



VARIATIONS OF CYPSELAS OF FIVE TAXA OF THE TRIBE –VERNONIEAE- (ASTERACEAE)

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Abstract

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Vernonieae is regarded as one of the plesiomorphic tribe of the family Asteraceae, containing of more than 100 genera and 1000 species. Present investigation has been carried out on the morpho-anatomical characters of cypselas of five species of the genus *Vernonia* Schreb. (*V. anthelmintica* Willd. *V. cinerea* (L.) Less. *V. galamensis* Less., *V. hymenolepis* A. Rich., *Vernonia stenolepis* Oliv.) Of the tribe Vernonieae have been studied in details. Combination of both these characters plays a paramount role in isolation of taxa at the species level. Morphological features of the apical part, surface hairs, location of vascular trace, structure of carpodium and their cellular arrangement, pappus bristles of cypselas are taxonomically significant. Anatomically presence of testal layer in the studied species has an important taxonomic parameter. In *V. stenolepis*, *V. hymenolepis*, testal layer made up of thick-walled, parenchyma cells, arranged in 3- 4 layers. except *V. anthelmintica*, In all the studied species, cellular variations absent in mesocarpic region. Presence of vellicular cavity in *V. cinerea*, *V. anthelmintica*, has some taxonomic significance. Secretary duct are important and has some taxonomic value. In *V. galamensis*, *V. stenolepis*, cells containing ergastic materials.

This character absent in other studied species. In all the studied species; except *V. cinerea*, testal epidermis addressed with pericarp. An indented dichotomous key is provided on the basis of macro-morphological and anatomical features of cypselas for identification of species.

INTRODUCTION

The tribe Vernonieae contains 118 genera and more than 1,000 species, mostly in tropical parts of the world. Vernonieae are part of subfamily Cichorioideae in the more restricted sense, with closest relationship to Arctotideae, Cichorieae, Liabeae and Moquinieae, all with usually calcarate anther base and stigmatic papillae across the whole inner surface of the style branches. The tribe differs from Arctotideae and Moquinieae by the narrow styles and long sweeping hairs, from Cichorieae by the usual lack of milky sap and the usually actinomorphic corollas, from Liabeae and Arctotideae- Eremothamneae by the usually bluish or reddish flowers and the lack of ray florets, and from Liabeae further by the usually alternate leaves, the solid bacula in the pollen and the often lophate pollen. Chemically, the genus *Vernonia* bears a unique constituent-vernolic acid (Harborne and Williams, 1977), along with different types of sesquiterpene lactones and flavonoid

compounds. Vernonieae are notable for the frequent extreme cymose forms of their inflorescences, involving seriate or scorpioid cymes. The Vernonieae is relatively more plesiomorphic than the 3 apomorphic tribes Eupatorieae, Senecioneae and Heliantheae of the subfamily Asteroideae (Bremer, 1987). Bremer (1996) has included it under subfamily Cichorioideae together with other 3 tribes in Vernonioid group which is characterised by their spiny pollen, long acute style branches and defetbn *jnddhF* gene. Jansen and Stuessy (1980) have pointed out that the Liabeae should be included within the Vernonieae, but the Liabeae has recognised as a distinct tribe from Senecioneae by Robinson (1983). Jones (*l.e.*) has mentioned that "the external features of cypselas including pappus under light microscope provide valuable information about the genus *Vernonia*". Similarly, structure of trichomes can be a diagnostic character in *Vernonia*. (Hunter and Austin, 1967; Urbatsch, 1972; Narayana, 1979 and Sahu,

1984).Rajashekara and Razi (1977) have discussed the role of morphology and described the morphology of cypsela in *Vernonia cinerea*. Carpopodium is a meristematic zone, which exists at the base of cypsela. Therefore, the available literature (Basak & Mukherjee, 2003; Mukherjee & Sarkar, 2001) provides us a meagre or fragmented information about the morphology and anatomy of cypselas characteristic of the genus *Vernonia*. Still it is essential to evaluate these diacritical characters for better understanding and classification of taxa at the species level. The present work is an attempt to bring out the cypselar characters of taxonomic value, those can be employed for the identification of the species either in cypselar stage or in association with other characters.

MATERIALS & METHODS

Mature cypselas of 5 species were procured from 1 herbarium and 2 specimens by 1st author.

Light microscopy procedures: Different parts of cypselas were cleared in 1% NaOH solution, stained with 0.1 % aqueous

safranin solution, mounted in phenol glycerin and dissected the different parts of cypselas with the help of 2 sharp needles under dissecting microscope and stereo dissecting binocular microscope. For anatomical study, free hand cross sections were done preferably from the middle part of cypselas with the aid of sharp razor blade. Selected sections were stained in safranin light green combination following standard method of staining. Properly stained good sections were observed under compound Research Microscope.

Scanning electron microscopy procedures: Cleaned dry cypselas were attached to brass holders by cellophane 'double -stick' tape. Cypselas were coated with gold. Coated specimens were observed and photographed using Hitachi SEM in Burdwan University. The description of the cypselar features follows the terminology of Dilcher (1974), Kynclova (1970) and Velez (1981).

RESULTS AND DISCUSSION

Vernonia anthelmintica

Morphology Figure (1 A-F, 2 A-D, 3 L-O)

Cypsela homomorphic, 4 mm x 1 mm with pappus, 3 mm x 1 mm without pappus, brownish, serrated, straight, upper part truncate whereas lower part tapered, more or less elliptic in cross section. Surface pubescent, containing 10 ribs, conspicuous, alternating with furrow, furrows wider than ribs. The distance between 2 ribs 0.02 mm. Surface hair villous type, ascending in orientation, non-glandular, biseriate forked type, made up of body and basal cell. Tips of the body cells of hair situated in different plane. Sessile hairs also present on the surface. Sessile hairs with 2 celled. At the upper portion of cypsela, stylopodium present, immersed in the nectar. At the upper part of cypsela, pappus present, homomorphic, persistent, represented by 20-22 serrulate setose type of pappus bristles, yellow white, unequal, represented in a single circle. At the basal region of cypsela, carpodium present; narrow than the base, symmetric, complete ring like. Carpodial cells thick-walled, rectangular, arranged in single row, distinguishable from other cells of cypsela.

Anatomy Figure (4 A)

Cypsela narrow elliptic in cross section. Ribs present, 10 in number, conspicuous.

Cypselar wall 0.01 mm and 0.03 mm wide at rib and furrow region respectively. Pericarp thin, on an average 0.02 mm wide, differentiated into two zones- epicarp and mesocarp. Epicarp uni-seriate, made up of thick-walled, rectangular, compactly arranged, parenchyma cells provided with cuticle. Internal to the epicarp, mesocarp present; made up of compactly arranged, thin-walled, penta-hexagonal, parenchyma cells and thin-walled, compactly arranged, hexagonal sclerenchyma cells, containing vascular bundle. Within the mesocarpic region, vellicular cavity present. Internal to the mesocarpic region, testal layer present, attached with pericarp, approximately 0.1 mm thick, uni-seriate, made up of horizontally placed, thick-walled, parenchyma cells. Internal to the testal layer, endosperm layer present, biseriate, made up of thick-walled, barrel shaped, parenchyma cells. Mature embryo occupies a major part of cypsela; cotyledons two in number, arranged at right angle to the axis of cypsela, containing 8 resin ducts (4 resin ducts in each cotyledon).

Vernonia cinerea

Morphology Figure (1 G-K, 2 E-G, 3 D-G)

Cypsela homomorphic, 2 mm x 1 mm with pappus, 0.5 mm x 1 mm without pappus, light brown, striated, oblong, straight, upper portion truncate whereas basal part tapered, trigonous in cross section. Surface pubescent, containing 2 ribs, conspicuous, alternating with furrow. Furrows wider than ribs. The distance between 2 ribs 0.021 mm. On the surface, vesicular body present. Surface hair sericeous- villous type, non glandular, biseriate forked type, made up of body and basal cells, tips of the body cells of hair situated in different plane, appressed- inclined in orientation with the surface. Sessile hairs not observed on the surface. At the upper part of cypsela, stylopodium present, inconspicuously arranged. Pappus present in the form of two circle. Outer circle represented by scaly pappus made up of 11-15 pappus bristles, light brown, unequally arranged whereas inner circle represented by 20-25 serrulate-setose type of pappus bristles, white brown, unequally arranged. At the basal region of cypsela, carpodium present, narrow than the base, complete ring like, made up of thick-walled, cubical cells.

Anatomy Figure (4 B)

Cypsela trigonous in cross section. Ribs present, 2 in number, conspicuous. Cypselar wall 0.1 mm and 0.31 mm wide at rib and furrow region respectively. Pericarp thin, on an average 0.2 mm wide, differentiated into two zones- epicarp and mesocarp. Epicarp uni-seriate, made up of thin-walled, compactly arranged, cubical parenchyma cells, provided with cuticle. Internal to the epicarp, mesocarp present, made up of thick-walled, compactly arranged, hexagonal, sclerenchyma cells containing vascular trace just below the ribs. Vellicular cavity present in the mesocarpic region, below the ribs. Internal to the mesocarpic region, testal layer present, attached with pericarp, made up of thin-walled, rectangular, parenchyma cells, 0.01 mm in diameter. Endosperm persist in mature cypsela, biseriate. Outer layer made up of thick-walled, horizontally placed, parenchyma cells whereas inner layer made up of thin-walled, horizontally placed, parenchyma cells. A layer exists between testa and endosperm layer. Mature embryo occupies a major part of the cypsela; cotyledons 2 in number, arranged oblique to the axis of cypsela, containing 6 resin ducts(3 ducts in each cotyledons).

V. *galamensis*

Morphology Figure (1 L-N, 2 H-J, 3 H-K)

Cypsela homomorphic, 6 mm x 1 mm, blackish, striated, obovate, straight, upper part truncate whereas basal part tapered, elliptical in crosssectional configuration. Surface pubescent, containing 2 ribs, conspicuous, alternating with furrow. Furrows wider than ribs. The distance between 2 ribs 0.01 mm. Surface hair sericeous, non glandular, biseriate forked type, made up of body and basal cells, appressed to ascending in orientation with the surface. Tips of the body cells of hair situated in different plane. Sessile hairs not observed on the surface. Pappus represented by small bristles. At the upper part of cypsela, stylopodium present, inconspicuous, fully immersed in the nectar. At the basal region of cypsela, carpodium present, narrow than the base, quadrangular, symmetric. Carpodium cells thick-walled, rectangular, horizontally placed, arranged in single row, distinguishable from other cells of cypsela.

Anatomy Figure (4 C)

Cypsela slightly elliptical in cross section. Ribs present, 2 in number, inconspicuous.

Cypselar wall 0.02 mm and 0.031 mm wide at rib and furrow region respectively. Pericarp thin, on an average 0.021 mm wide, differentiated into three zones-epicarp, mesocarp and endocarp. Epicarp uni-seriate, made up of thin-walled, compactly arranged, quadrangular-pentangular, parenchyma cells, provided with cuticle. In some region, epicarp with biseriate parenchyma cells. Internal to the epicarpic layer, mesocarpic layer present; made up of irregularly arranged, parenchyma cells. Within the mesocarpic region, ergastic material, secretory ducts present in some cells. Internal to the mesocarpic region, endocarpic region present, made up of thin-walled, uniseriately arranged, parenchyma cells. Testa attached with pericarp, approximately 0.1 mm thick, differentiated into outer and inner zone. Outer zone made up of horizontally placed, elongated parenchyma cells with swollen radial wall. Inner zone represented by a narrow layer of elongated parenchyma cells. Endosperm persists in mature cypsela, biseriate, made up of thin-walled, elongated parenchyma cells. Mature embryo occupies a major part of the cypsela; cotyledons 2 in number, placed

in right angle to the axis of cypsela, containing 6 resin ducts (3 ducts in each cotyledons).

V. Hymenolepis

Morphology Figure (1 O-Q, 2 K-M, 3 P-R)

Cypsela heteromorphic. Ray cypsela 4 mm x 1 mm, brownish, stripted, oblanceolate, margin entire, straight, upper part truncate whereas basal part tapered. Disk cypsela 3 mm x 1 mm, black, stripted, oblanceolate, straight, margin entire, upper part truncate whereas lower part tapered. More or less trigonous in cross section. Surface pubescent, containing 10- 13 ribs, conspicuous, alternating with furrow. Furrows wider than ribs. The distance between 2 ribs approximately 0.02 mm. On the surface, only sessile hairs present. Sessile hairs with 2 celled. At the upper part of cypsela, stylopodium present, unenlarged, partially immersed in the nectary. Pappus absent. At the basal region of cypsela, carpodium present, narrow than the base, pentangular. Carpodial cells thick-walled, circular, medium in size, arranged in 5-7 rows, distinguishable from other cells of the cypsela.

Anatomy Figure (4 D)

Cypsela trigonous in cross section. Ribs present 12-13 in number, conspicuous. Cypselar wall 0.03 mm and 0.05 mm wide at rib and furrow region respectively. Pericarp thin, on an average 0.04 mm wide, differentiated into two zones- epicarp and mesocarp. Epicarp uni-seriate, made up of thin-walled, rectangular, compactly arranged, parenchyma cells, provided with cuticle. Internal to the epicarpic region, mesocarpic region present; made up of thick-walled, circular, compactly arranged, sclerenchyma cells and a layer of thin-walled, round-ovoid, parenchyma cells present. Within the mesocarpic region, vascular trace present. Internal to the mesocarpic region, testal zone present, attached with pericarp, approximately 1.23 mm thick, parenchymatous. Endosperm persists in mature cypsela, uniseriate, made up of thick-walled, horizontally placed, parenchyma cells. Mature embryo occupies a major part of the cypsela; cotyledons 2 in number, arranged at right angle to the axis of cypsela, containing 10 resin ducts (5 ducts in each cotyledon).

V. stenolepis

Morphology Figure (1 R-V, 2 N-P, 3 A-C)

Cypsela homomorphic, 5 mm x 1 mm with pappus, 4.5 mm x 1 mm without pappus, brownish, stripped, narrow oblanceolate, straight, upper part truncate whereas basal part tapered. Circular in cross section. Surface pubescent, containing 10 ribs, alternating with furrow. Furrows wider than ribs. The distance between 2 ribs 0.02 mm. Surface hair tomentose type, made up of body and basal cell, non-glandular, ascending in orientation. Cypselar hair biseriate forked type; tips of the body cells of hair situated in different plain. On the surface, sessile hairs also present. Sessile hairs with 2 celled. At the upper portion of cypsela, pappus present; hetero-morphic, represented by 2 whorls. Outer whorl represented by bristellate pappus and inner whorl represented by 4 scaly pappus. At the upper part of cypsela Stylopodium present, inconspicuous. At the basal region of cypsela, carpopodium present, narrow than the base, symmetric, arranged in a ring.

Anatomy Figure (4 E)

Cypsela circular in cross section. Ribs present, 10 in number, conspicuous. Cypselar wall 0.02 mm and 0.04 mm wide at rib and furrow region respectively. Pericarp

thick, on an average 0.03 mm wide, differentiated into two zones- epicarp and mesocarp. Epicarp uni-seriate, made up of thin-walled, compactly arranged, horizontally placed parenchyma cells, provided with cuticle. Internal to the epicarpic region, mesocarpic region present; made up of thin-walled, compactly arranged, penta-hexagonal, parenchyma cells. Within the mesocarpic region, resin containing duct present. Internal to the mesocarpic zone, testal layer present, attached with pericarp, approximately 1.1 mm thick, arranged in 4-6 layer, made up of thick-walled, horizontally placed, parenchyma cells. Endosperm persists in mature cypsela, bi-seriate, made up of thin-walled, horizontally placed, parenchyma cells. Mature embryo occupies a major part of cypsela; cotyledons 2 in number, arranged oblique to the axis of cypsela, containing 6 resin ducts (3 ducts in each cotyledon).

The present study shows that diacritical cypselar features are distribution of vesicular bodies, structure of carpopodium and thickness of carpopodial cells, orientation of pappus bristles or scales, number of ribs and their relative thickness

with furrows, tissue distribution in mesocarpic zone, presence of vascular trace in pericarp and orientation of calcium oxalate crystals in pericarp etc. Morpho-anatomical study of cypsel of some species of the tribe Vernonieae has been done by Mukherjee & Sarkar (2001), Basak & Mukherjee (2003). The tribe Vernonieae has many primitive features such as-beak less cypsel, vesicular body, symmetric carpopodium, pappus in the form of scabrous bristles or scarious or setaceous scales, absence of phytomelanin layer, usually absence of resin canals or secretory canals, thick pericarp, absence of pitted parenchyma cells, absence of papilate hair and mucilage producing hairs. Therefore, this tribe can be regarded as one of the most primitive tribe in the Asteraceae. This view supports with the opinion of Augier and du Me'rac (1951) regarding its phylogenetic position in the Asteraceae.

The morpho-anatomical features of cypselas suggest that the separation of Rolandreae and Elephantopeae as distinct subtribes in Vernonieae as proposed by Cassini (1817) and supported by Philipson (1938) are justified. The value of cypselar characters can be employed for proper

evaluation of taxa together with other characters from different taxonomic sources. The present study reveals that cypselas are homomorphic, narrow oblanceolate in *V. stenolepis*, oblanceolate in *V. hymenolepis*, obovate in *V. galamensis*, oblong in *V. cinerea*, *V. anthelminthica*. Cypselar surface is usually addressed by numerous twin hairs and sessile hairs which are absent in *V. hymenolepis*. Stylopodium is an important character of cypsel. In *V. stenolepis*, stylopodium inconspicuously arranged; in *V. hymenolepis*, unenlarged and partially immersed in the nectar; in *V. galamensis*, inconspicuous, fully immersed in the nectar; in *V. cinerea*, inconspicuous; in *V. anthelminthica*, stylopodium present, immersed in the nectar. So, on the basis of stylopodium; we can separate the studied taxa easily. Mukherjee, made a study on stylopodial characters and discuss the different category of stylopodium. It is very essential for further study. In *Vernonia*, structure of stylopodium is not so much valuable for characterisation of taxa. At the basal region of cypselae, carpopodium present. Carpopodium is the basal meristematic tissue region of the cypsel.

Layers of carpopodial cells are also variable. In *V. anthelminthica*, carpopodial cells arranged in single row; in *V. cinerea*, 1-5 rows. So carpopodial features have definite systematic value for characterization of taxa. Aforesaid carpopodial features have been reported by Mukherjee & Nordenstam (2004). In *V. galamensis*, carpopodium absent (Basak and Mukherjee, 2003). So, our observation in the absence of carpopodium in *V. galamensis* is true. In *Vernonia anthelmintica*, pappus represented by serrulate-setose type of pappus bristles; in *V. ceneria*, scaly pappus and serrulate-setose type of pappus bristles; in *V. galamensis*, bristellate pappus; in *V. hymenolepis*, pappus absent; in *V. stenolepis*, scaly and bristellate pappus present. Observations regarding pappus fit well with the opinion of Jones (1977) and Bremer (1996). Regarding pappus structure Bremer (1987) has mentioned that it has been a principal source of information at the generic level but less important at the higher levels.

Anatomically (Except- *V. anthelminthica*) all the studied species, tissue differentiation in mesocarpic zone absent. More or less aforesaid types of mesocarpic zone have

been reported by Velez (1981). Epicarpic cells are commonly uniseriate but in *V. galamensis*, epicarpic zone is biseriate in some region. Presence of vellicular cavity in the mesocarpic region of *V. ceneria*, *V. anthelminthica* has some taxonomic value. Remaining studied species, vellicular cavity absent. Presence of ergastic matter and secretary cavity in the mesocarpic region of *V. galamensis*, containing some taxonomic significance. Testal features also important. Except *V. anthelminthica* and *V. cinerea*, remaining studied species, testa multiseriately arranged, parenchymatous.

The morpho-anatomical structures of cypselas suggest that the separation of Rolandreae and Elephantopeae as distinct subtribes in Vernonieae as proposed by Cassini (1817) and supported by Philipson (1938) are justified. The value of cypselar characters can be employed for proper evaluation of taxa together with other characters from different taxonomic sources.

Key to the species of Vernonia

- 1a. Pappus bristles absent
**Vernonia**
hymenolepis

- 1b. Pappus bristles present.....
..... (2)
- 2a. Surface containing vesicular
body.....**V.**
Cinerea
- 2b. Vesicular body absent in the
surface.....
(3)
- 3a. Secretary Duct present in mesocarpic
region.....**V. Galamensis**
- 3b. Secretary Duct absent in mesocarpic
region..... (4)
- 4a. Mesocarpic zone exhibit tissue
differentiation.....**V. Anthelmintica**
- 4b. Tissue differentiation absent in
mesocarp.....
(5)

- 5a. Presence of scaly
pappus.....**V.**
Stenolepis
- 5b. Scaly Pappus
absent.....
..... (6)

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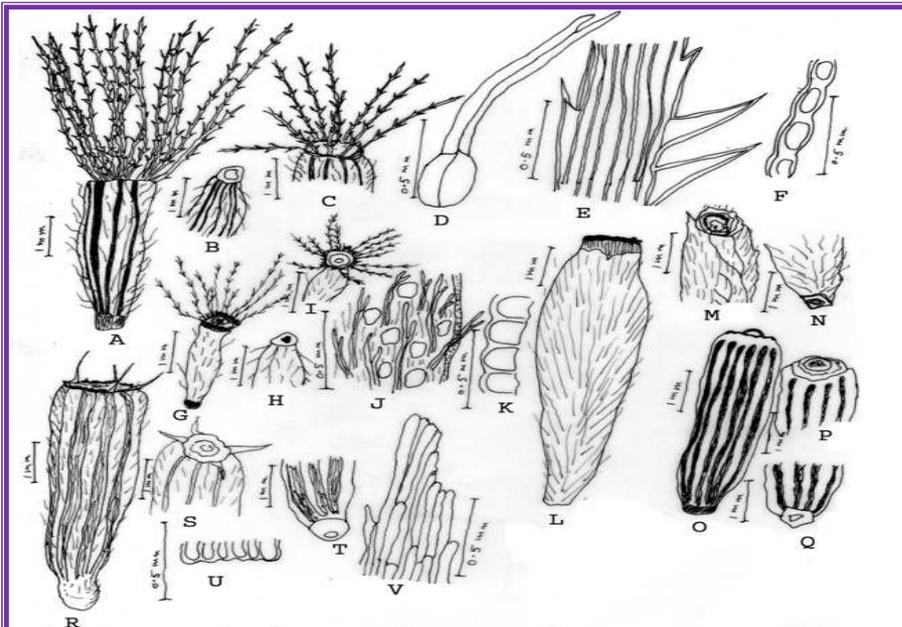


Fig.1 Morphology of cypselas

A-F-*Vernonia anthelmintica*: A-Cypselas; B-Basal part; C-Upper part; D-Surface hair; E-Part of pappus bristle; F-Carpopodial cells. G-K- *V. ceneria*: G-Cypselas; H-Basal part; I-Upper part; J-Surface; K-Carpopodial cells. L-N- *V. galamensis*: L-Cypselas; M-Upper part; N-Basal part. O-Q- *V. hymenolepis*: O-Cypselas; P-Upper part; Q-Basal part. R-V- *V. stenolepis*: R-Cypselas; S-Upper part; T-Basal part; U-Carpopodial cells; V-Scaly pappus.

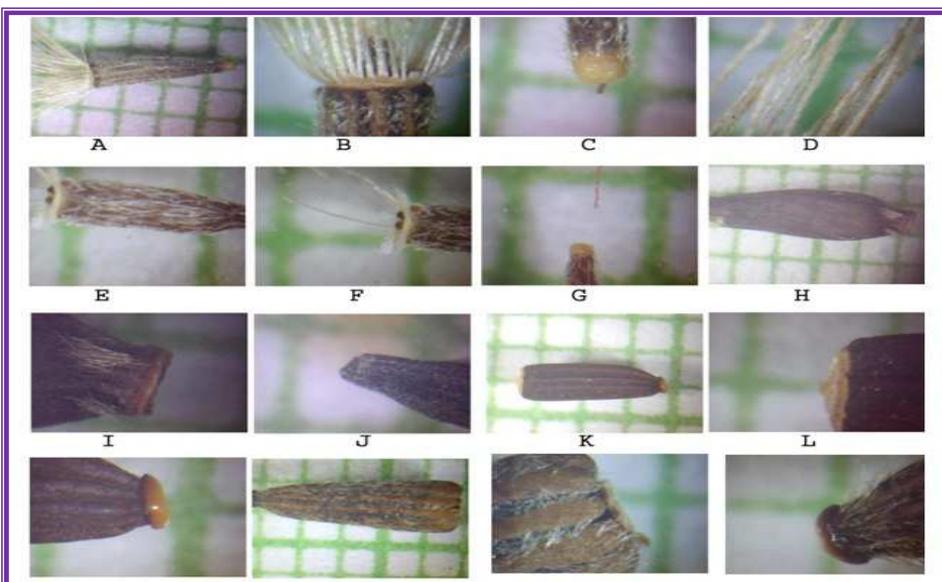


Fig.2 Photographs of different parts of cypselas

A-D- *Vernonia anthelmintica*: A-Cypselas; B-Upper part with pappus; C-Basal part; D-Part of pappus bristle. E-G- *V. ceneria*: E-Cypselas; F-Upper part with pappus; G-Basal part. H-J- *V. galamensis*: H-Cypselas; I-Upper part; J-Basal part. K-M- *V. hymenolepis*: K- Cypselas; L-Upper part; M-Basal part. N-P- *V. stenolepis*: N-Cypselas; O-Upper part; P-Basal part

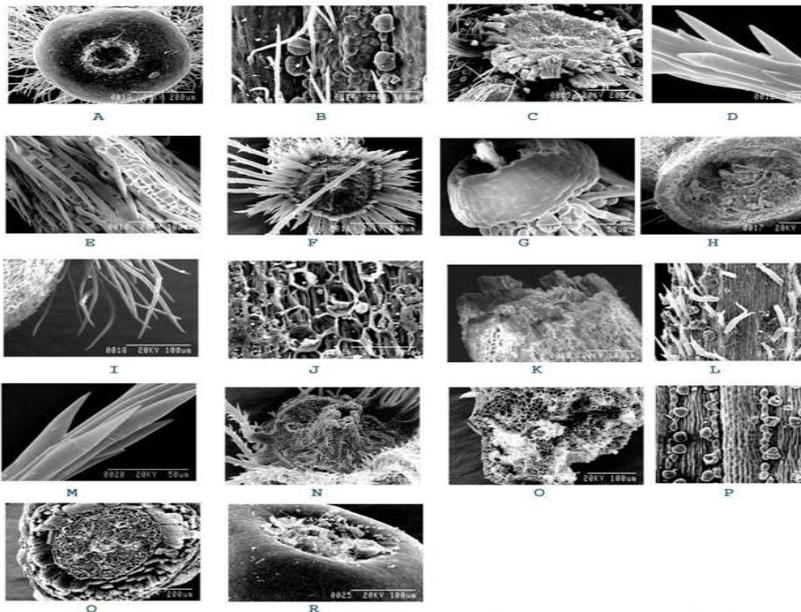


Fig.3 SEM photographs of different parts of cypselas
 A-C-*Vernonia stenolepis*: A-Basal part; B-Surface; C-Upper portion. D-G-*V. ceneria*: D-Portion of pappus bristle; E-Surface; F-Upper part; G-Basal part. H-K-*V. galamensis*: H-Upper part; I-Surface hair; J-Surface; K-Basal part. L-O-*V. anthelmintica*: L-Surface; M-Portion of pappus bristles; N-Upper part; O-Basal part. P-R-*V. hymenolepis*: P- Surface; Q-Upper part; R-Basal part.

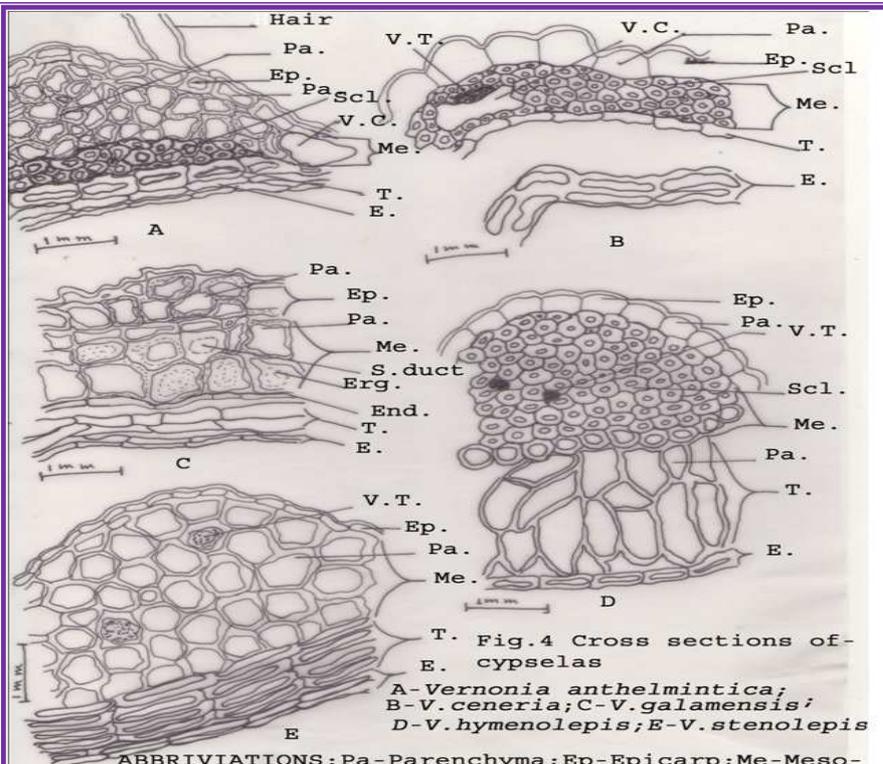


Fig.4 Cross sections of cypselas
 A-*Vernonia anthelmintica*;
 B-*V. ceneria*; C-*V. galamensis*;
 D-*V. hymenolepis*; E-*V. stenolepis*

ABBREVIATIONS: Pa- Parenchyma; Ep- Epicarp; Me- Meso- carp; T- Testa; E- Endosperm; En- Endocarp; V.C.- Vellicular- cavity; V.T.- Vascular trace; S. duct- Secretary duct; Erg.- Ergastic matter.

Table 1

List of taxa studied and their sources

NAME OF TAXA	SOURCE OF ORIGIN
<i>Vernonia anthelmintica</i> Willd.	Kalyani Township.
<i>V. cinerea</i> (L.) Less.	Kalyani Township.
<i>V. galamensis</i> Less.	NORTH CENTRAL REGIONAL, PLANT INTRO. STATION Ames, IA. 50011, PI 500003, LOT: 87 nc so 01, ORIGIN: Kenya, FOR: 100 CT Y MAINJARBOX, ORDER # 940162
<i>V. hymenolepis</i> A. Rich.	NORTH CENTRAL REGIONAL, PLANT INTRO. STATION Ames, IA. 50011, PI 312850, LOT: 88 nc po 01, CULT: 14533, ORIGIN: Ethiopia, FOR: 100 ct Y MAINJAR, ORDER # 940162
<i>V. stenolepis</i> Oliv.	NORTH CENTRAL REGIONAL, PLANT INTRO. STATION Ames, IA. 50011, Ames 8594, LOT: 88nt ao01, ORIGIN: UNCERTAIN, For: 100 ct Y MAINJAR, ORDER # 940162

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