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RESEARCH ARTICLE

Anatomical and Phytochemical Studies on *Ocimum basilicum* L. Plant (Lamiaceae)

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Abstract

The present study is concerned with histological features of Basil plant (*Ocimum basilicum* L.). Various organs of vegetative growth; namely, the main stem (represented by shoot apex, apical, median and basal internode) and different foliage leaves developed on the main stem and on lateral shoot; including lamina and petiole were investigated fortnightly throughout the whole growing season. Histological features of various vegetative organs of Basil plant were analysed microscopically and photomicrographed. Scanning electron microscope for the adaxial and abaxial surfaces of Basil leaf blade was also investigated. Moreover, volatile oil analysis of Basil herb at full blooming stage was carried out.

Introduction

The genus *Ocimum* Linn. belongs to the tribe Ocimeae, subfamily Nepetoideae, family Lamiaceae and the order Lamiales. It is one of the economically important groups of aromatic herbaceous plants extensively used in perfumery, flavouring and pharmaceutical products (Khosla,1993). There are about 150 species in this genus broadly dispersed over the warm regions of the globe (Evans, 2001 and Kumar,2009). Many species of *Ocimum* have been grown by local people as medicinal plants, culinary herbs and insect controlling agents (Grayer et al., 1996). *Ocimum* species differ in growth habit, physiological appearance and chemical and aromatic composition. They grow in wide variety of soil and climatic conditions. All *Ocimum* species yield essential oils which are responsible for the medicinal uses including antimicrobial, antioxidant, antifungal and antiinflammatory activities; yet their taxonomy and nomenclature are in a bit of muddle (Nahak et al., 2011). Therefore, any new botanical information about *Ocimum* plants are urgently to be wellcomed.

In this concern , *Ocimum basilicum* L. (Basil or Sweet Basil) called Rehan in Arabic was chosen to be the subject of the present investigation because of its economic importance as an ornamental, spice, culinary and medicinal herb; yet anatomical structure of Basil herb and its volatile oil composition are poorly investigated.

Thus, it is aimed in this study to bring to light more information about the anatomical structures of vegetative organs of Basil plant throughout the consecutive stages of its entire life span. Moreover, the analysis of essential oil of Basil herb at flowering stage was carried out. Such knowledge may fulfill the lack in information concerning the anatomical and phytochemical characteristics of such important economic species in the genus *Ocimum* of the family Lamiaceae.

MATERIALS AND METHODS

The present investigation was performed on *Ocimum basilicum* L. (Sweet Basil or Basil) of the family Lamiaceae (Labiatae). Seeds were procured from the Experimental Station of Medicinal plants, Faculty of Pharmacy, University of Cairo, Giza, Egypt.

The field work was carried out in the Agricultural Experiments and Researches Station, Faculty of Agriculture, Cairo University, Giza, Egypt during the summer growing season of 2011 to provide the experimental plant material. Date of cultivation was March 19th , 2011. The trial include five replicates, each represented by one plot. The plot was eight ridges, four meters long, 60 cm apart. Seeds were sown in hills spaced 20 cm, the plants were thinned to two plants per hill. All field practices were carried out as recommended for Basil in the vicinity.

Anatomical studies

A full microscopical study was carried out to investigate the histological structures of vegetative organs of Basil plant. Samples were taken fortnightly throughout the growing season of 2011. Specimens represented different plant organs of vegetative growth, including :

1- The shoot apex
2- The main stem, represented by terminal, median and basal internode.
3- The leaves developed on the main stem and on lateral branches, represented by the middle of the lamina and petiole.

Microtechnique practices were carried out at the Laboratory of Agricultural Botany Department, Faculty of Agriculture, Cairo University, Giza, Egypt, during the two successive years of 2011 and 2012. Microtechnique procedure given by Nassar and El-Sahhar (1998) were followed. Materials were killed and fixed for at least 48 hours in FAA (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). After fixation, materials were washed in 50% ethyl alcohol and dehydrated in a normal butyl alcohol series before being embedded in paraffin wax MP 56-58 °C. Transverse and longitudinal sections which were cut on a rotary microtome to a thickness of 20 µm were stained with crystal violet / erythrosine before mounting in Canada balsam. Slides were examined microscopically and photomicrographed.

Stomata and glandular trichomes were subjected to investigation using scanning electron microscopy (SEM) as a modern botanical discipline. The specimens were mounted after dehydration on the copper specimen holder stub with double-sides adhesive discs and coated with a thin layer of gold palladium using Edwards sputter coater unit S 150 A, England. The specimens were examined in different possitions using different magnifications by JXA-840A model Electron Probe Microanalyzer - JEOL, Japan. Scanning electron microscopy was carried out at the Central Laboratory, National Research Center (NRC), Dokki, Giza, Egypt.

Analysis of the volatile oil

A chemical analysis was carried out to gain information about the volatile oil of Basil herb at full blooming stage. Hydrodistillation of the volatile oil were conducted using the technique described by Denys and Simon (1990). Plant material was placed in a 2-liter roundbottomed flask with distilled, deionized water (400 ml for 200 g fresh material) and the volatile oil was extracted by water distillation using a modified Clevenger trap (ASTA, 1968) for smaller plant samples, the distillation period was 1 hour (fresh samples), and the volatile oil content was determined on an oil volume to tissue weight.

GC – MS technique was used to separate and detect the volatile oil constituents. Analysis was performed at Research Parks, Faculty of Agriculture, Cairo University.

Conditions used are as follows:

Instrument : Gas Chromatography Mass Spectrometry
(Hewlett Packard) HP 6890 series (Agilent).
Carrier Gas : Helium

Capillary Column : Thermo Scientific
TR – 5 MS (5% phenyl polysil phenylene siloxane).
30 m × 0.25mm ID × 0.25 um film

Conditions : Injector Temperature : 250 deg ˚C
Detector Temperature : 250 deg ˚C
Detector : MSD

Oven Programming :

Initial	Rate (˚C / min)	Temp (˚C)	Hold time (minutes)
-	-	50	3
Ramp	5	180	10

Gas Flow Rate (ml / min):
He : (1ml / min)
Mass Spectrometer HP 5973 (Agilent).

RESULTS AND DISCUSSION

I – Histological studies :

1- Structure of the main stem :

a- The shoot apex :

Longitudinal median section of the shoot apex of Basil, aged four weeks, as seen in Figure (1) exhibits that the shoot apex is clearly domed in shape. It is composed of two zones of tissues, the tunica (23 µm in depth) consisting of two peripheral layers of cells, and the corpus (106 µm in depth), a mass of cells enclosed by the tunica. The two layers of the tunica show predominantly anticlinal divisions; i.e., they are undergoing surface growth. Thus, the first layer gives rise to the protoderm. Whereas, the second layer contributes to the outermost layer of the ground tissue. In the corpus, the cells are relatively large, with arrangement and planes of cell division and the whole mass grows in volume. The outer layers of the corpus develop the inner layers of the cortex and procambium as well. The successive periclinal divisions of the inner corpus gives rise to rib meristem.

It is evident that the shoot apex above the first discernible leaf primordium measures 126 µm in height and 294 µm in diameter. The number of cells across this region ranges between 14 to 16. The distance below the tip where the procambium first differentiated was almost 298 µm. The first vascular element to differentiated was the phloem at 367 µm below the tip of the shoot apex. This was followed by xylem at 459 µm below the tip. Vaculation was observed at a distance of 224 µm below the tip.

Worthy to note that trichomes of various kinds are accompanied the longitudinal median section of the shoot apex of Basil plant (Figs. 1 and 2). The occurrence together of diverse kinds of non-glandular hairs and characteristic short – stalked glands with two or four cellular heads is characteristic of the section. The non-glandular hairs are uniseriate, pointed, straight or hook-like (sometimes curved), consisting of one to five thick-walled cells. The glandular hairs (Fig.2) consists of capitate and peltate (scales) hairs. The capitate hairs composed of one base cell, one stalk cell and a head of two broad cells. The peltate hairs have similar base and stalk cells but a broad four-celled head.

b-The apical internode :

The internode directly below the shoot apex was studied from the anatomical point of view at the age of 10 weeks as it represents the primary structure of the main stem. The transverse section shown in Figure (3A) reveals that the stem surface of Basil plant, directly below the shoot apex, is very frequently rectangular (tetragonal) in outline. The stem is strongly ridged and fluted. It has four ridges alternating with four furrows constituting its general outline and there is usually a considerable development of collenchyma in the angles (3 B).

The uniseriate epidermis, the outermost zone is consisting of tabular cells attached end on end without leaving intercellular spaces. The epidermal cells are vaculated and covered with a thin layer of cuticle. Stomata and trichomes of various kinds are observed. The non-glandular hairs are uniseriate consisting of three to five thick-walled cells (Figs 3 C and 4 A). The glandular hairs consist of capitate hairs which are composed of one base cell, one stalk cell and a head of either one elongated oval cell or two broad cells (Figs. 3 C and 4 B).

The ridges of the stem mainly consists of collenchyma, which constitute the chief mechanical tissue of the axis and form the ridges. The cortex at the furrows between the ridges consists of 8-9 layers of parenchymatous cells of which the outer 2 or 3 layers are chlorenchyma underlying the epidermis. The starch sheath, the innermost layer of the cortex, is difficult recognized.

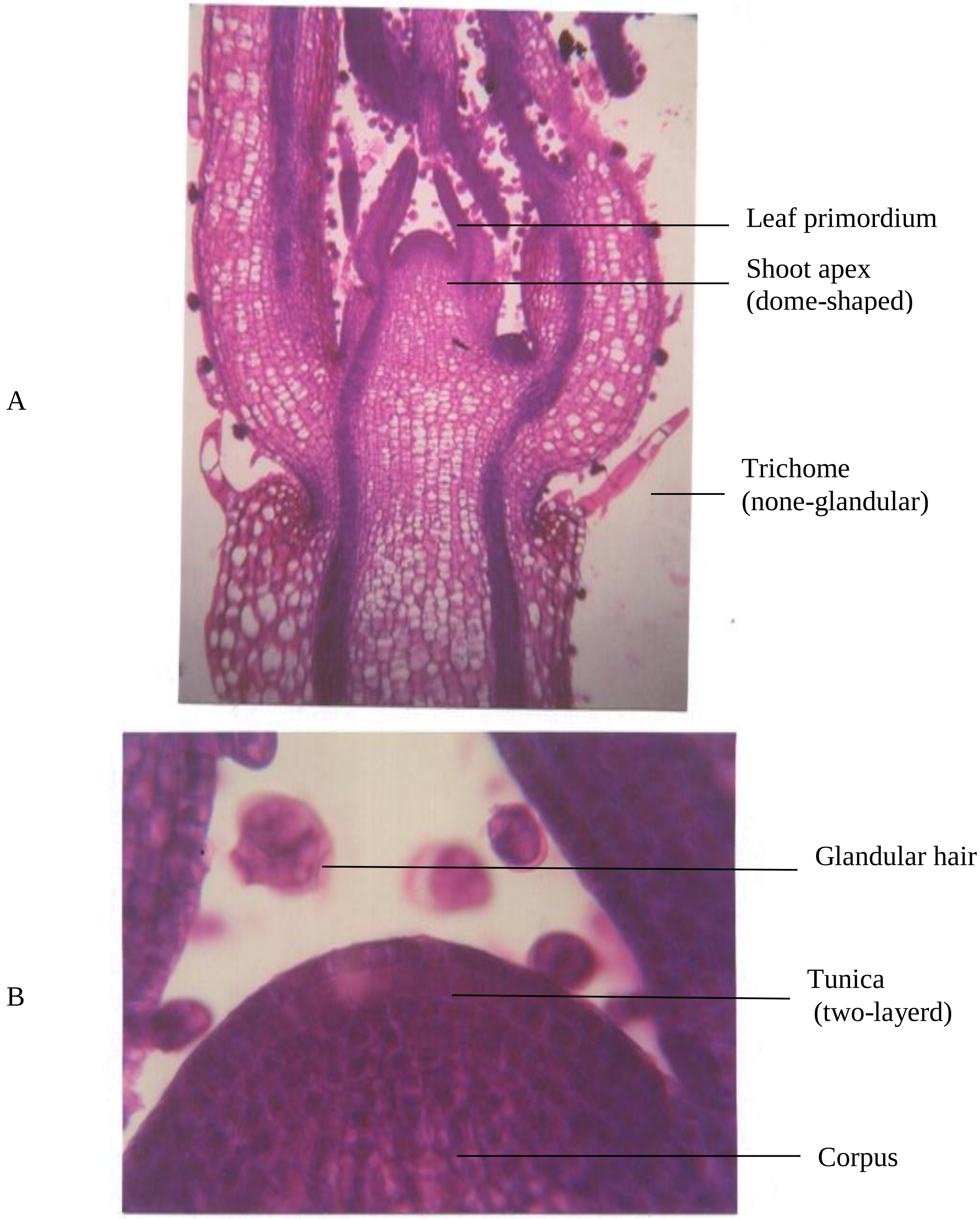


Fig 1. Longitudinal median section of the shoot apex of *Ocimum basilicum* L., at the age of four weeks, showing the two-layered tunica and corpus.

A- Whole section. (x 52)

B- Magnified portion of A. (x 360)

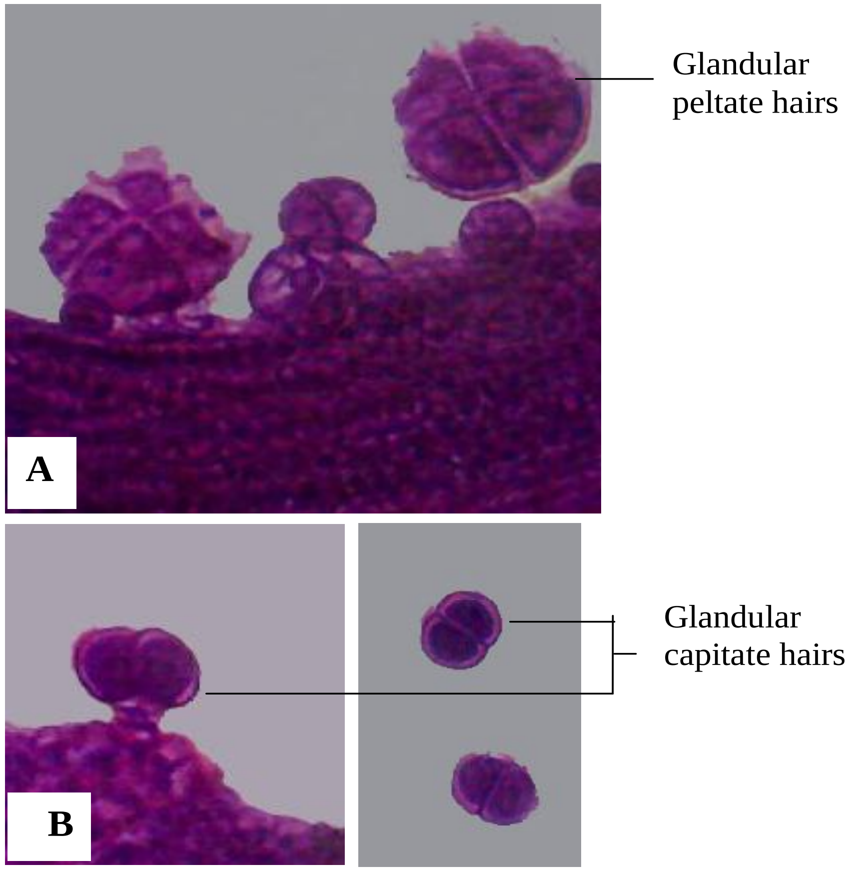
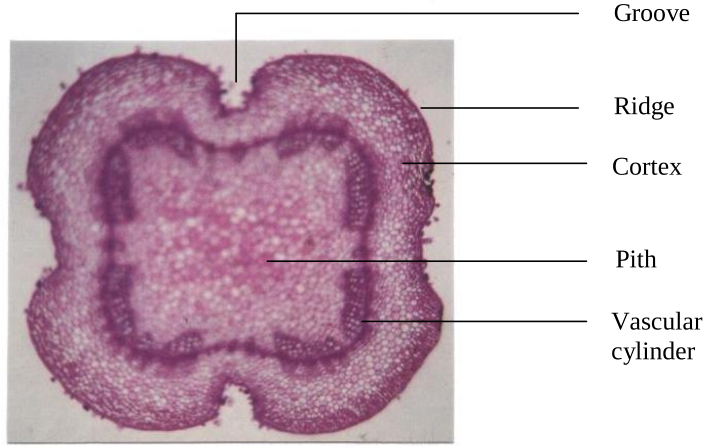


Fig 2. Glandular hairs accompanied longitudinal section of shoot apex of Basil plant.
A– Peltate hairs, four-celled head. (x 720)
B – Capitate hairs, two-celled head. (x 360)

The stele composed of collateral bundles arranged in a ring, being separated from each other by narrow panels of parenchyma tissue which are a part of the ground tissue. There are four arcs of large collateral bundles located opposite the ridges. Each arc consists of 8-9 continuous collateral bundles almost closed to each other (Fig. 3 B). In addition, there are one to three minor collateral bundles between any of two arcs lying opposite to the furrows. The cambium zone is easily recognized as continuous ring between phloem and xylem, being tetragonal in outline like apical internode in its circumference, and consists of 2-3 layers of thin-walled rectangular cells.

The pith occupies large portion in the center of the section and consists of relatively large polygonal parenchymatous cells with relatively small triangular intercellular spaces. The pith is connected with the cortex through medullary rays of 3-6 rows wide.

A



B

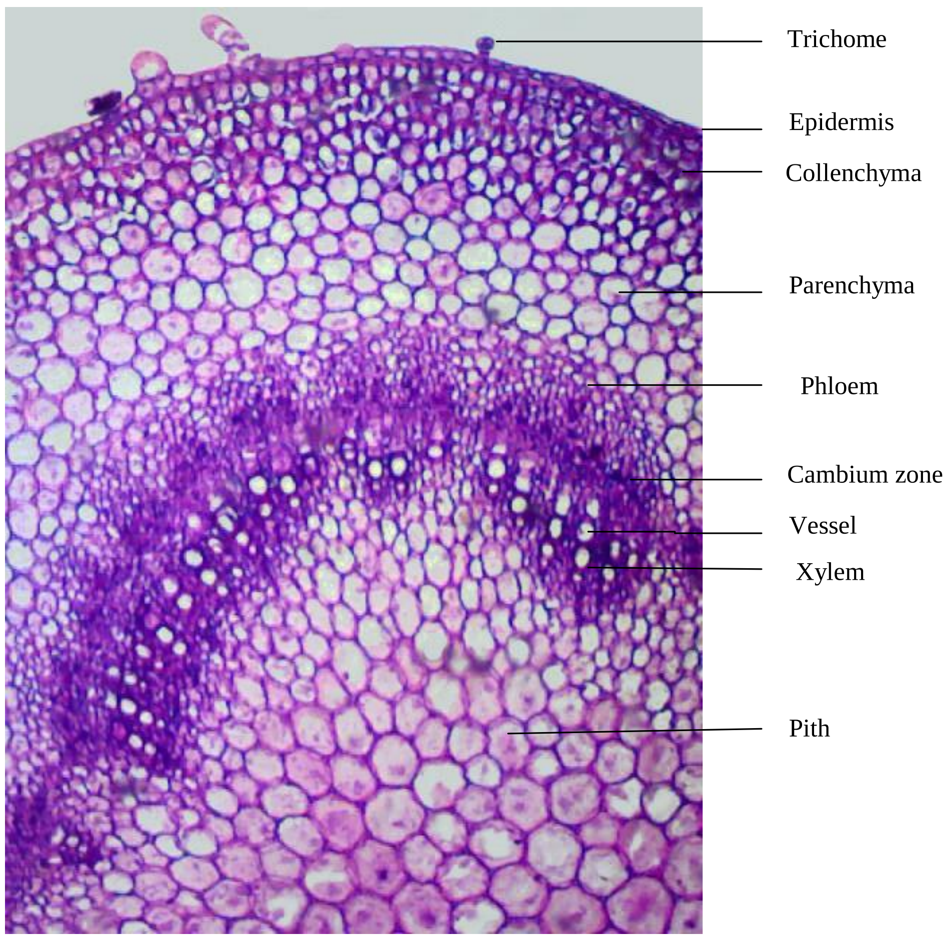


Fig 3. Transverse section of the apical internode of the main stem of *Ocimum basilicum* L.plant at the age of ten weeks.
A : Whole section. (x 36)
B : Magnified portion of A at the region of a ridge. (x 186)
(Cont).

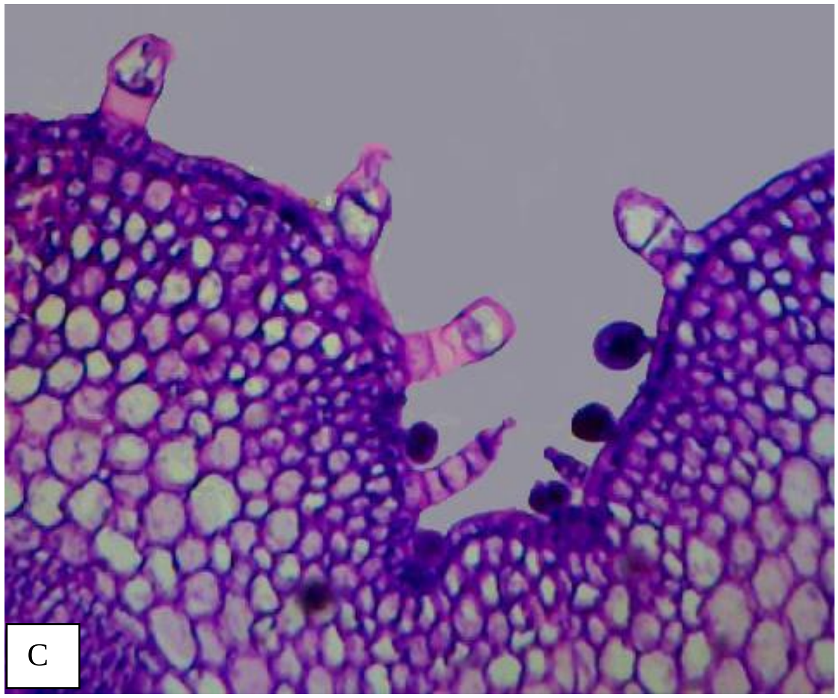


Fig 3. Cont.

C : Magnified portion of A at the region of the furrow showing various kinds of trichomes.

(x 186)

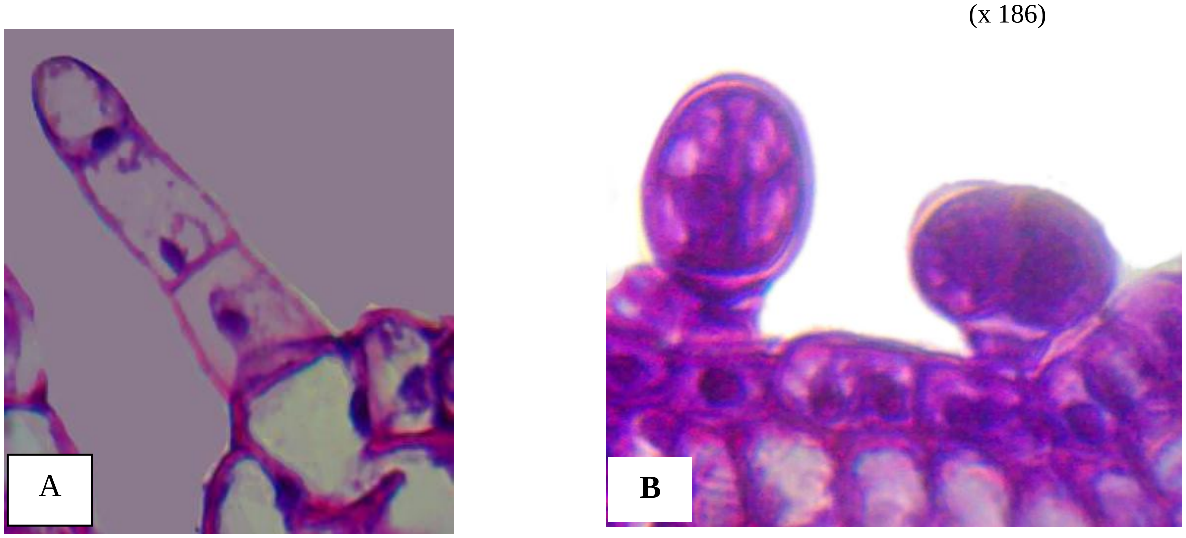


Fig 4. Some trichomes accompanied apical internode of the main stem of Basil plant at the age of ten weeks.

(x 720)

A : Non-glandular uniseriate hair of three cells.

B : Glandular capitate hairs with a head of one elongated oval cell (at left) or two broad cells (at right).

c - The median internode :

The transverse section through the median internode of the main stem of Basil plant at the age of ten weeks is shown in Figure (5). It is clear that the structure of the median internode is generally indifferent with that, previously investigated, of the apical internode. However, the diameter of the median internode is large than that of the apical one. The epidermal cells as well as the collenchyma and parenchyma cells of the cortex are keeping pace with the increase in the stem girth. The epidermal cells respond to the increase in stem diameter stresses by tangential enlargement and radial divisions. Also, many of both kinds of cortical cells show elongation in the tangential direction accompanied sometimes by radial divisions.

The transverse section shown in Figure (5) reveals that the stem surface of Basil plant at its median portion is strongly quadrangular in outline. The stem is decidedly ridged and fluted, composed of four ridges alternating with the same number of furrows constituting its general outline.

The epidermis consists of a single layer of nearly square parenchymatous cells covered with a thin layer of cuticle. Stomata are present and trichomes of various kinds are observed (Fig.6). The cortex underlying the angles composed of 10-12 layers of which the outer 3-4 layers are collenchyma cells abutting the epidermis and constitute the chief mechanical tissue of the axis and form the ridges, followed by one layer of chlorenchyma cells. The rest layers of cortex are parenchyma cells. The cortex in the furrows between the ridges consists of 6-7 layers of parenchymatous cells of which the outer two layers are chlorenchyma abutting the epidermis. The starch sheath, the innermost layer of the cortex, is hardly recognized.

The stele consists of 26 to 29 collateral bundles arranged in a ring, being separated from one another by the ground tissue. The bundles are relatively different in size. The large bundle has 28 to 32 vessels in 9 to 11 parallel rows, while the intermediate bundle has 19 to 23 vessels in 5 to 6 parallel rows and the small one has 7 to 11 vessels in 2 to 3 parallel rows. A complete cambial ring is observed between phloem and xylem.

The pith consists of large polygonal parenchyma cells with relatively small intercellular spaces.

d- The basal internode :

The transverse section through the basal internode of the main stem of Basil plant at the age of 12 weeks are shown in Figure (7). It is clear that the secondary growth takes place in a continuous cylindrical form. Therefore, the stem at its basal portion looses its distinct outline; i.e., disappearance of the ridges and furrows.

The epidermis which still having intact cells shows active dilation accompanied by radial division to accommodate with the increase in stem circumference. Glandular trichomes are observed, mostly of capitate hairs with a head of one elongated oval cell. The cortex consists of about 8 layers of thin-walled parenchyma cells.

Secondary thickening proceeds and secondary growth takes place in a continuous cylindrical form. The secondary xylem has an increased amount of vessels present in nearly radial rows, and the ground tissue where the vessels are embedded is formed of lignified parenchyma cells intermingled with small groups of fibers. Xylem rays are composed of 2 to 3 layers of well lignified parenchyma cells. The primary xylem is recognized abutting on the pith.

The pith consists of polygonal parenchyma cells which tend to decrease in size towards the periphery and enlarged in size at the center. Small triangular intercellular spaces are visible.

As far as the authors are aware no detailed study dealing with anatomical structure of Basil stem was carried out. However, Metcalfe and Chalk (1957) pointed out that stems in many genera and species of Labiatae are quadrangular in transverse section with well-defined groups of collenchyma in the four angles. In the young stems, xylem and phloem are in some species confined to collateral bundles, which are especially well developed in the angles of the axis. The phloem groups of the four large vascular bundles opposite to the four angles of the stem are occasionally connected to one another by sclerenchymatous elements. Xylem forming a continuous cylinder at a very early stage in most of genera and species. Four large vascular bundles in the angles of the stem separated from one another by sclerenchyma with small secondary vascular strands embedded in it. Pith commonly homogeneous. Many species are densely covered with hairs of various kinds, one of the most characteristic being the short-stalked glands with head of 1-16 or more cells. Non-glandular hairs are also frequent, and may be uniseriate, tufted or branched. In this connection, Kahraman, et al. (2009) studied the anatomical structure of *salvia indica* L. stem through transverse sections. It was found that epidermis is covered by a thin layer of cuticle.

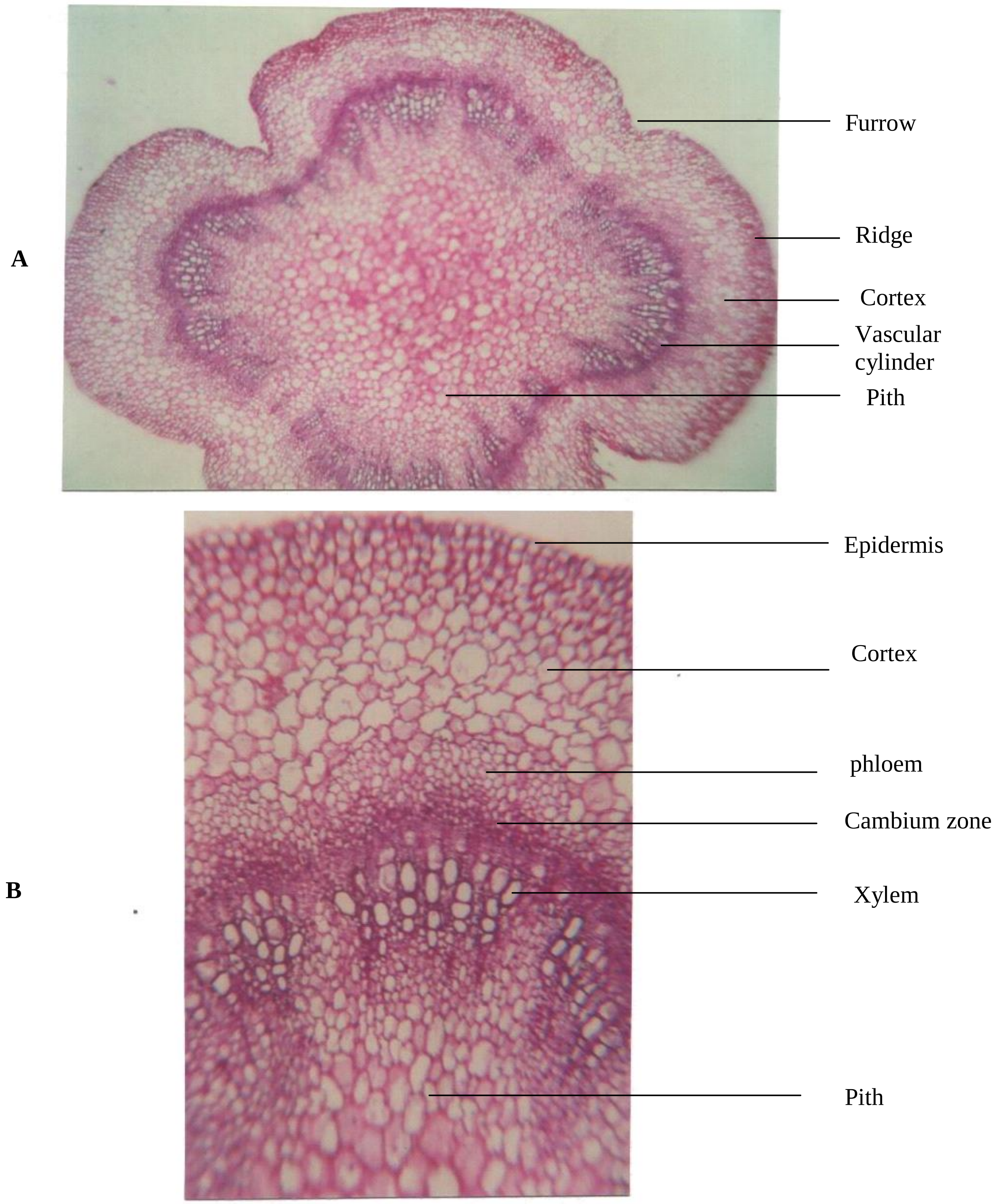


Fig 5. Transverse section through median internode of the main stem of *Ocimum basilicum* L. at the age of ten weeks.
A : Whole section. (x 36)
B : Magnified portion of A at the region of a ridge. (x 102)

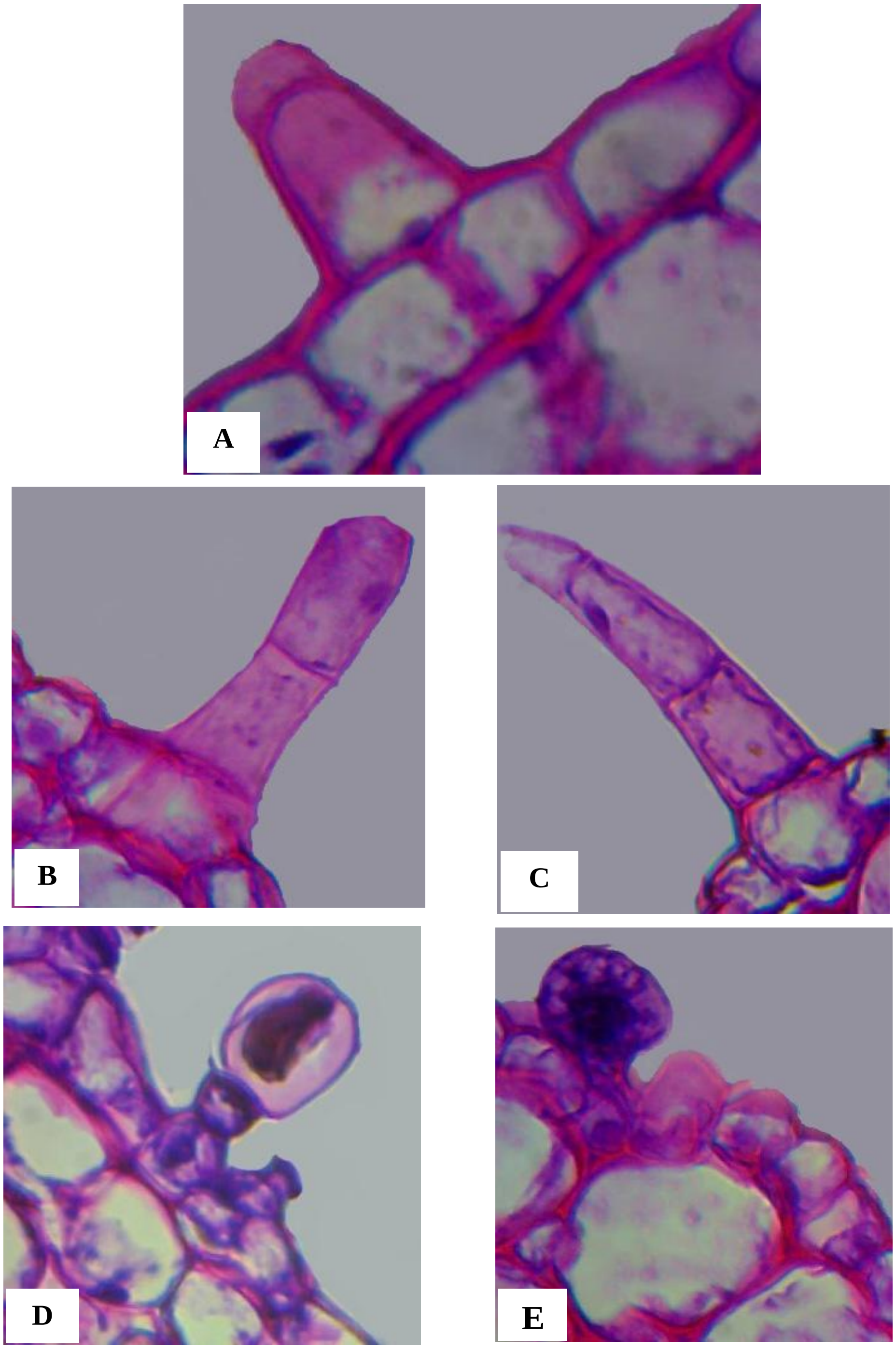


Fig 6. Kinds of trichomes accompanied median internode of the main stem of Basil plant aged ten weeks. (x 720)

A, B and C : Non-glandular uniseriate hairs of one, two or three cells; respectively.

D : Glandular capitate hair with a head of one elongated oval cell.

E : Glandular peltate or scale hair with a head of more than four cells.

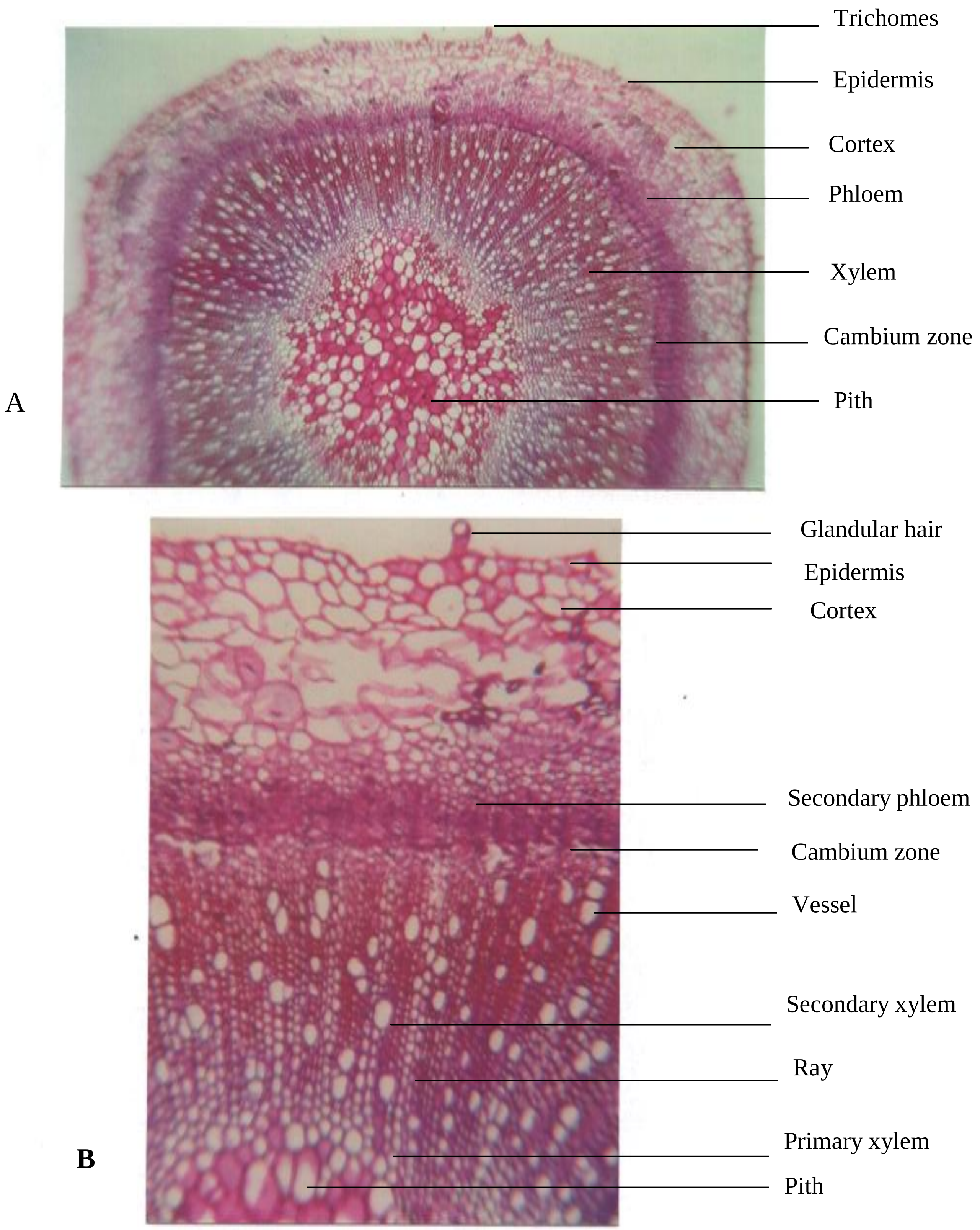


Fig 7. Transverse section of the basal internode of the main stem of *Ocimum basilicum* L. at the age of 12 weeks.

A : Most of the whole section. (x 36)

B : Magnified portion of A. (x 102)

Epidermis consists of uniseriate oval or rectangular cells and contains sparsely or densely eglandular or glandular trichomes . The collenchyma tissue , which is located immediately under the epidermis, is composed of regular cells. There are 3-5 layers of chlorenchymatous cortex with a lot of chloroplast below the epidermis in between the corners. The cortex consists of 2-6 layers of squashed oval or almost rectangular parenchymatous cells with intercellular spaces. Vascular bundles are often separated by parenchymatous cells. Sizes of the vascular bundles at the corners are more or slightly larger than the others. Sclerenchymatic sheat above the phloem comprises of 6-30 cells in between the corners which are more in the corners. Cambium is not distinguishable. The phloem and the xylem members are clear. The xylem elements occupy a larger region than the phloem. The pith is wide and consists of hexagonal or orbicular parenchymatous cells with intercellular spaces. In this respect, Ingole (2012) stated that transection of young stem of *Ocimum Americanum* L. is quadrangular in outline with prominent ribs at 4- corners with deep notches in between. 2-celled uniseriate non-glandular trichomes present on raised hair bases. Epidermis 1-layered, cells barrel- shaped, with outer and inner walls arched or slight angular. Cuticle moderate thick. Hypodermis 1-3 layered, collenchymatous, discontinuous at shallow portions ; interrupted by chlorenchyma. Inner cortex narrow, 3-5 layered, parenchymatous. Cells regular , thin–walled, more or less even, horizontally slight elongated. Vascular cylinder with outer continuous ring of phloem, 4-5 cells high; xylem inner to cambium traversed by 1-rowed medullary rays. Vessels in radial multiples of 2 or solitary , oval-circular in outline. Older xylem with solitary vessels. Pith 12-17 layered wide, homogeneous, parenchymatous. All, generally, being in harmony with the present findings .

3 - Structure of the leaf :

a - Leaf blade (lamina) :

The anatomical structure of leaf blades representing different simple foliage leaves developed on the main stem and on lateral branches of Basil plant throughout successive ages of its entire life span was investigated in form of transverse sections.

It is clear that all investigated blades have almost the same structure. Leaves are dorsiventral (Figs. 8, 9 and 10); i.e., the palisade tissue is located on the adaxial side of the blade (upper side) and the spongy tissue on the abaxial one (lower side).

There are two epidermal layers on adaxial and abaxial surfaces of the leaf blade, each is uniseriate and composed of a row of compactly-set tabular cells. Some regions of both upper and lower epidermises are papillose consisting of specially large oval cells. The outer walls are cutinised and possess thin cuticle. Stomata occur on both surfaces, being more numerous on the lower epidermis than on the upper one. They are of anomocytic or irregular-celled type (ranunculaceous type); i.e.,no subsidiary cells are present, several ordinary epidermal cells irregularly surround the stoma (Figs. 11 , 12 ,13 and 14). Trichomes are present. The occurrence together of diverse kinds of non-glandular hairs (uniseriate) and characteristic short-stalked glands with uni-or multicellular heads (capitate and peltate hairs) is characteristic of Basil leaf epidermis (Figs. 8, 9, 13 and 15). In this concern, Werker et al. (1993) stated that leaves of *Ocimum basilicum* L. bear non-glandular and glandular hairs on both sides. The non-glandular hairs are uniseriate, pointed, straight or hook- like, consists of one to five thick-walled cells. The glandular hairs consist of small capitate hairs and larger peltate hairs or scales. The capitate hairs are composed of one base cell, one stalk cell and a head of either one elongated , oval cell or two broad cells. The peltate hairs have similar base and stalk cells but a broad four-celled head. Very rarely the head consists of more than four cells, being in good agreement with the present findings.

The palisade tissue consists of one layer of rectangular cells which elongated perpendicularly to the surface of the blade being characterized by abundance of chloroplasts (Fig.10). The palisade tissue occupies one-third of the whole thickness of the mesophyll. Whereas, the spongy tissue is composed of almost four layers of chlorenchymatous loosely arranged cells with many wide intercellular spaces. The mesophyll is slightly extended in the midrib region.

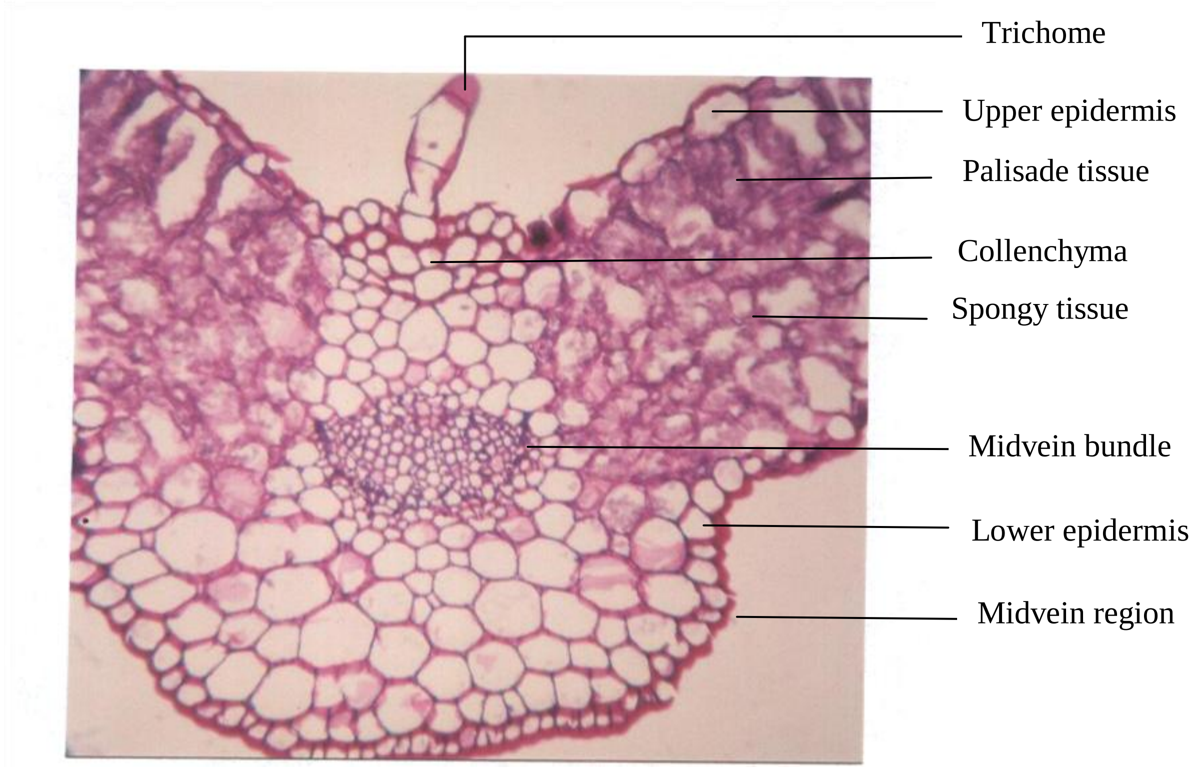


Fig 8. Transverse section through the blade of foliage leaf developed on the third node of the main stem of *Ocimum basilicum* L. plant aged six weeks. (x144)

At the midrib region, the upper epidermis is almost flate, while the lower one is convex. There is a mass of collenchyma cells below the adaxial (consists of 2-3 layers) and abaxial (consists of 2 layers) epidermises at the midrib region. Therefore, the included bundle, the principle one, is not directly embedded in the mesophyll as do the smaller ones. The principle vascular bundle of the midvein is accompanied from above and below by a parenchyma cells of the ground tissue and the bundle is oriented with the xylem directed toward the adaxial surface and the phloem toward the abaxial one. The xylem consists of about 15 to 20 parallel rows depending on the size of the bundle according to the age of the leaf of which the section was investigated, each xylem row comprised of 6 to 9 vessels.

Fig 9. Transverse section through midvein portion of a simple foliage leaf developed on the six node of the main stem of *Ocimum basilicum* L. plant aged ten weeks. (x 52)

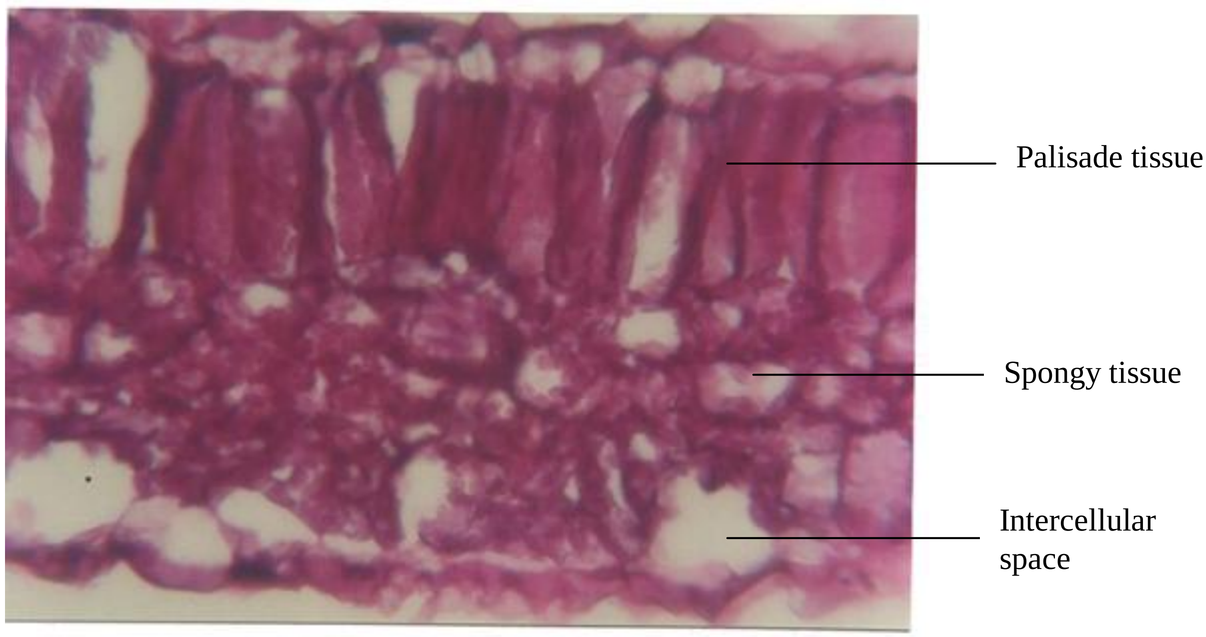


Fig 10. Transverse section through marginal portion of a simple foliage leaf developed on the six node of the main stem of *Ocimum basilicum* L. plant aged ten weeks. (x144)

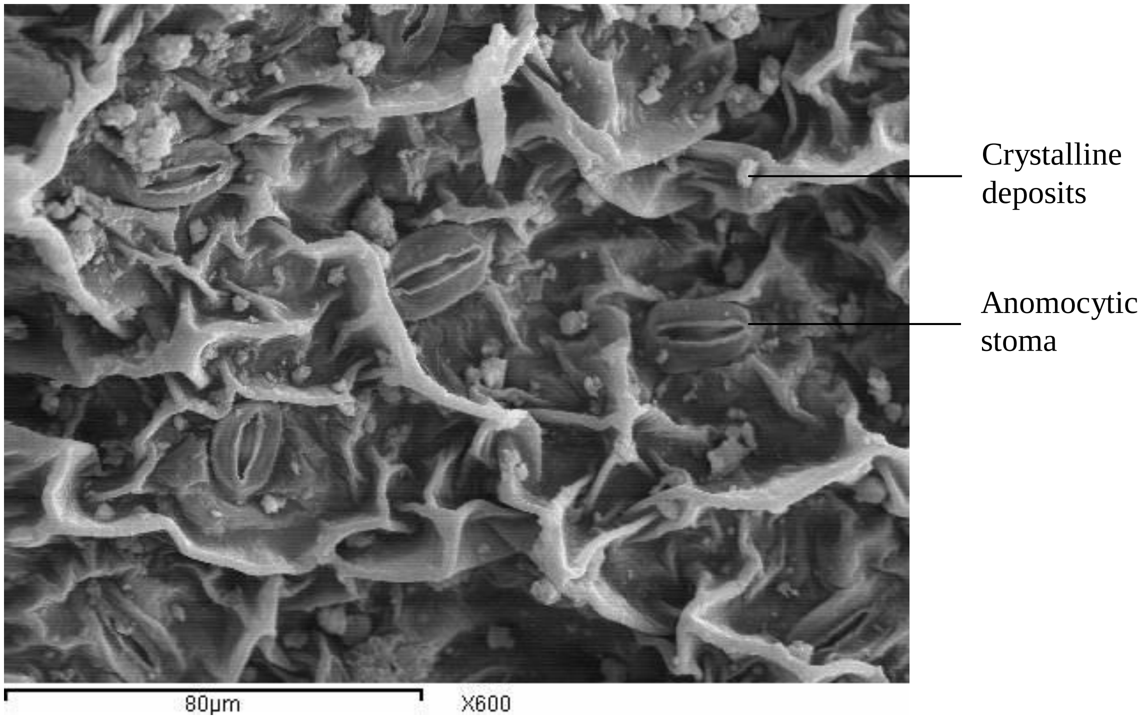


Fig 11. A scanning electron micrograph of the adaxial surface in Basil leaf blade showing stomata of anomocytic type (Ranunculaceous). Scale bar = 80 µm.

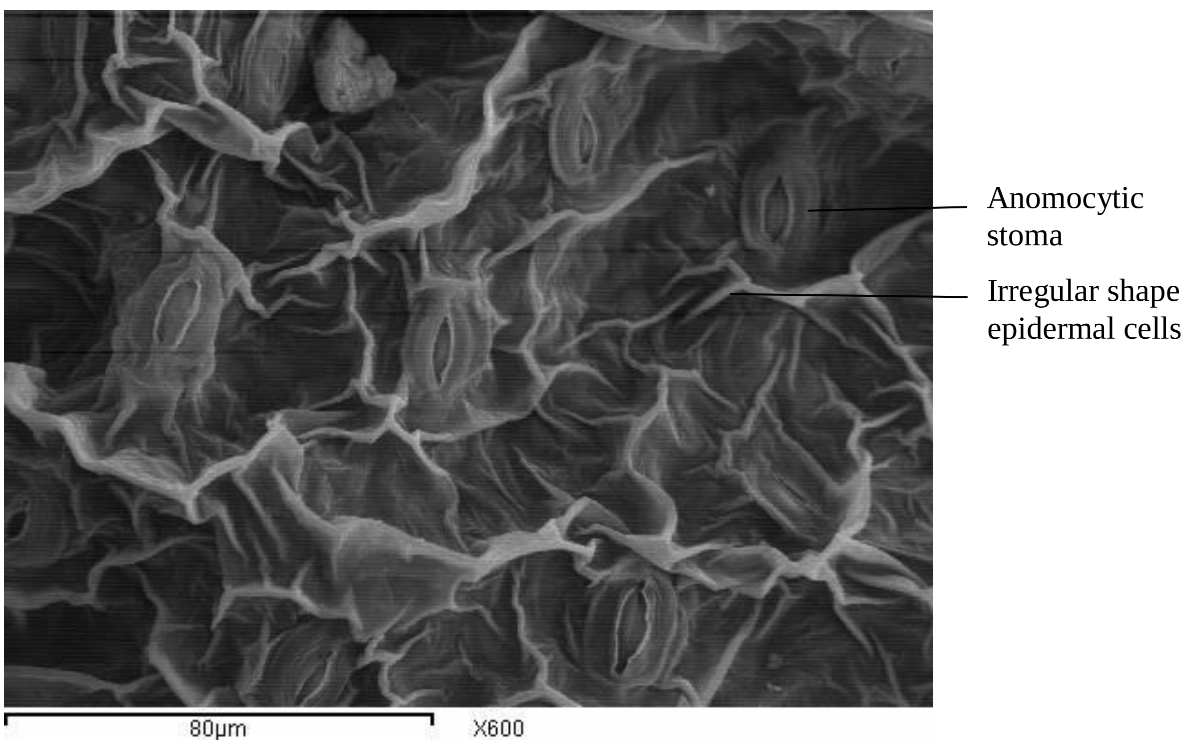


Fig 12. A scanning electron micrograph of the abaxial surface in Basil leaf blade showing stomata of anomocytic type. Scale bar = 80 µm.

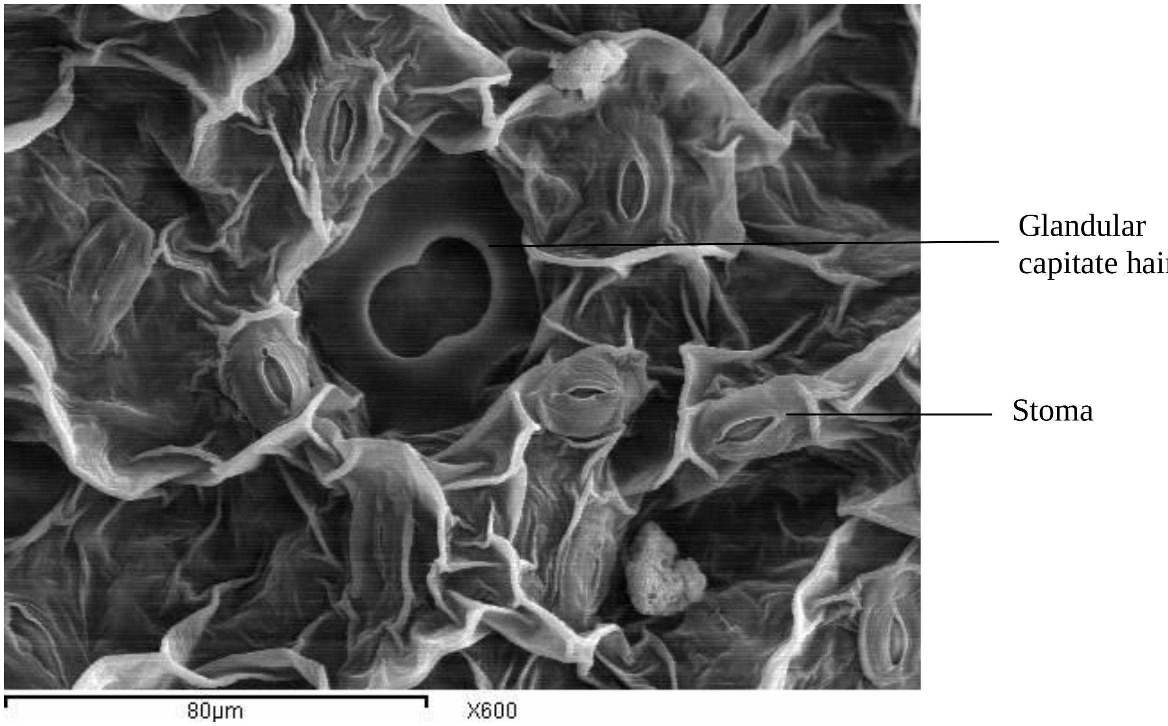


Fig 13. A scanning electron micrograph of the abaxial surface in Basil leaf blade showing glandular capitate hair. Scale bar = 80 µm.

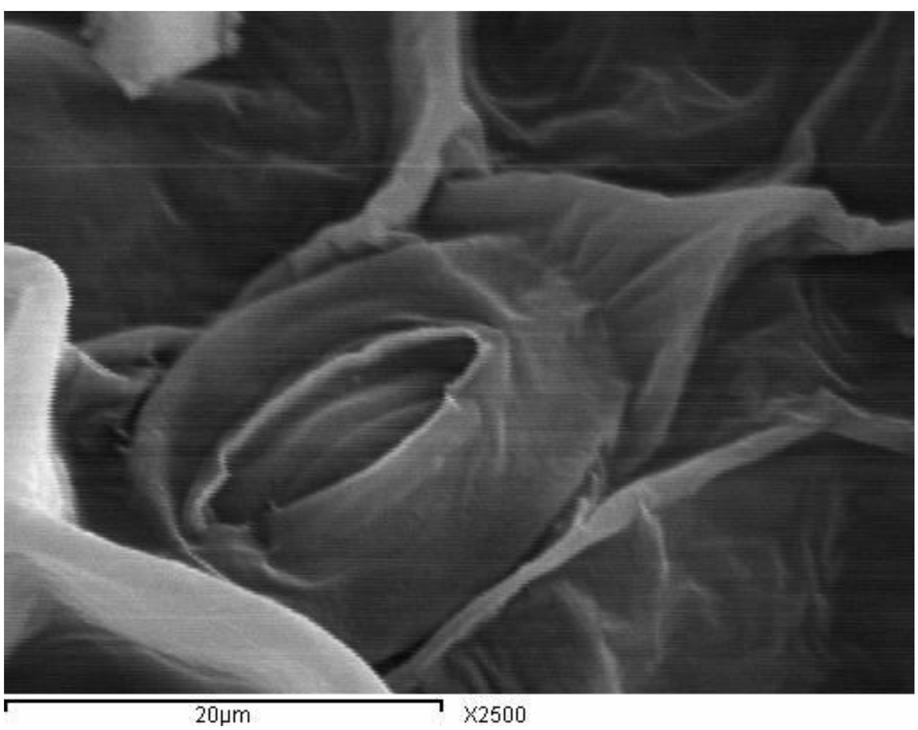


Fig 14. A scanning electron micrograph showing magnified anomocytic stoma present on abaxial surface of Basil leaf blade. Scale bar = 20 µm.

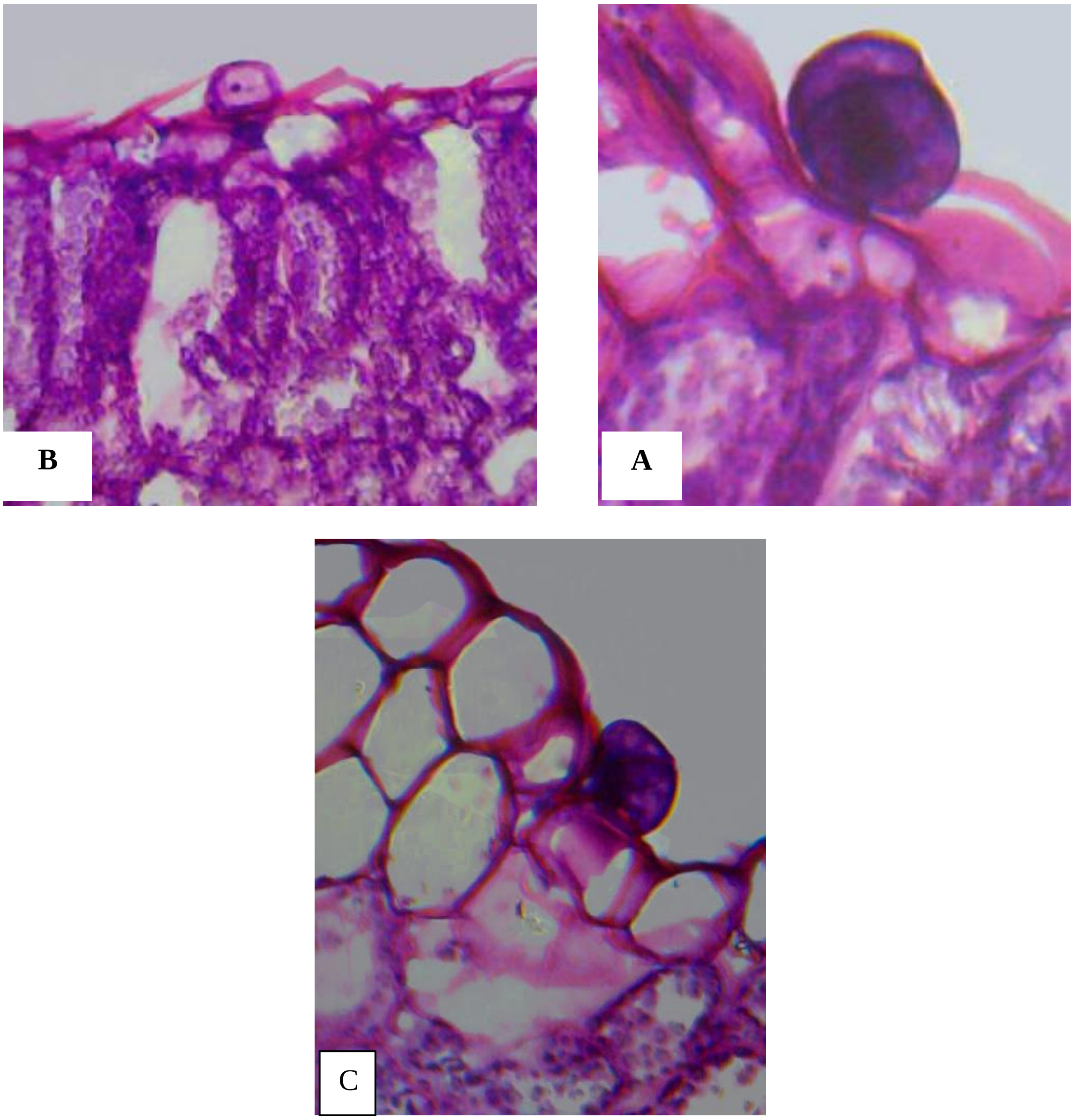


Fig 15. Certain glandular hairs accompanied leaf blades of *Ocimum basilicum* L. plant.

A- Glandular capitate hair with a head of one rounded cell. (x 360)

B- Glandular capitate hair with a head of two broad cells. (x 720)

C - Glandular peltate (scale-like) hair with a head of multicellular. (x 720)

b- Leaf petiole :

The petiole of Basil leaf as seen in the transverse section (Fig.16) is reniform in shape; i.e., transectional outline through middle part of the petiole is roundish abaxially and shallowly depressed in central region adaxially. It is bounded by uniseriate epidermis of nearly square-shaped cells. The outer walls of the epidermis cells are somewhat thickened and covered with a thin layer of cuticle. Stomata and trichomes of various kinds are present (Fig.17). Beneath the epidermis there is one layer of collenchyma cells followed by another layer of chlorenchyma cells. The ground tissue consists mostly of relatively large polygonal parenchyma cells with small triangular intercellular spaces.

At the center of the petiole transection there is a large wide collateral bundle embedded in the ground tissue, beside two small traces located in the two lateral wings each embedded in the ground tissue of each wing. The main large vascular bundle is shallow arc in shape and oriented with the xylem directed toward the adaxial surface and the phloem toward the abaxial one. The xylem consists of about 26 parallel rows each comprised of 3-6 vessels.

Ingole (2012) described The internal structure of young petiole of *Ocimum Americanum* L. leaf and pointed out that the transectional outline through middle region, roundish abaxially, shallowly depressed in central region adaxially, margin regular. Adaxial and abaxial epidermis-1-layered, discontinuous at few places, cells larger, roundish, and rectangular, outer and inner walls roundish, cuticle thick. Multicelluar uniseriate, non–glandular trichomes present. Hypodermis-indistinct, discontinuous at few places, collenchymatous, 1-layered adaxially, 1-layered abaxially and 2-3 layered under wings; cells smaller, inner larger, uneven sized. Ground tissue broad, parenchymatous, enclosing small intercuticular spaces; cells polygonal, roundish; inner layers compact and chlorenchymatous patches below wings. Pattern of vascular configuration in middle region- displaying single median shallow crescent with simple widely placed ends, xylem directed toward adaxial concave side, phloem towards abaxial side of crescent, vessels circular in outline, in radial multiples of mostly 4. Apical and basal regions-exhibiting similar median vascular patterns as in middle region. Perivascular sclerenchyma -absent. Collateral, additional accessory vascular bundles in wings 1-each locted below hyopdermis; xylem adaxially faced; perivascular sclerenchyma absent; vessels in radial multiples of 3; in middle region of petiole 1-per wing; in apical region and in basal region-wing bundles absent, being generally in harmony with the present findings.

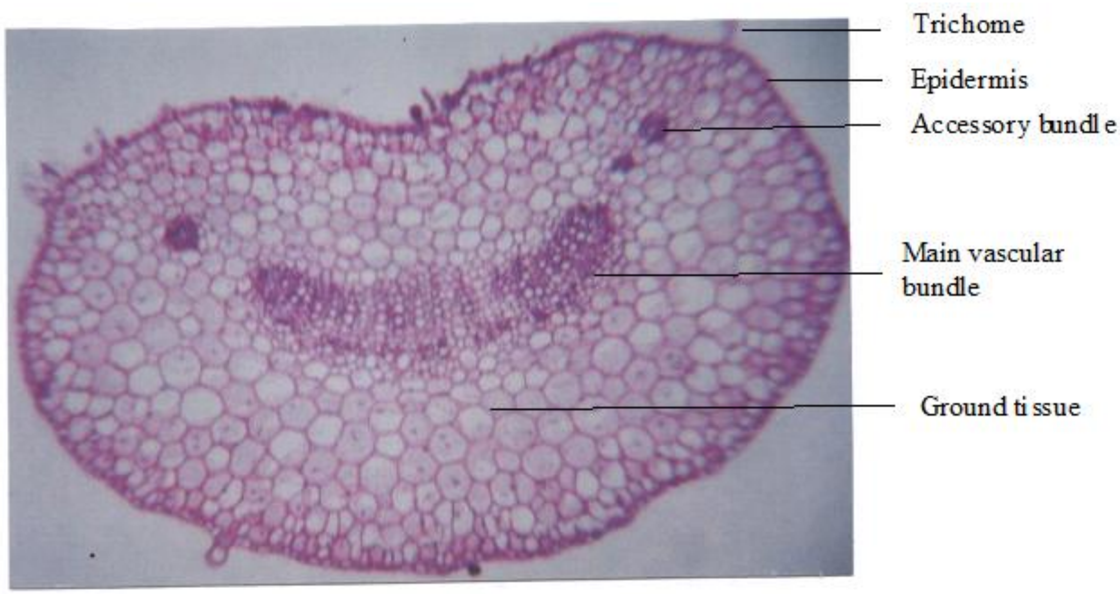


Fig 16. Transverse section throught the petiole of a simple foliage leaf developed on the six node of the main stem of *Ocimum basilicum* L. plant aged 10 weeks . (x 52)

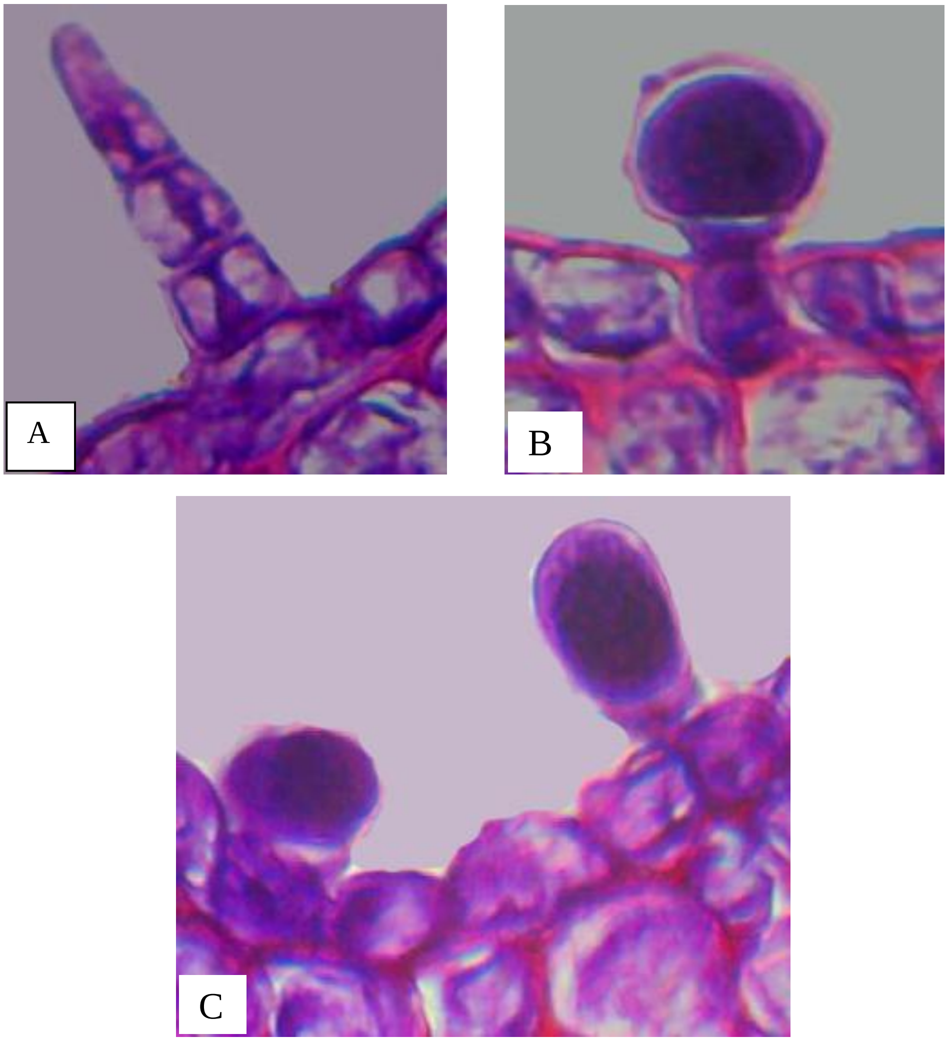


Fig 17. Some trichomes accompanied epidermis of leaf petiole of O*cimum basilicum* L. plant.
A : Uniseriate non-glandular hair. (x720)
B and C : Glandular capitate hairs . (x720)

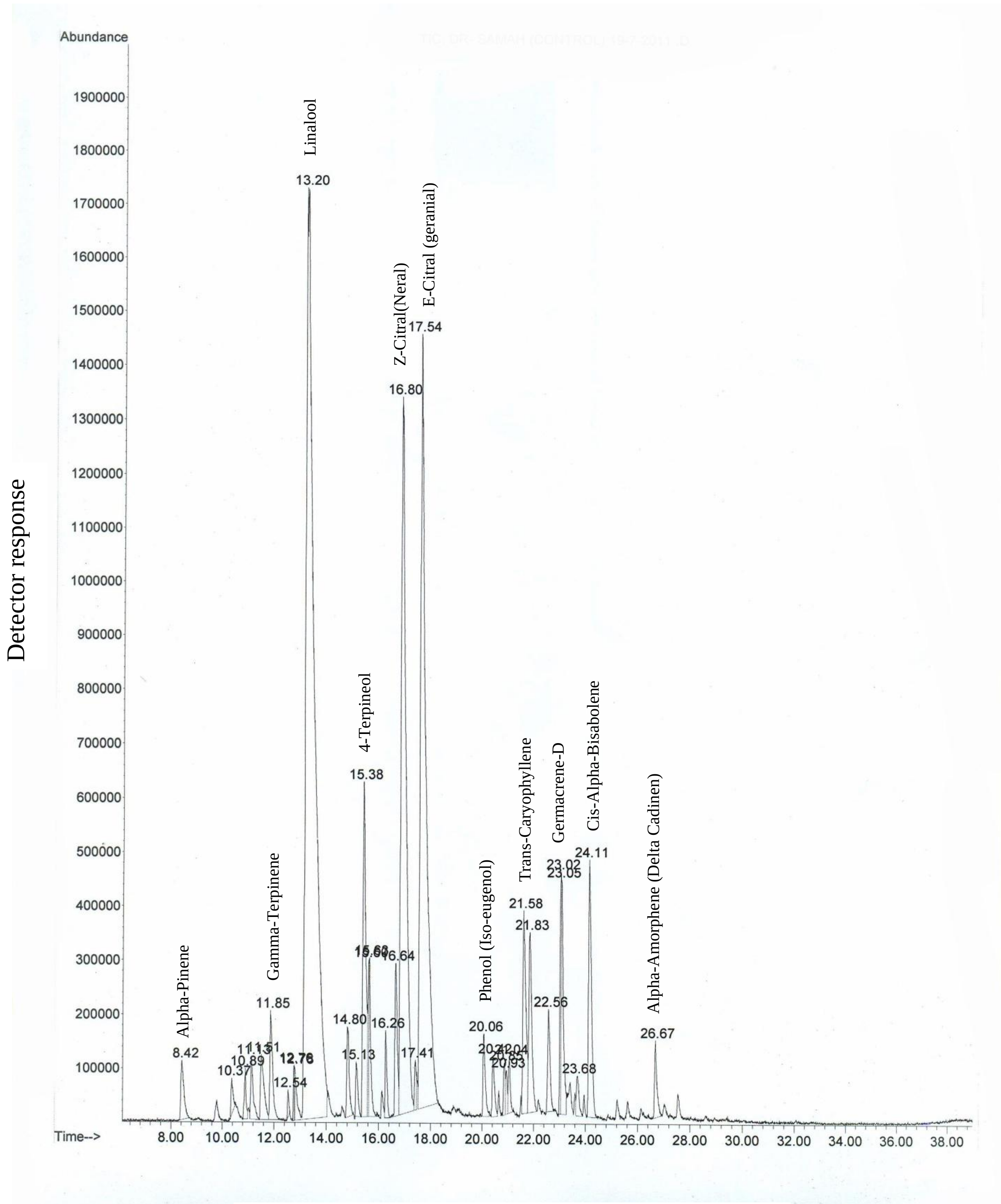
II- Analysis of the volatile oil :

The volatile oil of Basil herb at full blooming stage was obtained by means of water-steam distillation. Basil herb at flowering stage yielded 0.6 % of volatile oil.

Using GC-MS technique in analyzing volatile oil of Basil herb (Fig.18) proved the presence of 39 components. Data presented in Table (1) clearly show that the major constituents present are linalool (constitute 32.69% of the volatile oil), geranial (constitute 17.41% of the volatile oil) and neral (constitute 14.77% of the volatile oil) followed by 4-terpineol (4.02%), germacrene–D (3.27%) ,cis-alpha–bisabolene(3.06%), trans-caryophyllene (2.60%) and bicyclo (3.1.1) heptane (2.28%). In addition, some constituents were detected at the percentage of 1.17 to 1.78% such as cyclofenchene (1.78%), nerol (1.55%), gamma- terpinene (1.24%) and alpha-caryophyllene (1.17%). Other constituents (the remainder which comprised 27 components) were found at the rate of less than 1.0% (from 0.13% , beta –bisabolene , to 0.98%, eucalyptol).

Table 1 .Volatile oil of *Ocimum basilicum* L. herb at flowering stage, retention time, components and their percentages

Peak No.	Retention time, min.	Components	%
1	8.42	Alpha-Pinene	0.83
2	9.79	Sabinene	0.24
3	10.37	Beta-Pinene	0.70
4	10.88	Alpha-Terpinene	0.38
5	11.12	Limonene	0.71
6	11.50	Eucalyptol (1,8-cineol)	0.98
7	11.86	Gamma-Terpinene	1.24
8	12.54	Alpha-Terpinolene	0.20
9	12.76	Cis-Sabinene Hydrate	0.74
10	13.17	Linalool	32.69
11	14.81	2,2-Dimethylocta-3,4-dienal	0.97
12	15.14	Cyclohexane	0.53
13	15.39	4-Terpineol	4.02
14	15.62	Cyclofenchene	1.78
15	16.12	Linalyl Propionate	0.21
16	16.26	Acetic Acid	0.64
17	16.64	Nerol	1.55
18	16.81	Z-Citral (neral)	14.77
19	17.41	Geraniol	0.57
20	17.55	E-Citral (geranial)	17.41
21	20.05	Phenol (Iso-eugenol)	0.83
22	20.42	Neryl Acetate	0.59
23	20.65	Alpha-Copaene (Alpha-cubebene)	0.15
24	20.85	1,2,6-Octadienol	0.37
25	20.93	Germacrene-D	0.31
26	21.04	Beta-Elemene	0.52
27	21.59	Trans-Caryophyllene	2.60
28	21.83	Bicyclo[3.1.1]Heptane	2.28
29	22.56	Alpha-Caryophyllene	1.17
30	23.03	Germacrene-D	3.27
31	23.41	Junipene	0.48
32	23.60	Beta-Bisabolene	0.13
33	23.68	Naphthalene	0.49
34	23.94	Gamma-Cadinene	0.15
35	24.12	Cis-Alpha-Bisabolene	3.06
36	25.21	1,6,10-Dodecatrienol-3	0.21
37	25.62	Farnsyl Acetate,2	0.71
38	26.67	Alpha-Amorphene	0.76
39	27.54	Trans-Beta-Farnesene	0.28



Constituents and their retention time, min.

Fig 18. GC / MS of volatile oil of Basil herb (*Ocimum basilicum* L.) at full blooming stage

From the aforementioned results it could be stated that Basil herb at full blooming stage yielded 0.6 % of volatile oil. The main constituents are linalool which comprised 32.69% of the volatile oil followed by geranial which comprised 17.41% of the volatile oil and neral which comprised 14.77% of the volatile oil. Such three main components comprised 64.87% of the volatile oil of Basil herb. The rest 36 components comprised 34.65% of the volatile oil of Basil herb. In this respect, Hiltunen (1999) reported that Basil herb (*Ocimum basilicum* L.) contains 0.5 -1.5% essential oil of varying compositions. Özcan and Chalchat (2002) identified 49 components , using GC-MS technique, accounting 88.1% of the essential oil isolated by hydrodistillation of the overground parts of *Ocimum basilicum* L. from Turkey. It was found that the essential oil of *Ocimum basilicum* L. was characterised by its high content of methyl eugenol which comprised 78.02% of the essential oil. At the same time, lee et al. (2005) stated that the major aroma constituents of Basil essential oil were linalool (39.8%), estragole (20.5%) , methyl cinnamate (12.9%), eugenol (9.1%) and 1,8-cineole (2.9%). Likewise, Ismail (2006) using GC-MS in analysing the essential oil of *Ocimum basilicum* L. grown in Egypt and reported that the major terpenes present are linalool (44.18%), cineole (13.65%), eugenol (8.59%), α-cubebene (4.97%), methyl cinnamate (4.26%) and isocaryophyllene (3.10%). In this connection, Sajjadi (2006) found that the yield of the essential oils obtained from aerial parts of *Ocimum basilicum* L. cv. purple and *Ocimum basilicum* L. cv. green (cultivated in Iran)were 0.2 and 0.5 % (V/W); respectively. Twenty compounds of the oil of Basil cv. purple and twelve components of Basil cv. green oil were identified (98.5 and 99.4% of the total essential oils ; respectively). The main constituents found in the oil of basil cv. purple were methyl chavicol (52.4%), linalool (20.1%), epi-α-cadinol (5.9%), trans-α-bergamotene (5.2%) and 1,8-cineole (2.4%). In the oil of Basil cv. green, methyl chavicol (40.5%), geranial (27.6%), neral (18.5%), caryophyllene oxide (5.4% and humulene epoxide II (1.8%) were the major components. In this respect, Al-maskari et al. (2011) reported that Omani Basil herb contains 0.17% of essential oil. Linalool was identified as the major component comprised 69.9% of the essential oil of Omani Basil herb. All, being partially, in accordance with the present findings. Worthy to mention that the observed differences in composition of volatile oils of Basil genotypes recorded in the literature may be probably due to different environmental and genetic factors, different chemotypes and the nutritional status of the plants as well as other factors that can influence the oil composition.

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