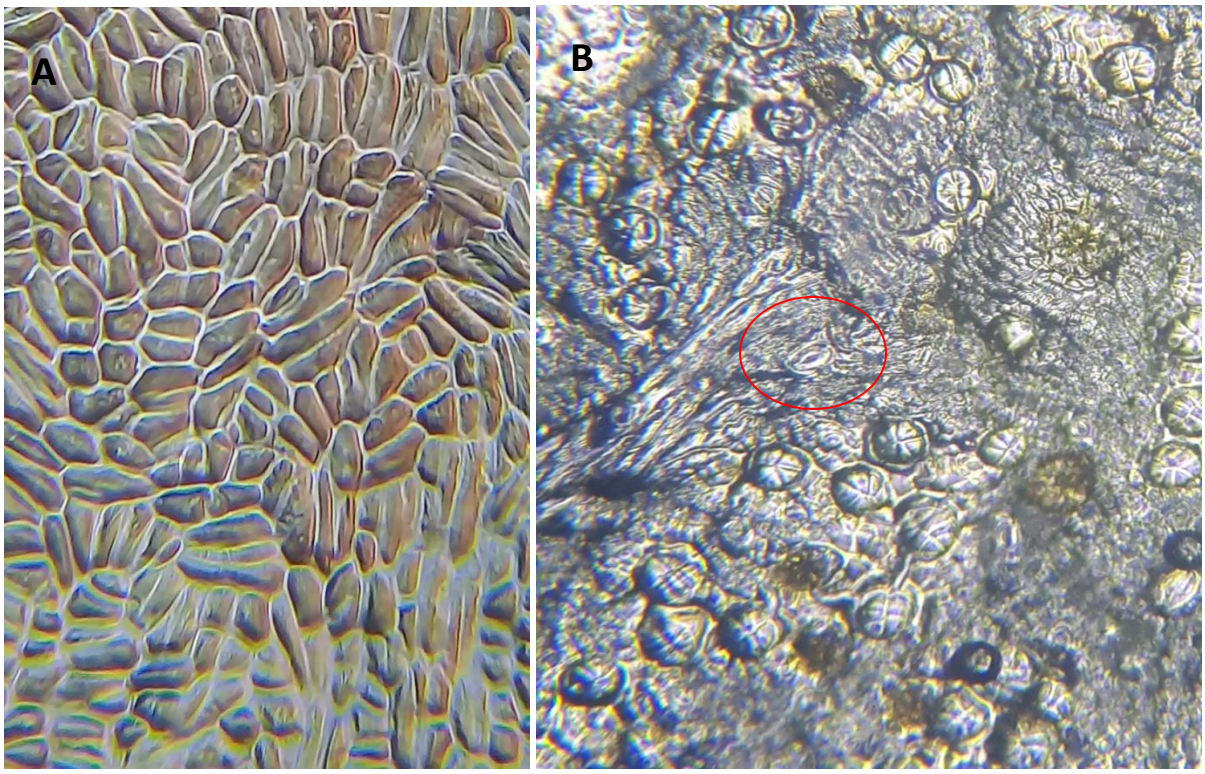


Fig. 25. A) Nail-polish imprint of surface of the chocolate-brown septum dividing fruit into two longitudinal halves. The cells in TS are thick-walled, closely-fitting and variable in shape. B) Stoma (in circle) and peltate glands, generally 8-celled on the surface of fruit.

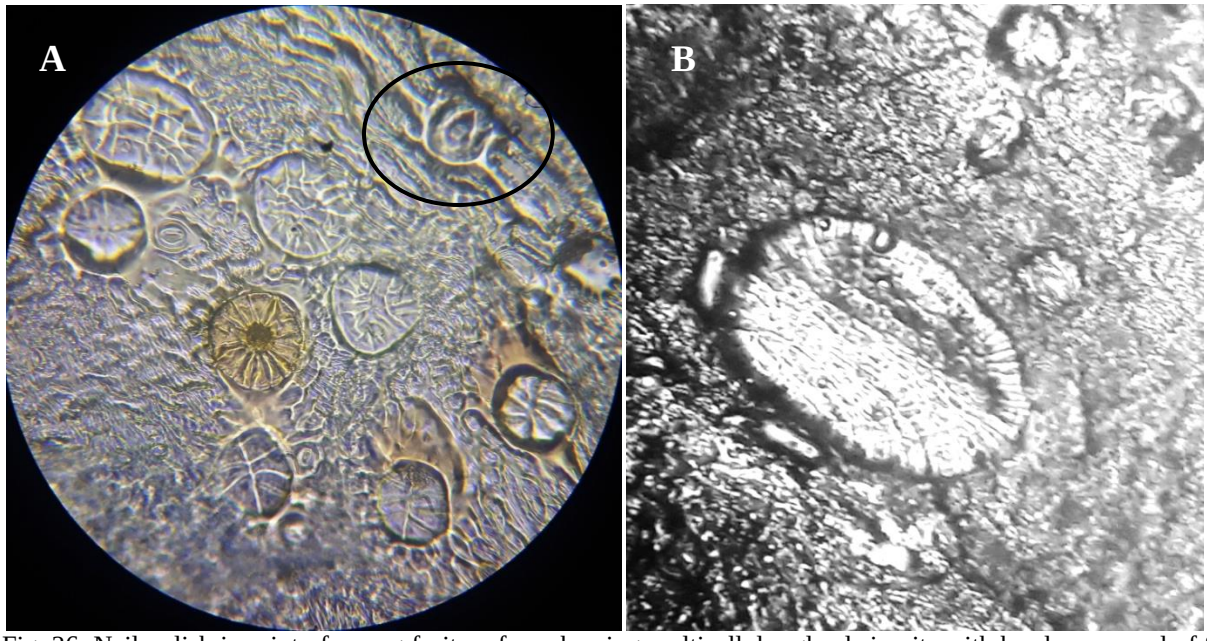


Fig. 26. Nail polish imprint of young fruit surface showing multicellular glands in pits with head composed of 8 or more cells and a stoma (within circle) (A). The yellowish exudates are presumably phenolic in nature. Large multicellular gland with head of numerous cells present in shallow pit (B) – probably it is the young stage of the type D capitate GT described by Mehra and Kulkarni (1989), see Fig. 32.

Burelo-Ramos *et al.* (2011) have investigated some species of Pithecocteniinae (Fam. Bignoniaceae) and reported three types of tracheoids - 1) Tracheoids with no ornamentation (genera *Amphilophium, Glaziovia and haplolophium*), 2) Tracheoids with true helices (genera *Distictella* and *Disticlis*) and 3) tracheids with pseudohelics

(genus *Pithecoctenium*). A remarkable range of wing tracheoids wall patterns have been reported by Lersten *et al.* (2002) such as annular, helical, and reticulate and pitted. Of the 20 Bignoniaceae species investigated, they found *Campsis radicans* to be the only species representing almost all types of ornamentations. Some of the species showed no ornamentation at all.

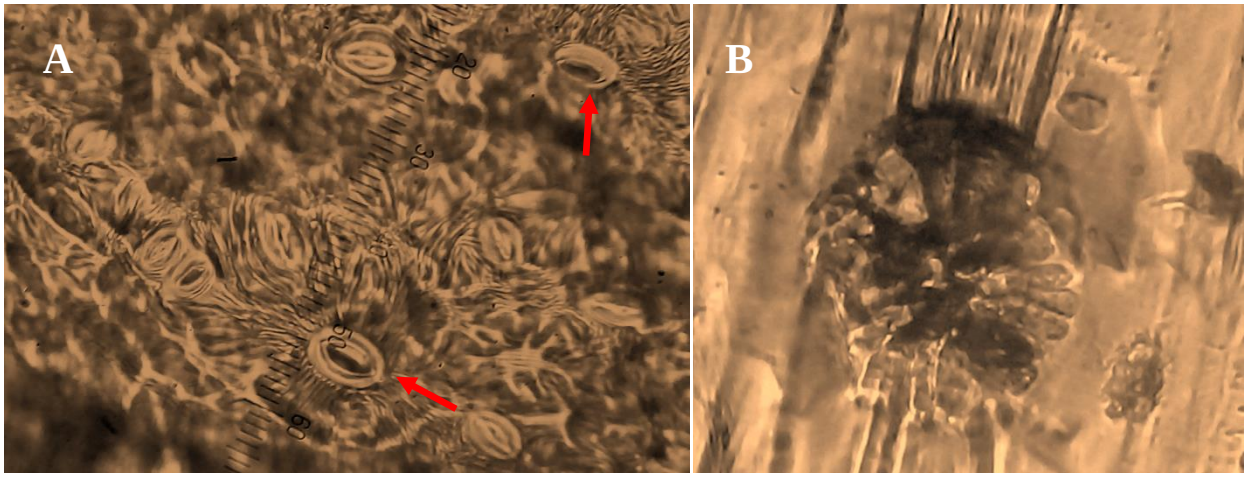


Fig. 27. A, Stomata on the fruit surface – two stomata without guard cells are also visible. B, A glandular peltate trichome (16-celled head) present on the mature fruit surface.

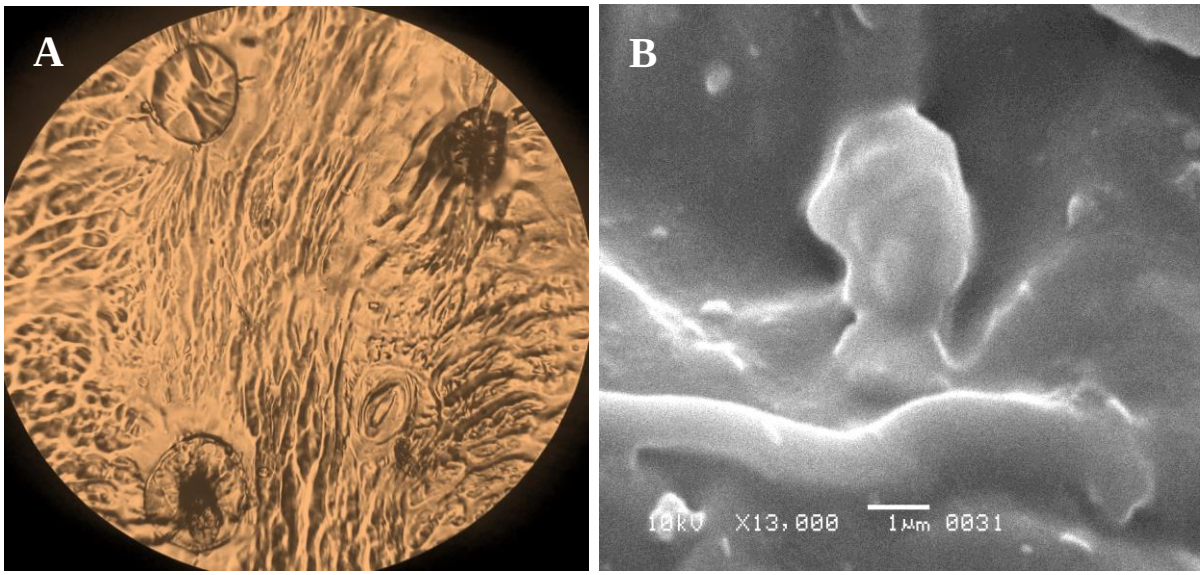


Fig. 28. The surface of maturing fruit showing 8-celled young peltate trichome (gland), two mature glands and a stoma (A). SEM view of a capitate trichome at very high magnification of 13,000 X (B). The multicellular head is supported with a short wide stalk – the trichome resembling the statue of a lady).

The wings of *M. hortensis* seeds appear to be the dispersal organ of seed. Such function to wings is also attributed by Clobert *et al.* (2012) in Bignoniaceae. On dehiscence, such seeds flutter and spin in the air so that may be carried to short distances by the winds (pitchaudiculum-herbarium.org/contents/plfeb99.htm) – a novel strategy in the heterogeneous environment). Wind is considered to be the best agent of dispersal. Such intrinsic characters as smallness and light-seededness of seeds provided with accessory structures like wings and feathers keep the seeds afloat in air currents (Werker, 1997). Lersten *et al.* (2002) called such seeds "sail flyer seeds".

The embryo region of seeds on storage, particularly in case of the relatively younger seeds, turned dark brown that may presumably be attributed to the glands present on the younger seeds (Fig. 30). Biological and physiological changes in *Tabebuia roseoalba*, a member of Bignoniaceae, on storage leading to increased phenolic contents of seeds has been reported by Abbade and Takaki (2014).

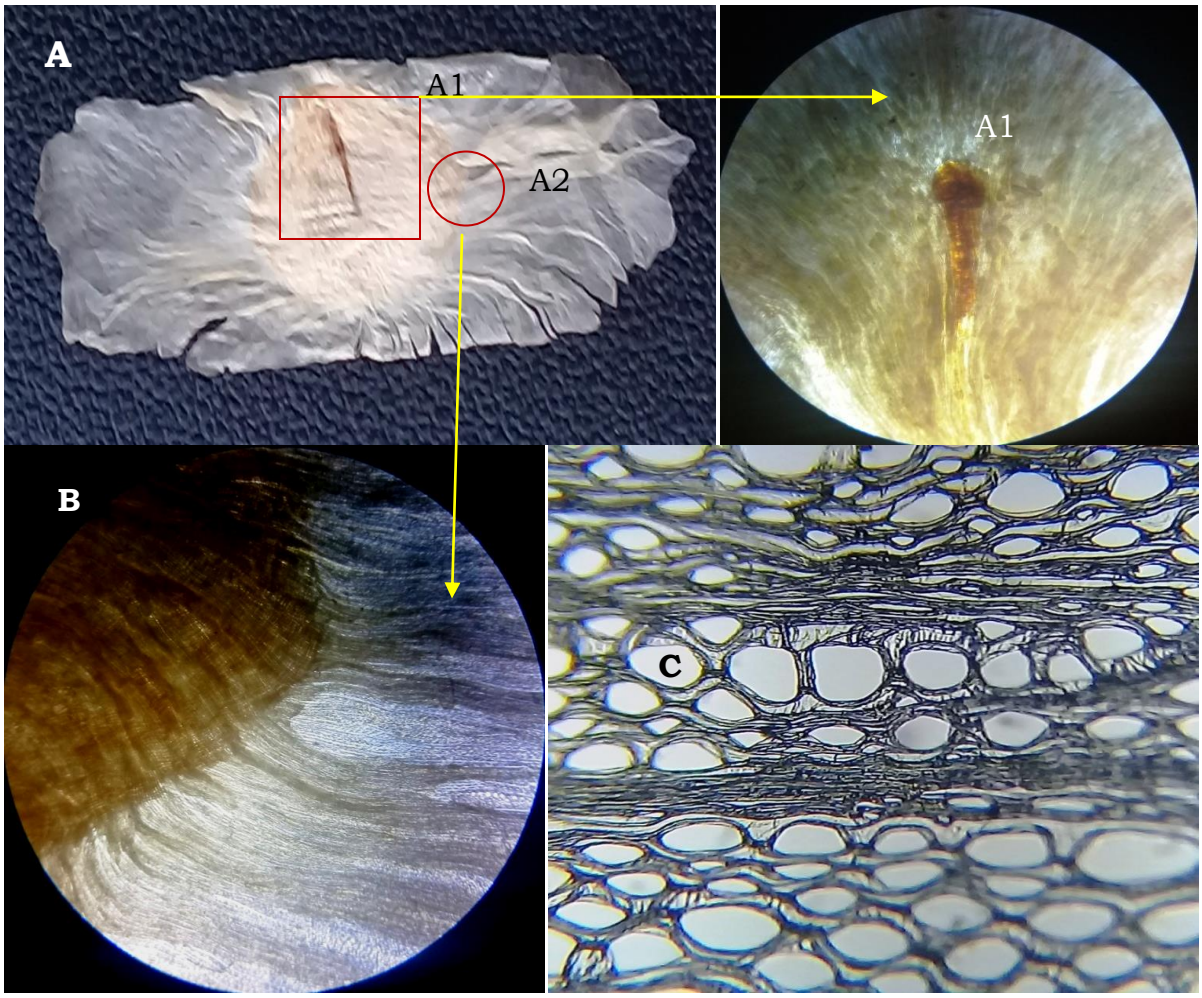


Fig. 29. A) general view of seed; A1) Embryo region showing linear brown embryo; A2) Wing region; B) Several tracheoids extend from the embryo region to the wing margin. Numerous large air spaces bounded with tracheoids may be observed and C) A close up view of tracheoids showing helical thickening at some places. The air spaces presumably facilitate seeds in dispersal as they flutter and spin in the air.

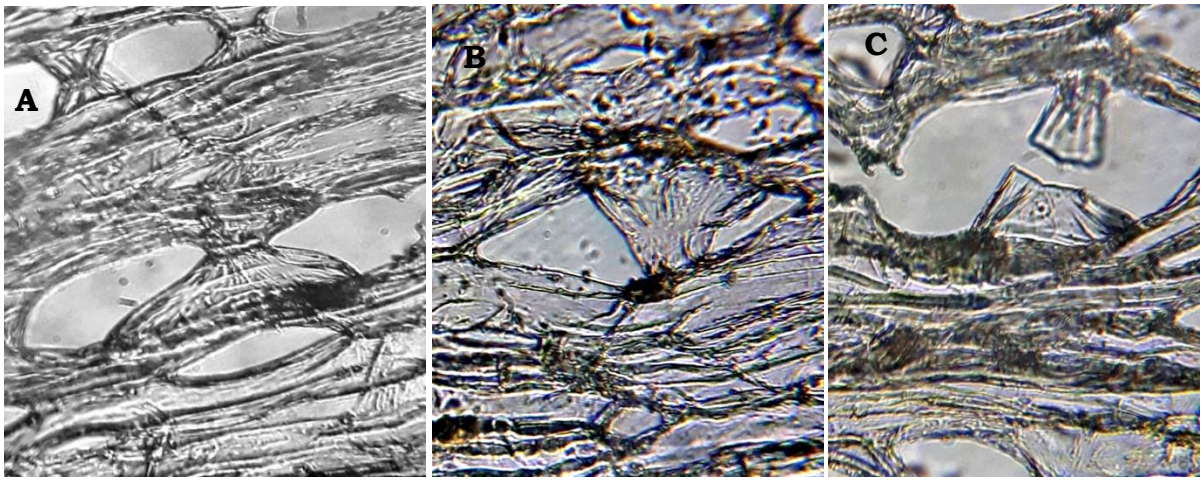


Fig. 30. The ingrowths of tracheoids in large air spaces form knots of various shapes and sizes. Presumably these bodies are meant for mechanical strength to the wing.

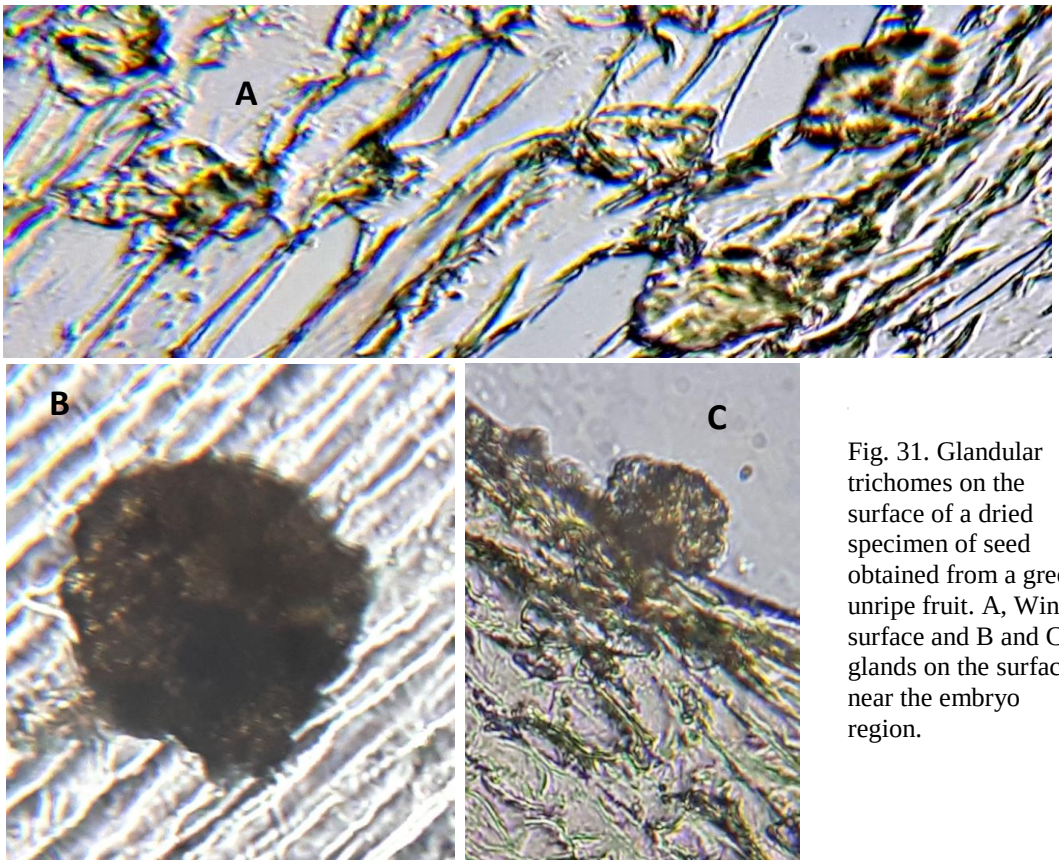
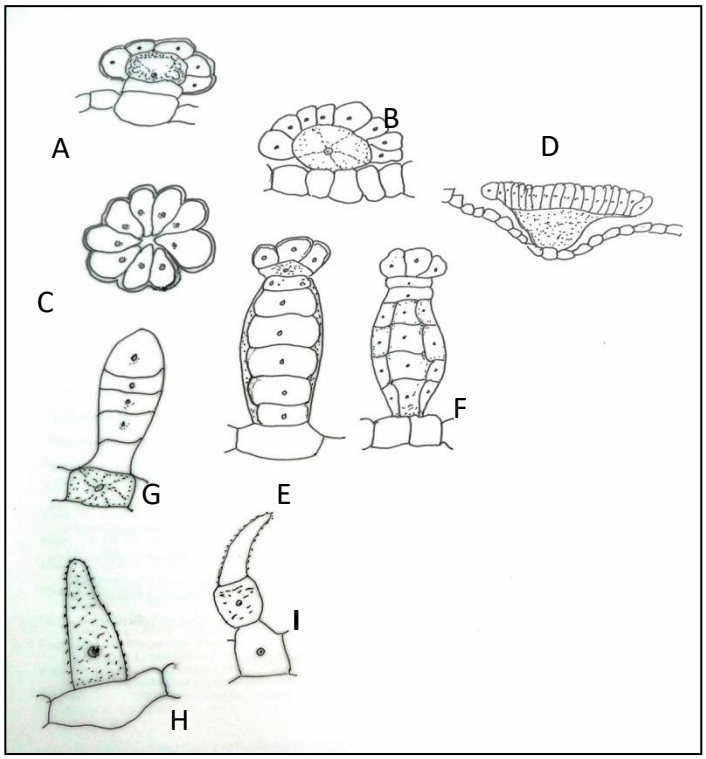


Fig. 31. Glandular trichomes on the surface of a dried specimen of seed obtained from a green unripe fruit. A, Wing surface and B and C, glands on the surface near the embryo region.



TRICHOMES

PELTATE TYPE (A, B and C) – A & B, sectional view and C, surface view. Present on peduncle, outer and inner side of calyx, corolla, ovary and young and maturing fruits.

SUNKEN TYPE (D) – Large and glandular. Present on ovary and fruits.

CAPITATE TYPE (E and F) E, broader in the middle & uniseriate; F, Multiseriate. Both glandular. Present on stalk, outer and inner sides of corolla and filaments.

FLEXIBLE TYPE (G) Provide velvety touch to petals.

STIFF & BRISTLY (H and I) Eglandular. Present on peduncle and outer surface of calyx.

Fig. 32. Free drawings of trichome types in *Millingtonia hortensis* [Drawn from Mehra and Kulkarni (1989)].

Taken together the results pertaining to trichomes in *M. hortensis*, it is obvious that trichomes are differentially profuse on all organs of *M. hortensis* – both vegetative and floral. Historically, earlier account of trichomes in Bignoniaceae was given by Bureau (1884). Later two important papers were published – one by Mehra and Kulkarni (1989) on some species of Bignoniaceae of India (including *M. hortensis*) and other by Muravnik *et al*. (2019) on *M. hortensis*. Mehra and Kulkarni (1989) recognized four GTs (differing in number of cells in the head) and two types of NGTs in *M. hortensis* (Fig. 32). Comparison of data of the present studies with that of Mehra and Kulkarni (1989) indicated our inability to record uniseriate and multiseriate capitate GTs (Type E and F; Fig. 32) from herbarium material (flower or floral parts like filament) that may probably be owing to the paucity of fresh material. Although not conclusive, type F like GT (Fig.32) was probably present on the calyx (Fig. 21B). However, some trichomes were found in this study, perhaps not described earlier to my knowledge, for instance:

1) Branched moniliform trichomes on petals imparting velvet touch to the petal (Fig. 20 B, F, D).
2) Multicellular uniseriate conical and stiff NGT with curved apical cell (Fig. 17B) on peduncle.
3) Unicellular soft trichome, NGT (Fig. 20) on petal.
4) Glandular trichome present in pit and attached laterally with epidermis of the leaf (Fig. 12).
5) Club shaped GT on pedicel (Fig. 14B).

There is, however, need to rework with floral parts of *M. hortensis* with fresh material to further elucidate the trichome diversity in this species.

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