EVALUATION OF MORPHO-ANATOMICAL VARIATIONS IN *Ocimum* Spp.

BY

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THESIS

Submitted in partial fulfilment of the
requirement for the degree of

Master of Science in Agriculture

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1996

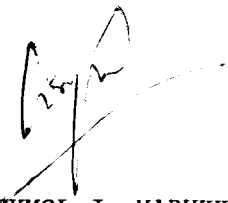
IN LOVING MEMORY

OF

MY MOTHER

DECLARATION

I hereby declare that this thesis entitled ***Evaluation of Morpho-anatomical variations in Ocimum spp.*** *is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.*



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CERTIFICATE

Certified that this thesis entitled ***Evaluation of Morpho-anatomical variations in Ocimum spp.*** *is a record of research work done independently by Ms.* ***LISYMOL J. VADUKKOOT,*** *under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.*

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LISYMOL J. VADUKKOOT

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Introduction

INTRODUCTION

Ocimum is an important genus which includes several species yielding essential oil that is valued in medicine and perfumery. Oil of some like sweet basil has insecticidal properties also. A few like Karpurthulasi is an excellent alternative to camphor and can be grown as pure crop or intercrop in coconut and arecanut gardens.

The nomenclature of *Ocimum* is complicated and confused and hence, eventhough different species yield essential oil, it is difficult to classify oils according to botanical nomenclature. Further the plants are collected by people who base their identification on a few characters only. In many cases samples are adulterated with plant organs of similar morphology. There is, at present, no scientific control at any stage of collection and it passes through so many hands that indiscriminate adulteration/ substitution is possible.

The anatomical markers like trichomes, crystal, xylem vessels etc and the protein banding pattern along with morphological features will serve as ready reckoner for the correct identification of the species. These have also great relevance to our understanding of the phylogenetic trends and systematic relationships of different species of *Ocimum*.

Hence this project entitled `Evaluation of Morpho-anatomical variations in *Ocimum* spp' was undertaken with the following objectives of:

1. evaluating and correlating the morphological and anatomical features existing in different species of *Ocimum*.

2. making a comparative evaluation of protein banding pattern and essential oil content of leaves of different species and

3. preparing a key for identification of the different species.

Review of Literature

REVIEW OF LITERATURE

2.1 Morphoanatomical aspects

The study of dermatotypes of 10 species of *Ocimum* by Gupta and Bambie (1978a) revealed that the 16 types of trichomes observed, fall under four categories, such as capitate glandular, non-glandular, uniseriate filiform, non-glandular biseriate filiform and non-glandular capitate. They also suggested that on the basis of trichome types, different species of *Ocimum* can be identified.

Another investigation on venation pattern of the species of *Ocimum* by Gupta and Bambie (1978b) supported the proposition that venation pattern did not have much significance in the delimitation of species in the genus *Ocimum*.

In a comparative study of normal and tricotylous seedlings, Gupta and Bambie (1978c) reported that they showed triarch root and unilacunar two-trace cotyledon. Tricotyly in *O. sanctum* is the result of the differentiation of supernumerary primordia during embryogenesis.

Moreno *et al.* (1987) reported large variations in plant morphology, particularly in shoot architecture and types of inflorescences, flower, fruit and leaf among seventeen introductions of the genus *Ocimum* including four

cultivars of *O. basilicum*, two of *O. minimum*, two of *O. gratissimum*, two of *O. micranthum* and seven of *O. americanum*.

In eugenol type *O. gratissimum*, Colson *et al.* (1991) reported glandular hairs which are four celled headed and two celled headed capitate as well as non-glandular trichomes.

Werker *et al.* (1993) reported that glandular hair density was very high on young meristematic leaves and on the meristematic regions of older leaves. As leaf expansion occurred, no new glandular hairs were produced and their density declined. Leaf essential oil content decreased as leaves expanded.

2.2 Origin and distribution

Kirtikar and Basu (1918) suggested that *Ocimum* is cultivated throughout India, but doubtfully indigenous to Malay Archipelago, Australia, West Asia and Arabia. According to the reports of CSIR (1966), *Ocimum* is a genus of aromatic herbs, undershrubs or shrubs, distributed in the tropical and warm temperate regions of the world. Sobti *et al.* (1976) studied the geographical distribution of 160 reported species of the genus *Ocimum* and found that the genus is well distributed in the warmer parts of both hemispheres from sea level to 6000 ft. Considering Vavilov's view, they reported that

Central and West Africa are the primary regions of origin of *Ocimum* species. Their study on distribution of various species in India revealed that *O. canum* is confined mostly to southern parts, *O. basilicum* occur throughout India, *Ocimum americanum* spread in North Western regions of India, *O. gratissimum* and *O. adscendens* have distributional range within southern and south eastern regions of the Indian subcontinent, *O. sanctum* is cultivated in almost every part of India and *O. kilmandscharicum* introduced to India does not have a natural distribution.

2.3 Biochemical aspects

Dickerson (1972) has pointed out that proteins are valuable taxonomic characters because they exhibit conservatism in evolution.

Guenther (1974)has pointed out that even though different species of *Ocimum* yield essential oil, it is difficult to classify oils based on botanical nomenclature of plants from which they are derived.

Piechura and Fair Brothers (1983) reported that analysis of protein extracts of 12 representative taxa of Oleaceae family by electrophoresis yielded taxonomic informations.

Xason *et al.* (1983) could get a large amount of mixture of sterols like stigma sterol, sitosterol etc. and triterpenes like oleanolic acid, maslinic acid and 3-epi-maslinic acid from the leaves and flowers of *O. spicatum*.

Dey and Choudhuri (1984) found that in *O. tenuiflorum* among the different plant parts, the leaf contained the highest percentage of oil (0.97%), followed by inflorescence (0.28%) and stem (0.09%) but root was devoid of essential oils. Gas chromatographic analysis of oil showed the presence of ten components of which eugenol, methyl eugenol and caryophyllene were the major components.

Modawi *et al.* (1984) found that in *O. basilicum* var. *thyrsiflorum*, the yield and quantitative composition of the essential oil were found to vary with the plant habitat. The main oil constituents were methyl chavicol and linalol; cineole and eugenole was also found.

Scheffer and Ntezurubanza (1984) found that the oil samples of *O. kilmandscharicum* analyzed contained neither camphor nor eugenol but 1,8-cineole was present as the main component.

Ntezurubanza *et al.* (1985) found that the essential oil from *O. canum* was characterized by a high linalool content (60-90%).

Philip and Damodaran (1985) identified and classified *Ocimum sanctum* types having purple leaves and green leaves in the local cultivars of *Ocimum sanctum* having methyleugenol as the major constituent and another green type having eugenol as the major constituent.

Tripathi *et al.* (1985) found that the constituents of clocimum oil are myrecene, beta-pinene, p-cymene, camphor, alpha-terpineol, alpha-terpinyl acetate, linalool, linalylacetate, geraniol, citronellol and eugenol.

Verma *et al.* (1989) has reported great variability among different *Ocimum* species in morphological traits and essential oil content. The essential oil content varied between 0.16% in *O. basilicum* EC 112807 and 0.43% in *O. basilicum* Indian Basil.

Charles and Simon (1990) extracted essential oils from leaves, flowers and stems of *O. basilicum, O. kilimandscharicum* and *O. micranthum* by solvent extraction, hydro-distillation and steam distillation for essential oil content and the oil analyzed by GC and GC/MS for composition. While the yield of essential oil was consistently higher from steam distillation than hydrodistillation, a similar number of compounds was recovered from both methods. Essential oil content and composition varied by plant species and plant parts. Essential oil content was highest in flowers of

O. basilicum and in leaves for *O. micranthum*. No significant differences were observed in essential oil yield and relative concentration of major constituents using fresh or dry samples and using samples from 75 g to 10 g of dry plant tissue.

Charles *et al.* (1990) observed that in *O. micranthum* the essential oil content between plant parts varied significantly with 1.54, 0.63 and 0.08 (per cent volume/ fresh weight) stems respectively. Essential oil composition also varied by plant part. Eugenol, the major constituent in leaves, was present only in trace amounts in flowers and stems.

Singh and Gulati (1990) studied the essential oils from the wild and cultivated plants of *Ocimum americanum*, from their seeds by GLC, GC/MS for their chemical composition and reported the presence of pinene, camphene, β-pinene, myrcene, p-cymene, 1-8 cineole, terpinolene, linalool etc.

Gupta and sobti (1991) studied the inheritance of linalool and camphor found as major constituent in the oil of *O. canum*. A number of intraspecific hybrids between these two chemotypes were evolved which on selfing segregated into plants either rich in linalool or camphor.

Charles and Simon (1992a) reported that the essential oil which were obtained from the leaves, flowers and stems of geraniol chemotype of *O. gratissimum* were in concentrations of 1.34%, 1.49% and 0.14% respectively. Fifteen constituents were identified in the essential oils of *O. gratissimum* with geraniol (83.7 - 88.8%) as the major constituent.

Charles and Simon (1992b) analyzed the essential oil constituents of *O. kilmandscharicum* by GC/MS. Though the essential oil content varied between the leaves (0.77 - 1.12% dry weight basis) and the flowers (1.96 - 2.8% dry weight basis), the composition was similar with linalool as the major constituent.

Demissew (1993) reported that the chemical composition of the essential oil of *Ocimum americanum* - linalool 15.3%, camphor 15.1% and terpinen 4-ol 17.6%, *Ocimum gratissimum* - eugenol 57.4%, *Ocimum forskolei* - linalool 17.3%, methyl chavicol 19.3% and (E)-methyl cinnamate 33.0% or myrcene 24.2% and eugenol 25.0%.

Werker *et al.* (1993) have reported that in *Ocimum basilicum* leaf essential oil content decreased as leaves expanded. The percentage of linalol and β-caryophyllene in the essential oil decreased and that of methyl chavicol increased as leaves expanded.

Materials and Methods

MATERIALS AND METHODS

Four different species of *Ocimum* viz., *O. tenuiflorum, O. gratissimum, O. basilicum* and *O. canum* were selected for comparison of morphological, anatomical and biochemical characters. Plants were raised in medium sized pots, filled with potting mixture in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during 1994-1995.

3.1. Morphological studies

Observations were recorded on the following morphological characters and a descriptor was prepared (Radford *et al.,* 1974).

1. General habit
2. Root type
3. Stem type
4. Bark type
5. Branching pattern
6. Leaf type
7. Internodal length
8. Leaf shape
9. Leaf size

10. L:W ratio
11. Leaf arrangement
12. Leaf attachment
13. Venation
14. Leaf surface
15. Vestiture
16. Leaf texture
17. Type of inflorescence
18. Position of inflorescence
19. Length of inflorescence
20. Number of whorls in the inflorescence
21. Floral characters
22. Fruit characters

3.2. Anatomical studies

3.2.1. Stem anatomy

Free hand transverse sections of the third internode of vegetative first primary branches from each species were taken and made permanent following the procedure described by Prasad and Krishna Prasad (1970). Sections were stained for five minutes in 1 per cent aquous saffranine solution and washed in distilled water until excess stain was removed. Sections were dehydrated by

passing through graded concentrations of alcohol (30, 50, 70, 90 and 100 per cent ethyl alcohol for five minutes, two minutes, one minute, one minute and one minute respectively). The sections were then counterstained with light green SF in clove oil (1:1) for two minutes, passed through xylene and mounted in Canadabalsm.

3.2.2. Study of xylem vessels

For studying the features of xylem vessels, the internodal segments were macerated by treating with Jeffrey's fluid (Prasad and Krishna Prasad, 1970). Jeffrey's fluid was prepared by mixing equal volumes of 10 per cent chromic acid and 10 per cent nitric acid. The treatment time was 48 hours. The macerated tissue was washed and stained with 1 per cent aquous saffranine solution. Then it was mounted on clear slides and examined under microscope. The pattern of thickening and proportion of different types of xylem vessels were observed. The length and diameter of xylem elements of each species were measured using ocular micrometer. For each species observations were taken from ten different fields.

3.2.3. Leaf anatomy

The leaves in the third node of vegetative first primary branches were selected for taking transverse sections. Sections stained with 1 per cent aquous

solution of saffranine were used to study features of trichomes, mesophyll tissue and vascular architecture.

3.2.4. Study of stomata

Epidermal peelings were taken from upper and lower surfaces of the mature leaves with the help of 'Quickfix' (adhesive) and mounted on slides. Stomatal Index (SI) was worked out as number of stomates per unit leaf area. For each species stomatal counts were taken from 10 different fields. Stomatal size was also measured.

3.2.5. Vein angle

The angle between the midrib of leaf blade and primary vein (third primary vein from base of leaf blade) was noted. Ten mature 'cleared' leaves were observed in each species for measuring vein angle with the help of Camera Lucida. For clearing the leaves, a quick method described by Payne, W.W. (1969) was followed.

3.2.5.1. Payne's method for clearing leaves

Fresh leaves from the third node of primary branches were used for the study. The leaves were boiled in alcohol until chlorophyll was removed. Then

they were put in five per cent NaOH and placed in an oven at 37°C for two days. When the tissue became transparent leaves were washed in water. Then the leaves were passed through 30, 50, 70, 95 per cent and absolute ethyl alcohol for five minutes each until tissues were completely dehydrated. The dehydrated leaves were passed through a solution of 50 per cent absolute alcohol and 50 per cent xylene for a few minutes. Finally the leaves were cleared using xylene and mounted on slides using canada balsam.

3.3. Biochemical studies

3.3.1. Essential oil extraction

The essential oil from the leaves of *Ocimum* species was extracted by hydrodistillation, using clevenger trap. 150 grams of fresh leaf samples of each species were distilled for two hours and the volume of oil was recorded. The essential oil content was expressed as volume per 100 g of leaf sample. The optimum temperature for distillation, appearance and quantity of oil collected were noted for each sample. The essential oil content was estimated during two seasons - before and during monsoon.

3.3.2. Gas Chromatography

The constituents of the essential oils from the four species were analysed by gas chromatography (Neucon 5700) and the relative peak area for individual

constituents was found out and the essential oil constituents were identified based on retention time. The analytical condition of the Gas Chromatography was as given below:

Column used	:	SE 30
Temperature range	:	80-180°C
Programme	:	4°C/minute
Volume of oil injected	:	0.1 μl
Carrier gas	:	Nitrogen

3.3.3. Polyacrylamide gel electrophoresis(PAGE)

The Polyacrylamide gel electrophoresis (vertical electrophoretic unit) was performed according to the procedure discussed by Ornstein and Davis (1962), to compare the protein banding pattern of the four *Ocimum* species.

3.3.3.1. Preparation of gel

i) Electrode buffer solution

Six grams of Tris buffer and 28.8 grams of glycine dissolved in 1000 ml of water (pH 8.6)

ii) Stock solutions

a) Stock A(pH 9): 38.3 grams of Tris buffer and 0.46 ml of TEMED was dissolved in 48 ml of 1 N HCl and made upto 200 ml.

b) Stock B: 30 grams of acrylamide and 0.9 grams of bisacrylamide were dissolved in water and made upto 100 ml.

c) Stock C: 0.14 grams of crystalline ammonium persulfate was dissolved in 100 ml of water. It was prepared afresh each time.

iii) Preparation of gel slab

Stock A, B and C were pipetted out (in that order) in the ratio 1:1:2, mixed well and the solution was allowed to polymerise in the electrophoretic gel plates.

3.3.3.2. Fixing solution

Fifteen per cent TCA ie., 150 grams of Trichloroacetic acid in one litre of water.

3.3.3.3. Preparation of commassie blue

Added 10 ml of commassie blue prepared in methanol (one gram commassie blue in 100 ml methanol) to 100 ml of 15 per cent TCA.

3.3.3.4. Preparation of destaining solution

Seven per cent hot acetic acid was used for destaining process.

3.3.3.5. Preparation of extraction buffer

Extraction buffer contained hydroxymethyl amine methane (35 μ M), citric acid (2.5 μ M), ascorbic acid (6μ M), Cysteine - HCl (6 μ M) and sucrose (0.5 μ M). Insoluble PVP (1000 ppm) was added to avoid the interference of phenolics.

3.3.3.6. Preparation of sample

The samples were prepared by grinding five grams of leaves with five ml extraction buffer in a chilled mortar and pestle. The extract was centrifuged in a refregerated centrifuge at 5°C and the supernatent (5 μl) used for electrophoresis.

Electrophoresis was performed using LKB electrophoretic unit at 5°C for four hours. Subsequently fixing of proteins with 15 per cent TCA and staining with commassie blue was done. The gel was destained with seven per cent hot acetic acid. Repeated destaining was carried out for two days to get bands. The relative mobility (Rm values) and the size of bands were recorded.

Calculation:

$$\text{Relative mobility of proteins} = \frac{\text{Distance travelled by protein}}{\text{Distance travelled by solvent}}$$

3.3.4. Chlorophyll estimation

Chlorophyll estimation was done as described by Sestak *et al.* (1971). Chlorophyll was extracted in 80 per cent chilled acetone and the absorption at 663 nm and 645 nm were read in spectrophotometer. Using absorption coefficients, the amount of chlorophyll was calculated. Fresh and mature leaves from selected twigs with inflorescences were used for chlorophyll extraction. The samples were extracted with 20 ml of 80 per cent acetone. The absorbance of the clear acetone extract was read at 645 nm and 663 nm against the solvent (80 per cent acetone) blank.

Calculation:

The amount of chlorophyll present in the extract, mg chlorophyll per gram tissue:

$$\text{Milligram chlorophyll a/g tissue} = \frac{[12.7(A_{663}) - 2.69(A_{645})] \times V}{1000 \times W}$$

$$\text{Milligram chlorophyll b/g tissue} = \frac{[22.9(A_{645}) - 4.68(A_{663})] \times V}{1000 \times W}$$

$$\text{and Milligram total chlorophyll/g tissue} = \frac{[20.2(A_{645}) + 8.02(A_{663})] \times V}{1000 \times W}$$

where A = Absorbance at specific wave length.

V = Final volume of chlorophyll extract in 80 per cent acetone and

W = Fresh weight of tissue extracted

Chlorophyll Index (CI) was also calculated as grams of chlorophyll present in a stand per unit ground area.

Results

RESULTS

4.1. Morphological observations

The various morphological characters of four different species (Plates I to VII) of *Ocimum* are presented in Table 1

Table 1 Morphological features of different species of *Ocimum*

Species / Characters	*O. tenuiflorum*	*O. gratissimum*	*O. basilicum*	*O. canum*
General habit	Perennial under shrub	Perennial shrub	Annual herb	Annual herb
Root type	Primary	Primary	Primary	Primary
Average height	60-105 cm	80-120 cm	60-90 cm	50-75 cm
Stem	Usually purplish, subquadrangular in T.S., woody below	Green, grooves and ridges are prominent, sub quadrangular in T.S., woody below	Green or sometimes purplish in colour, round and smooth except one or two longitudinal grooves, woody below	Green, smooth, slightly hairy, subquadrangular stem, woody below
Branching pattern	Falsely dichotomous	Falsely dichotomous	Falsely dichotomous	Falsely dichotomous
Leaf type	Unifoliate	Unifoliate	Unifoliate	Unifoliate
Internodal length	2-2.5 cm	3-4 cm	2-4 cm	2 cm
Leaf shape	Ovate	Ovate	Ovate	Ovate
L/W ratio	3:2	3:2	2:1	2:1
Leaf arrangement	Opposite decussate	Opposite decussate	Opposite decussate	Opposite decussate
Leaf attachment	Petiolate	Petiolate	Petiolate	Petiolate
Leaf margin	Crenate, undulate in mature leaves, hairy	Crenate	Serrulate	Serrulate

Length of petiole	1.5-2.5 cm	3-4 cm	1-1.5 cm	3 cm
Leaf apex	Acute	Acuminate	Acuminate	Acuminate
Leaf base	Obtuse	Obtuse	Acute	Acute
Venation	Pinnately netted. 1 primary vein, 10-14 secondary vein	Pinnately netted. 1 primary vein, 11-12 secondary vein	Pinnately netted. 1 primary vein and 11-14 secondary veins	Pinnately netted. 1 primary vein, 13-16 secondary vein
Leaf texture	Herbaceous	Herbaceous	Herbaceous	Herbaceous
Leaf surface upper	Subglabrate	Glabrous	Glabrous	Glabrous
Leaf surface lower	Glandular, veins ribbed and hairy on lower surface	Glandular, veins hairy and ribbed on lower surface	Glandular, veins ribbed otherwise lower leaf surface smooth	Glandular, veins are hairy and ribbed on lower surface
Inflorescence type	Verticillaster	Verticillaster	Verticillaster	Verticillaster
Position of inflorescence	Terminal	Terminal	Terminal	Terminal
Number of whorls	7-9	20-24	8-10	10-14
Number of flowers/whorl	6	6	6	6
Length of inflorescence	7-10 cm	16-19 cm	10-15 cm	10-14 cm
Hairy covering over inflorescence	Hairy throughout	Minute hairs throughout	Hairy throughout	Hairy throughout
Colour of inflorescence stalk	Purple	Green	Purple	Green
Bract	Two persistent hairy bracts per whorl	Two persistent bracts/whorl	Two very prominent-persistent and hairy bracts/whorl	Two persisten bracts/whorl, bracts have long hairs on margin

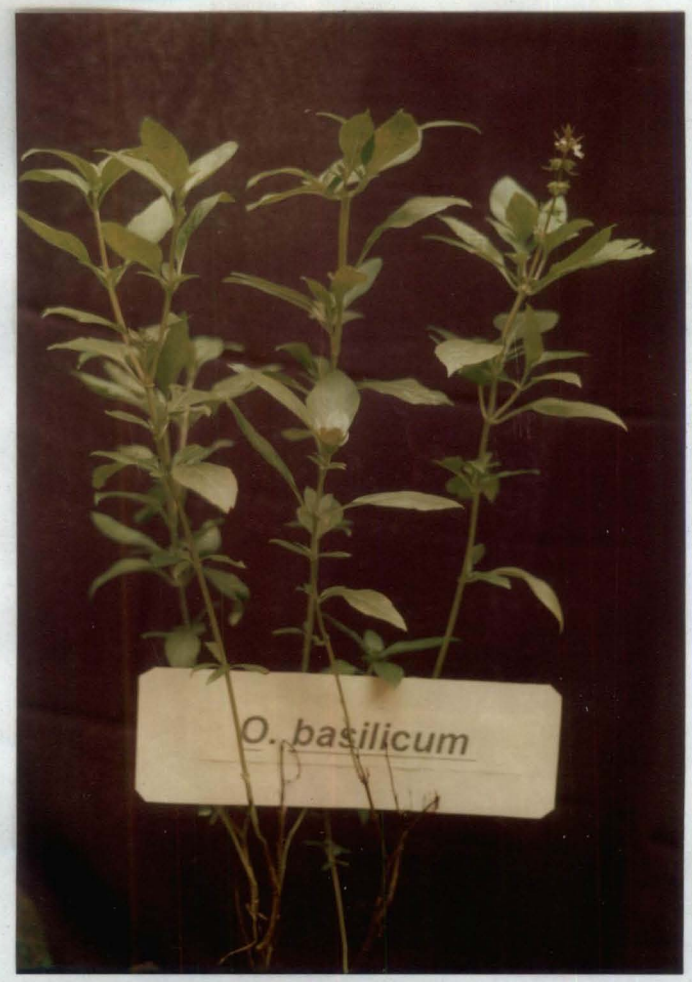
Arrangement of bract	Opposite decussate	Opposite decussate	Opposite decussate	Opposite decussate
Size of bract	0.2 cm	0.4-0.6 cm	1 cm	0.7 cm
Flower opening	From base upwards	From base upwards	From base upwards	From base upwards
Flower sex	Perfect	Perfect	Perfect	Perfect
Flower type	Pedicellate	Pedicellate	Pedicellate	Pedicellate
Symmetry	Zygomorphic	Zygomorphic	Zygomorphic	Zygomorphic
Calyx				
Nature	Persistent	Persistent	Persistent	Persistent
Sepals	Five lobed, fused basally, lower lip longer	Five lobed, fused basally, lower lip shorter	Five lobed, fused basally, lower lip longer	Five lobed, fused basally, lower lip longer
Colour	Green and hairy	Green and hairy	Green with purple tinch and slightly hairy	Pale green with very long hairs
Corolla				
Size of petals	0.4 cm	0.5 cm	0.8 cm	0.5 cm
Petals	Five lobed	Five lobed	Five lobed	Five lobed and hairy
Colour	Pale violet	White with a pale greenish tinch	White with purple stripes on the lower lip	White
Androecium	Apostemonous	Apostemonous	Apostemonous	Apostemonous
Stamen structural type	Filantherous	Filantherous	Filantherous	Filantherous
Colour of anther	Yellow	Yellow	Pale yellow	Pale yellow
Colour of filament	Violet	White	White	Pale violet
Size of stamen (filament + anther)cm	(0.4+0.1)=0.5	(0.5+0.1)=0.6	(0.7+0.1)=0.8	(0.4+0.1)=0.5

Features of stamen	Didynamous Epipetalous	Didynamous Epipetalous	Didynamous Epipetalous	Didynamous Epipetalous
Anther attachment	Basifixed	Basifixed	Basifixed	Basifixed
Anther shape	Reniform	Reniform	Reniform	Reniform
Pollen	Monad	Monad	Monad	Monad
Pollen shape	Circular	Circular	Circular	Circular
Symmetry	Radialsymmetry	Radialsymmetry	Radialsymmetry	Radialsymmetry
Pollen dehiscence	Longitudinal	Longitudinal	Longitudinal	Longitudinal
Colour of pollen	Creamy yellow	Creamy white	White	White
Gynoecium				
Ovary	Superior, tetralocular 2 carpeled, 4 ovules in basal placentation. Anatropous ovary	Superior, 4 lobed, 2 carpels, 4 locules, 4 ovules, anatropous and basal placentation	Superior, 4 lobed, 2 carpels, 4 locules, 4 ovules, anatropous and basal placentation	Superior, 4 lobed, 2 carpels, 4 locules, 4 ovules, anatropous and basal placentation
Style	Violet in colour Gynobasic	White in colour Gynobasic	White towards tip, basally violet Gynobasic	Light violet in colour Gynobasic
Stigma	Bifid	Bifid	Bifid	Bifid
Fruits	4 nutlets enclosed within persistent calyx	4 nutlets enclosed within persistent calyx	4 nutlets enclosed within persistent calyx	4 nutlets enclosed within persistent calyx
Seeds	Small dark brown	Subglobose dark brown	Ellipsoid large black mucilagenos when wetted	Small black ellipsoid mucilagenos when wetted

Plate I General habit of different species of *Ocimum*

c) *Ocimum basilicum*

d) *Ocimum canum*


O. basilicum

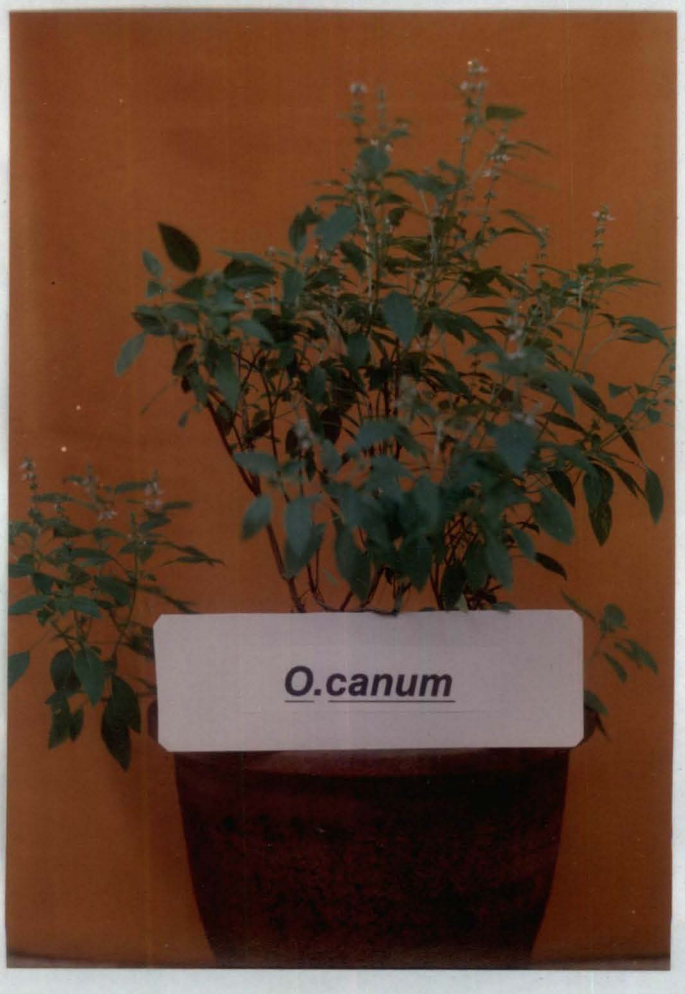

O.canum

Plate I General habit of different species of *Ocimum*

a) *Ocimum tenuiflorum*

b) *Ocimum gratissimum*

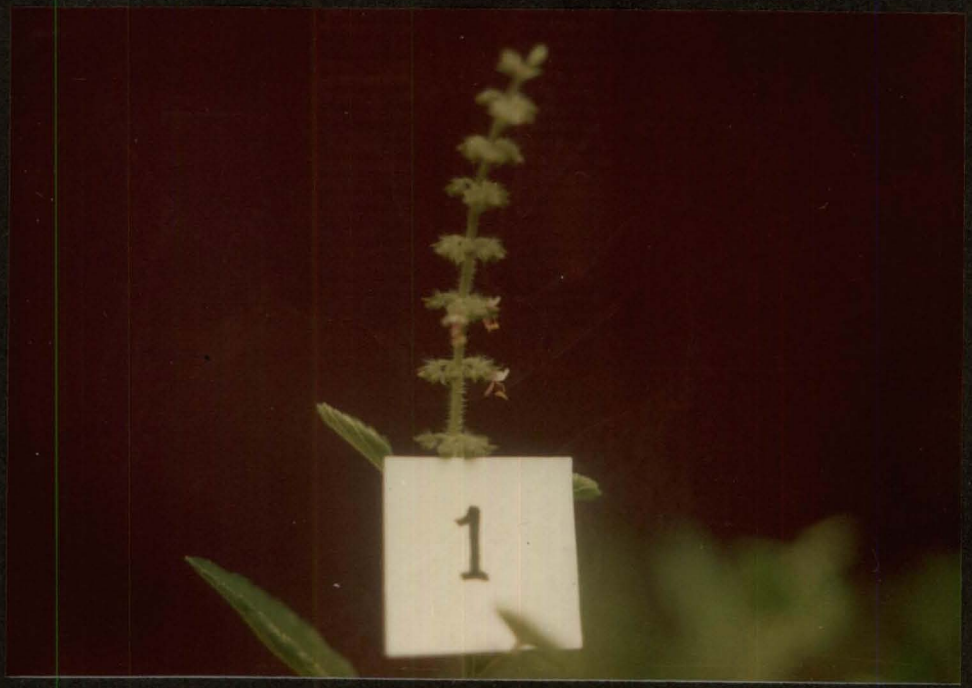

O. tenuiflorum


O. gratissimum

Plate II Twig with inflorescence of different species of *Ocimum*

a) *Ocimum tenuiflorum*

b) *Ocimum gratissimum*


1


O. gratissimum

Plate II Twig with inflorescence of different species of *Ocimum*

c) *Ocimum basilicum*

d) *Ocimum canum*


3


4

Plate III Leaves of different species of *Ocimum*

1. *Ocimum tenuiflorum*

2. *Ocimum gratissimum*

3. *Ocimum basilicum*

4. *Ocimum canum*


1
2
3
4

Plate IV Inflorescence of different species of *Ocimum*

1. *Ocimum tenuiflorum*

2. *Ocimum gratissimum*

3. *Ocimum basilicum*

4. *Ocimum canum*

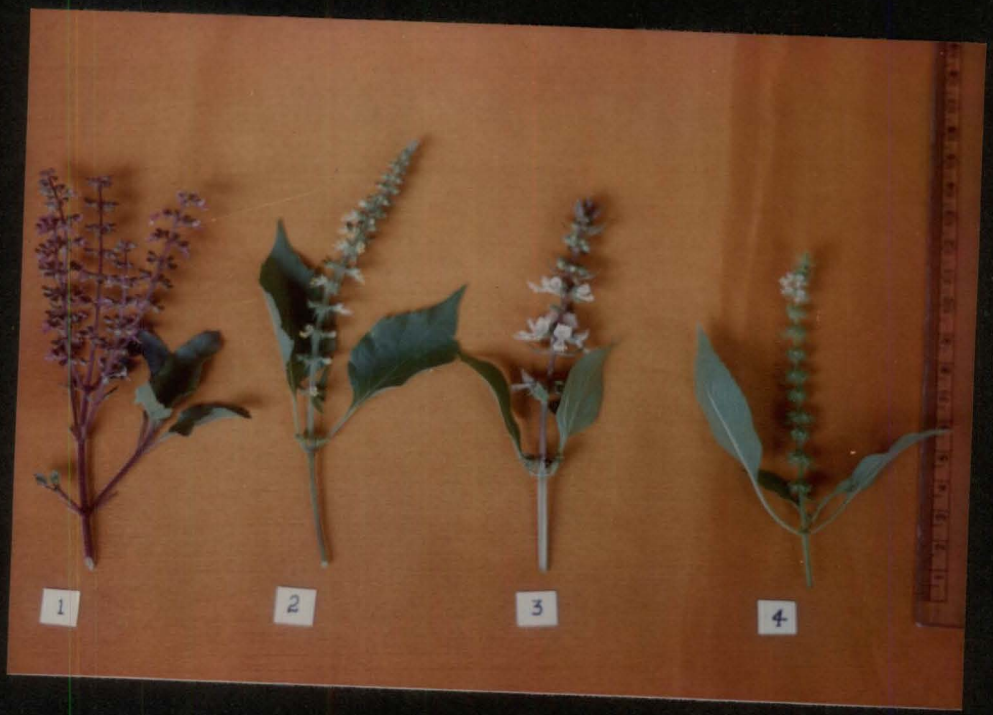

1
2
3
4

Plate V Flowers of different species of *Ocimum* (x 25)

1. *Ocimum tenuiflorum*

2. *Ocimum gratissimum*

3. *Ocimum basilicum*

4. *Ocimum canum*

Plate VI Seeds of different species of *Ocimum* (x 60)

1. *Ocimum tenuiflorum*

2. *Ocimum gratissimum*

3. *Ocimum basilicum*

4. *Ocimum canum*

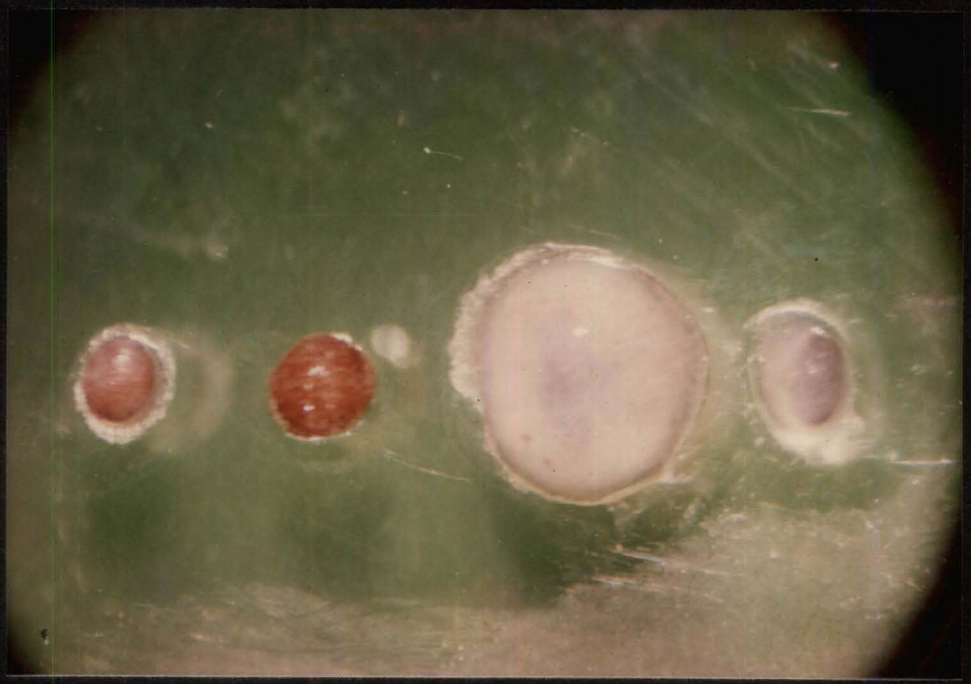
Plate VII Wetted seeds of different species of *Ocimum* (x 35)

1. *Ocimum tenuiflorum*

2. *Ocimum gratissimum*

3. *Ocimum basilicum*

4. *Ocimum canum*



4.2.1. Stem anatomy

Anatomical features of the third internode of unflowered primary branches of four different *Ocimum* species are presented in Table 2.

Table 2 Anatomical features of stem of *Ocimum* species

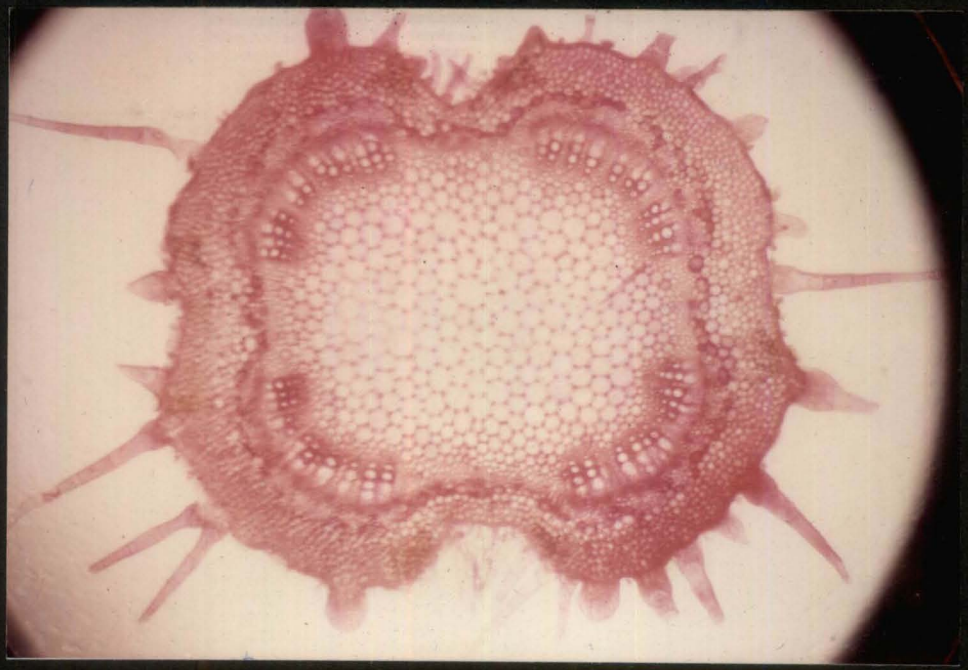
Species / Characters	*O. tenuiflorum*	*O. gratissimum*	*O. basilicum*	*O. canum*
I OUTLINE OF T.S.	Subquandrangular, four lobed with two deep constrictions	Subquadrangular and four lobed	Almost round, four lobed, constrictions not so deep	Subquadrangular four lobed with two deep constrictions
II EPIDERMIS				
i) Degree of cutinization	0.0042 mm thick	0.0042 mm thick	0.0042 mm thick	0.0042 mm thick
ii) Size of Epidermal cells LxW (in mm)^2	0.0167 x 0.0125	0.0208 x 0.0292	0.0208 x 0.025	0.025 x 0.0208
iii) Epidermal appendages	Plenty of hairs and oil glands are present	Hairs only a few, plenty of glands are present	Hairs are rarely seen. A number of oil glands are present	Plenty of hairs and oil glands are present
a) Non-glandular trichomes	Long, multicellular (7 to many celled), uniseriate filiform hairs and swollen four celled base. 3-4 celled short uniseriate hairs with round ends and unswollen base.	Long, multicellular (7-8 celled) uniseriate filiform hairs with pointed tip and two celled base. Also 2-3 celled short uniseriate hairs with pointed tip and two celled base.	Rarely 2-3 celled, short uniseriate hairs with pointed tips seen.	Most of the hairs are short, 2-3 celled, uniseriate with smooth round ends and bicelled base. Some hairs are longer (5-6 celled) uniseriate with pointed tip.

b) Glandular trichomes	Short stalked and multicelled globular glands which are sparkling yellow in colour. Small stalked glands are also present. Triangular multi cellular structures with round and pointed tips are also seen.	Short stalked multicelled globular colourless glands are present.	Short stalked multicelled globular colourless glands are present.	Short stalked multicelled globular colourless glands are present.
III CORTEX				
i) Collenchyma	3-4 layers towards corner	5-6 layers towards corners.	3-4 layers towards corners.	3-5 layers towards corner
ii) Endodermis	Well defined continuous ring	Well defined continuous ring	Well defined, not continuous	Well defined, not continuous
iii) Extra steeler secondary thickening	-	Started	-	-
iv) Pericycle	Ill defined	Ill defined	Discontinuous patches of 3-4 layers forming a ring	No pericycle
IV STEEL				
i) Cambium	Prominent ring present	Prominent ring present	Prominent ring present	Prominent ring present
ii) Vascular tissue	Secondary thicking has started. Xylem vessels have affinity towards tracheids	Secondary thicking has started. Xylem vessels have affinity towards tracheids	Secondary thicking has started. Xylem vessels have affinity towards tracheids	Secondary thicking has started. Xyle vessels have affinity towar tracheids
iii) Pith Extent	0.92 mm^2	0.753 mm^2	1.269 mm^2	0.481 mm^2
iv) Crystals	Absent	Absent	Absent	Absent

Plate VIII T.S. of stem of different species of Ocimum (x 140)

a) Ocimum tenuiflorum

c) Ocimum gratissimum



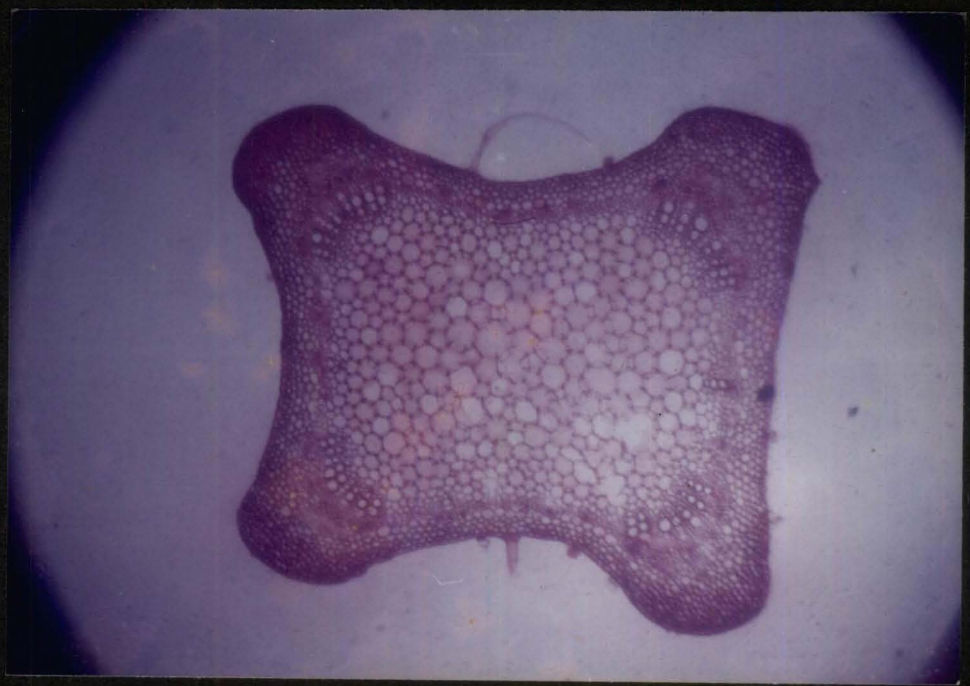
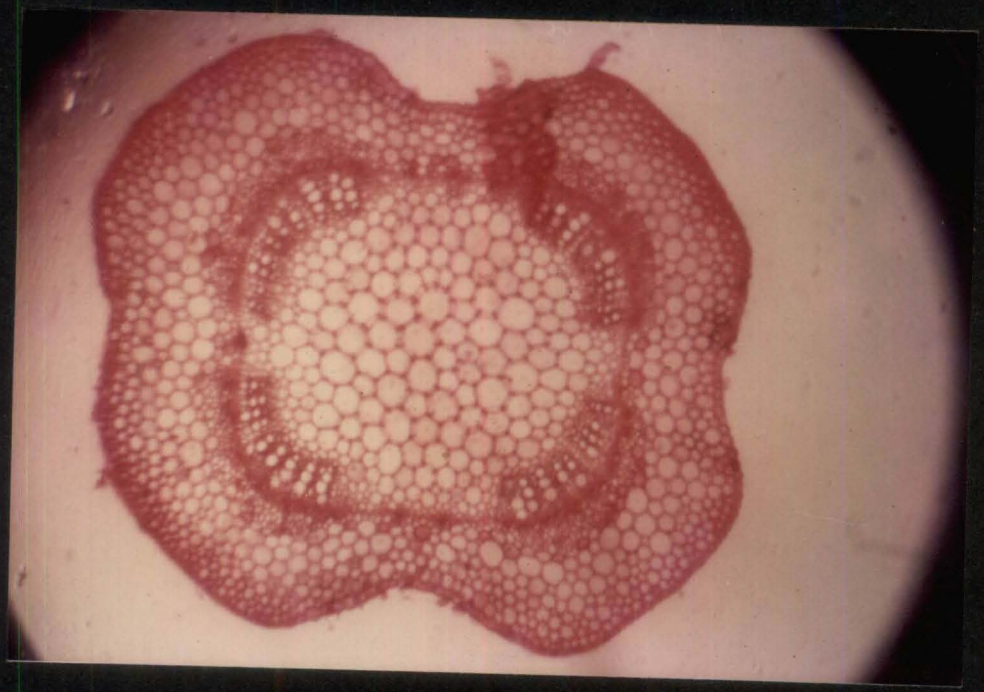


Plate VIII T.S. of stem of different species of Ocimum (x 140)

c) Ocimum basilicum

d) Ocimum canum



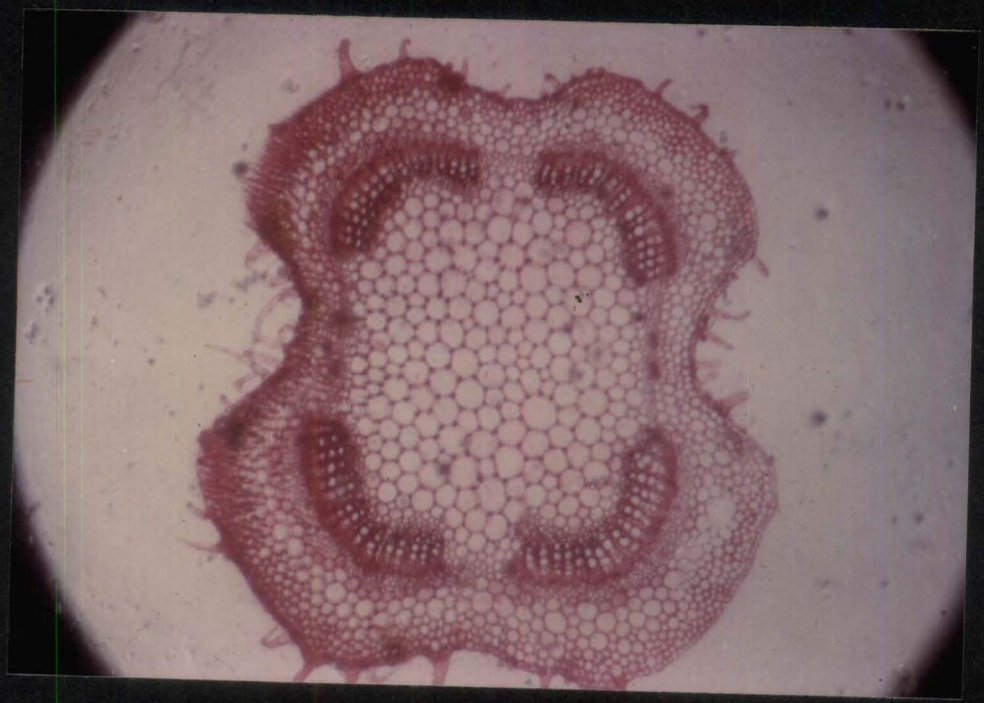
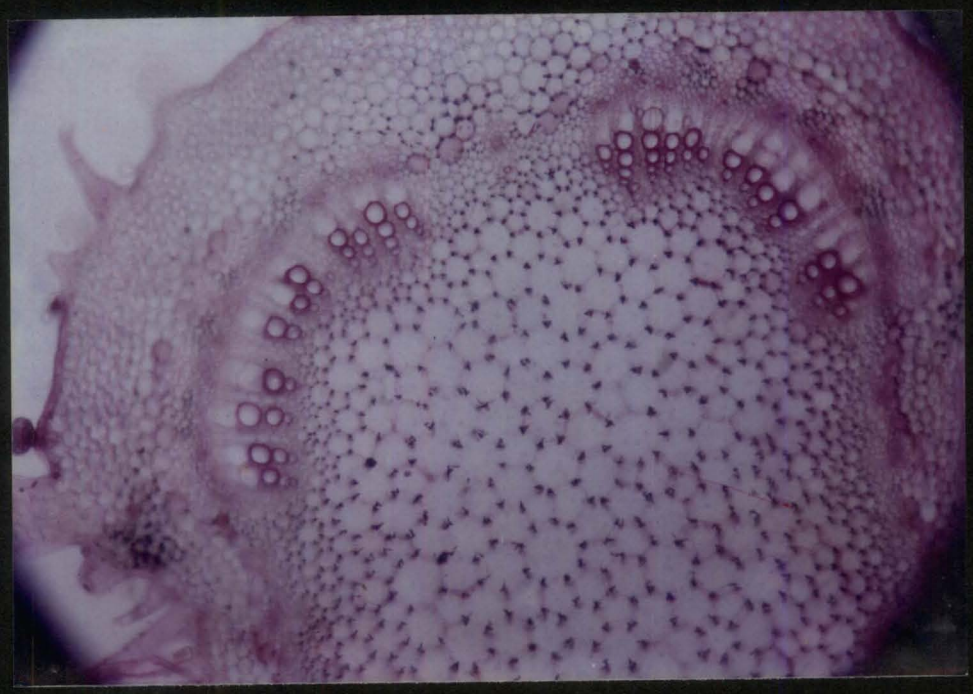


Plate IX T.S. of stem of different species of Ocimum - a portion enlarged (x 280)

a) **Ocimum tenuiflorum**

b) **Ocimum gratissimum**



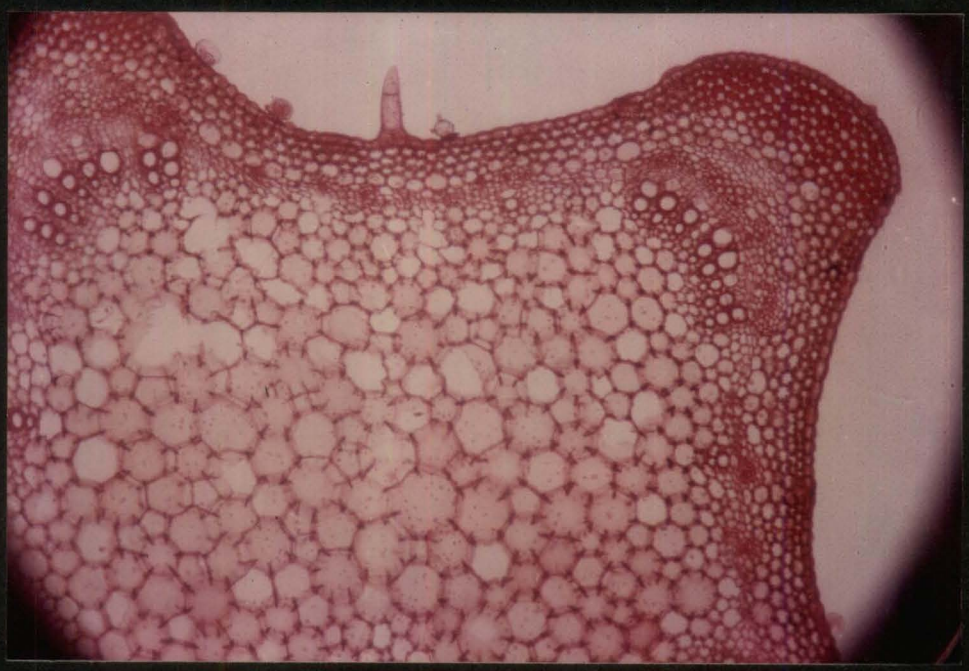


Plate IX T.S. of stem of different species of Ocimum - a portion enlarged (x 280)

c) Ocimum basilicum

d) Ocimum canum

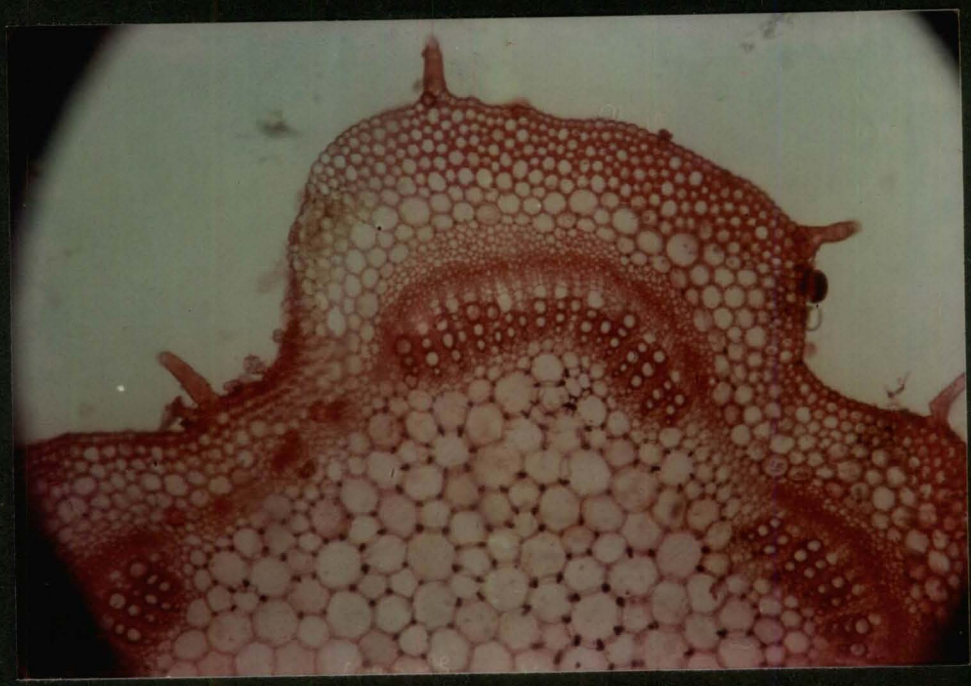
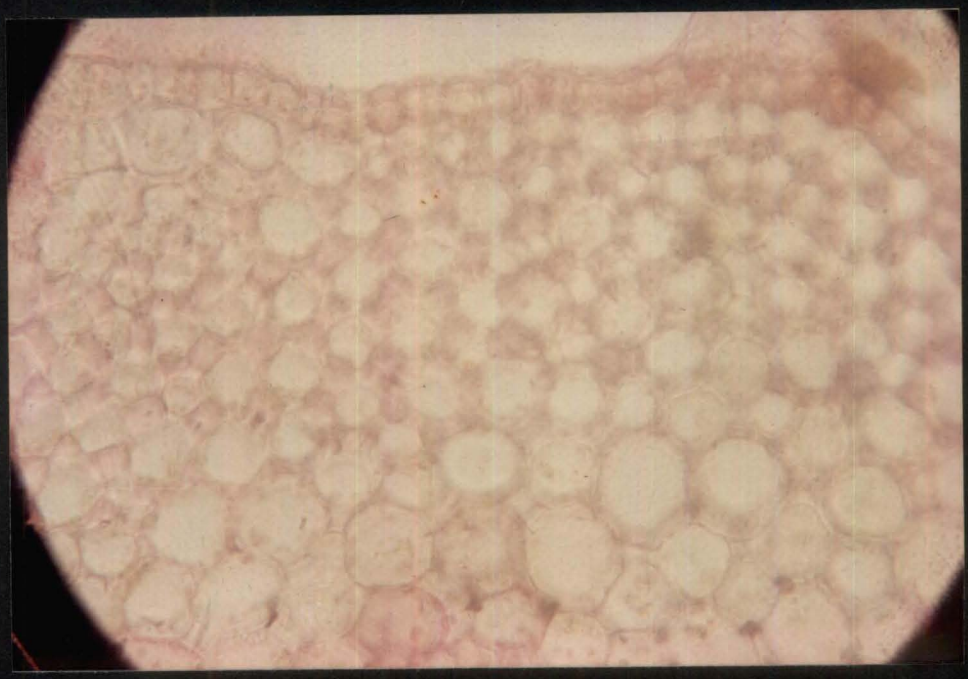


Plate X *T.S. of cortical region of stem in different species of Ocimum (x 1260)*

a) Ocimum tenuiflorum

b) Ocimum gratissimum



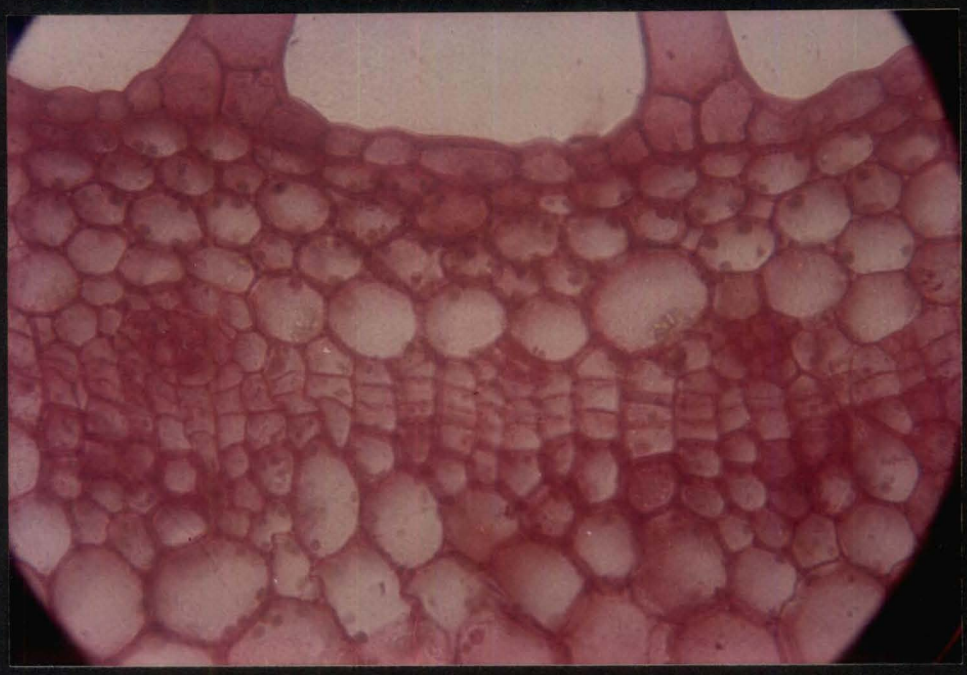
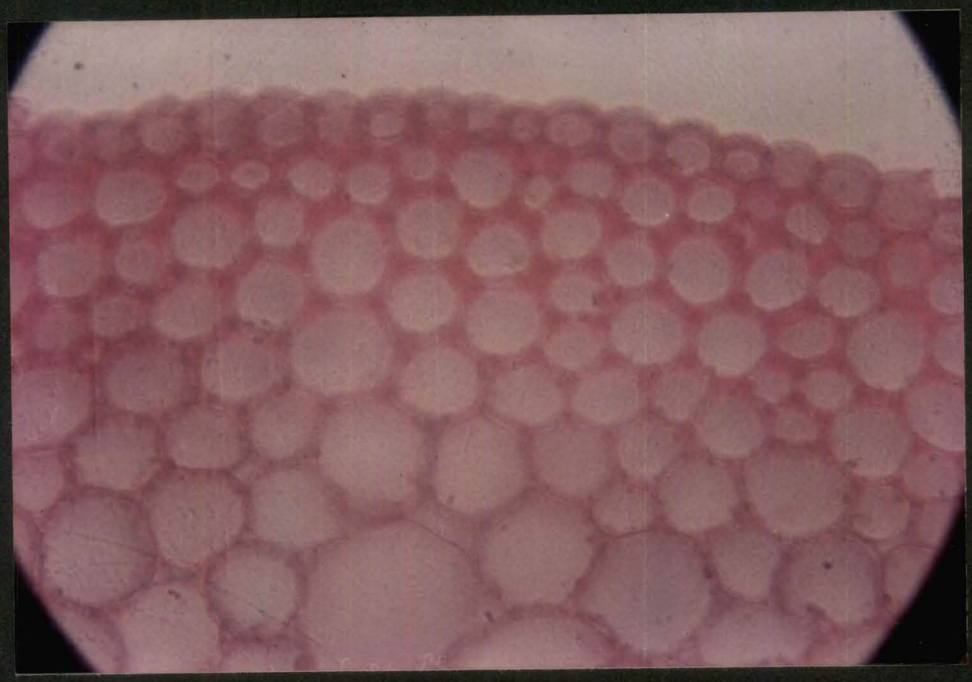


Plate X *T.S. of cortical region of stem in different species of Ocimum (x 1260)*

c) Ocimum basilicum

d) Ocimum canum



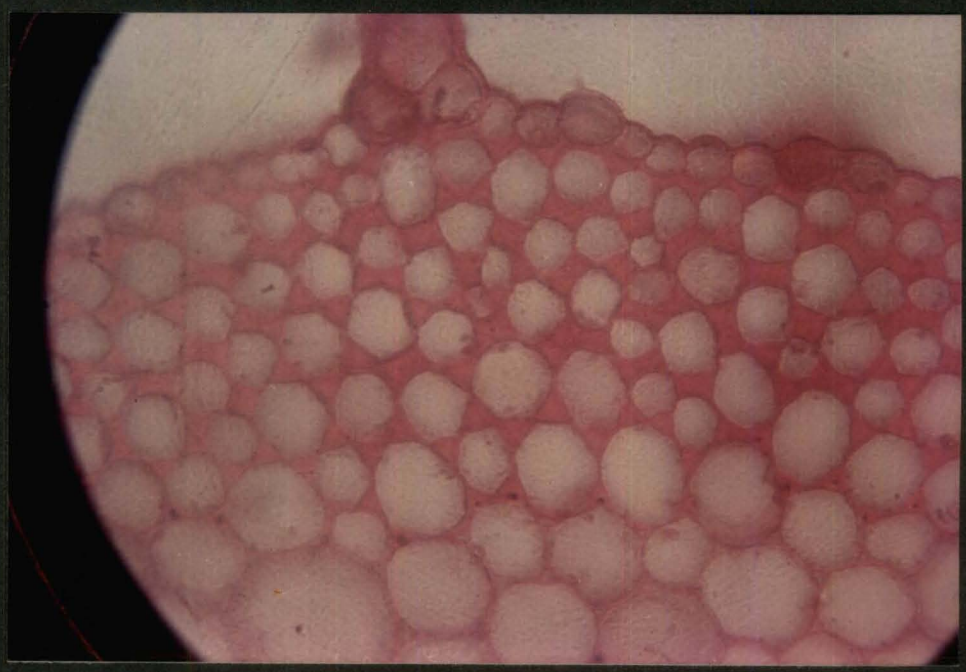
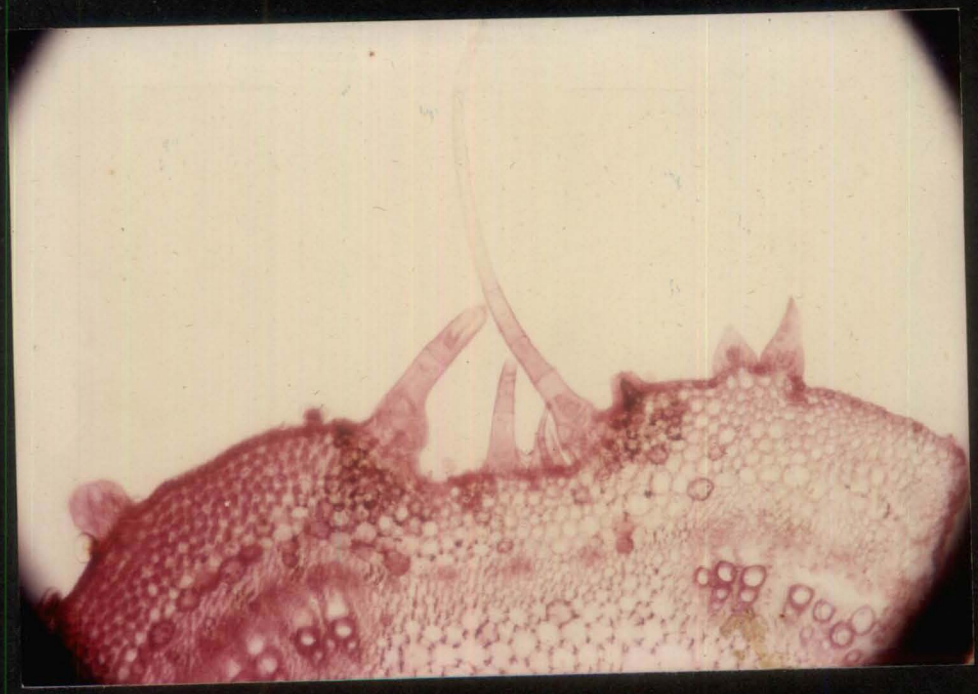


Plate XI. Epidermal appendages in different species of Ocimum

a) Ocimum tenuiflorum (x 280)

b) Ocimum gratissimum (x 280)



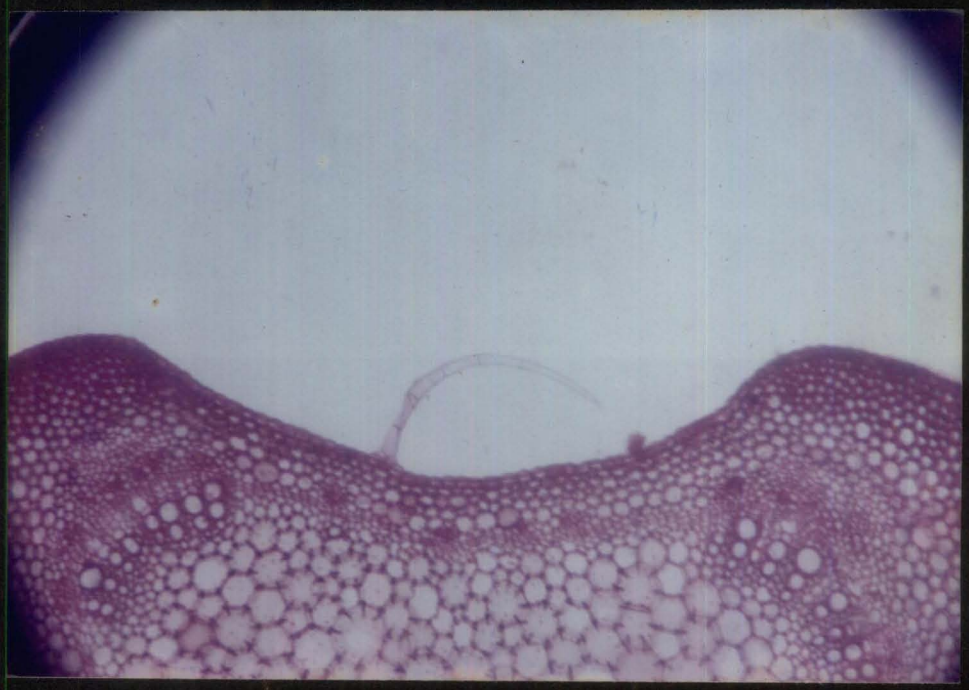
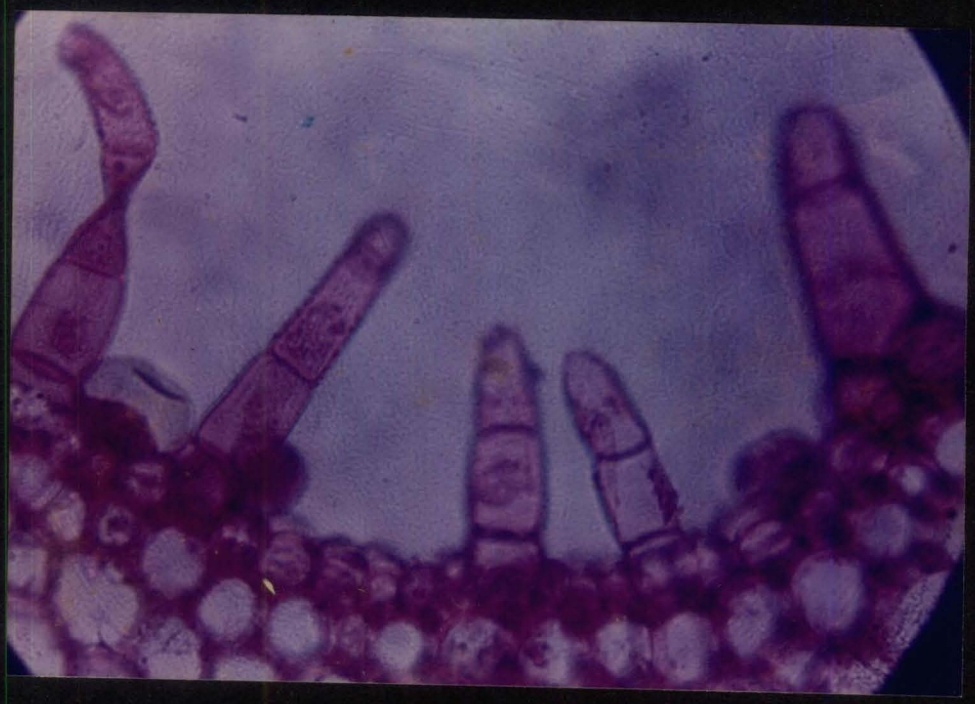


Plate XI Epidermal appendages in different species of Ocimum

c) Ocimum canum (x 1260)



4.2.2 Xylem vessel elements

The xylem vessel elements of the four species were examined (Plates XII and XIII) to and the details are furnished below. Table 3 shows the range of different types of vessel elements in different species (under magnification 45).

Table 3 Range of different types of xylem vessel elements in four species of *Ocimum*

Species	Range					
	Annular	Helical	Spiral	Scalari-form	Reticu-late	Pitted
O. tenuiflorum	0-8	1-5	2-10	0-2	0-1	0
O. gratissimum	0-4	0-5	2-8	0-1	0-2	0
O. basilicum	0-4	1-6	1-6	0-2	0-7	0-3
O. canum	0-3	0-8	3-9	0-1	0-3	0-2

The proportion of different types of xylem vessel elements in different species of *Ocimum* are presented in Table 4 and Fig.1.

Plate XII Types of xylem vessel elements commonly found in different species of Ocimum (x 1260)

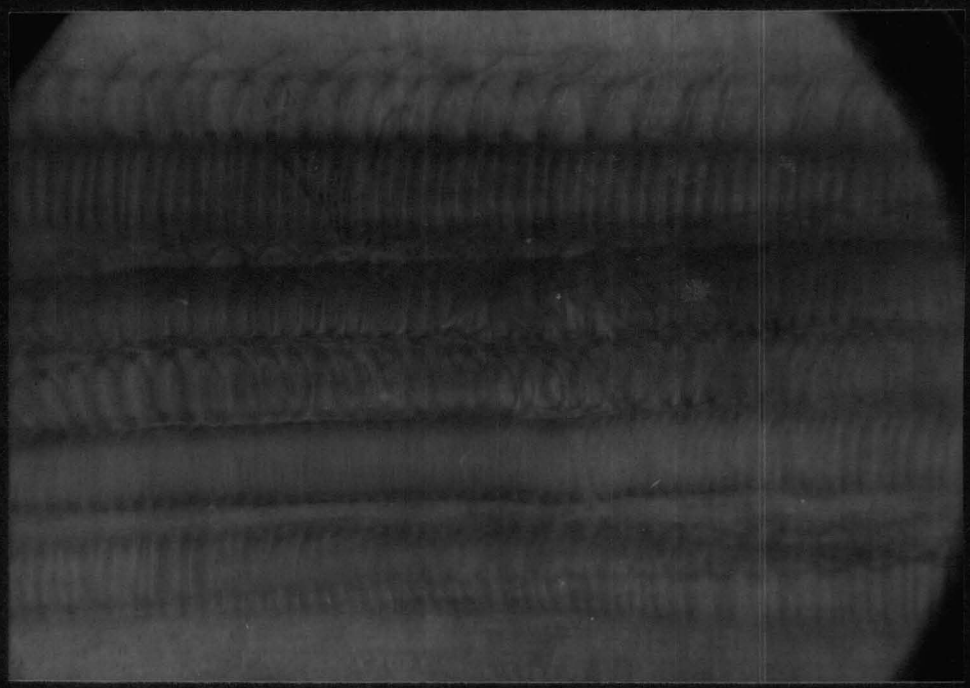
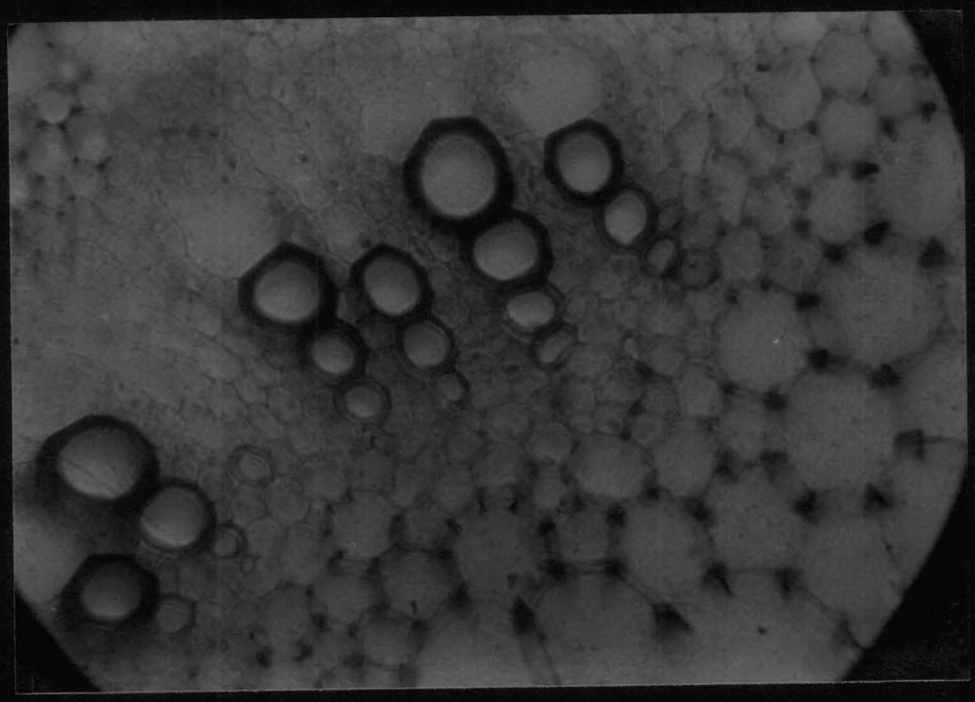


Plate XIII *Xylem vessel elements in stem T.S. (x 1260)*

a) *Ocimum tenuiflorum*

b) *Ocimum gratissimum*



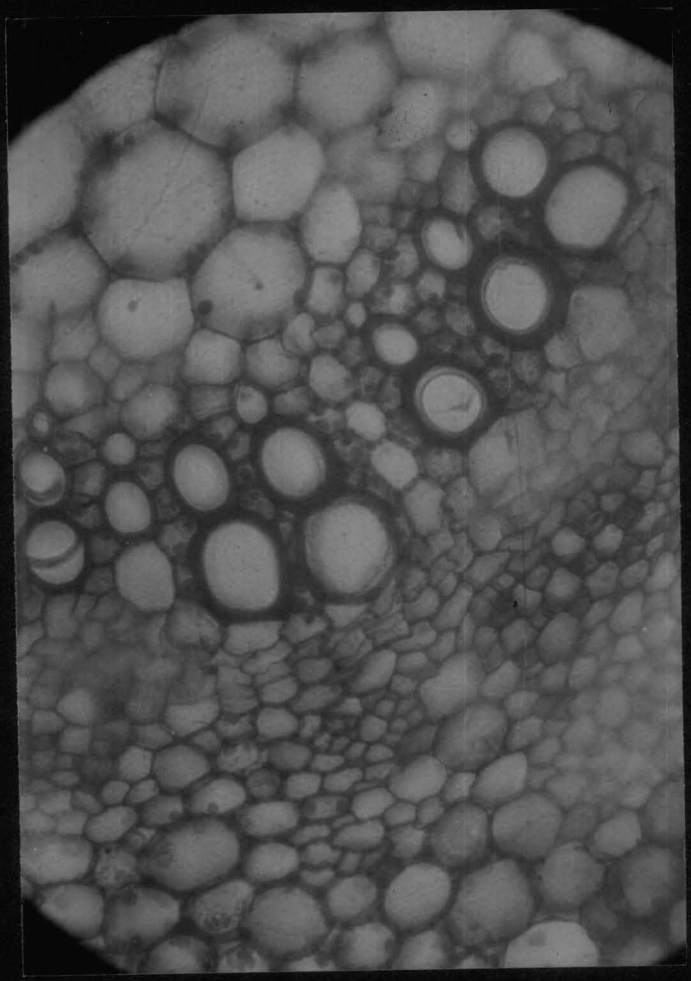
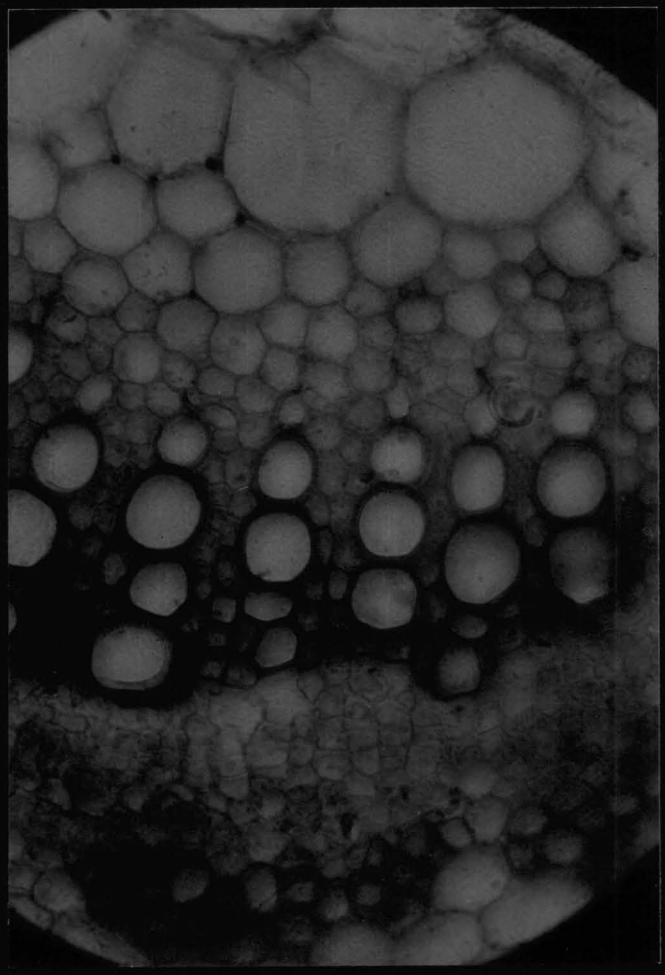
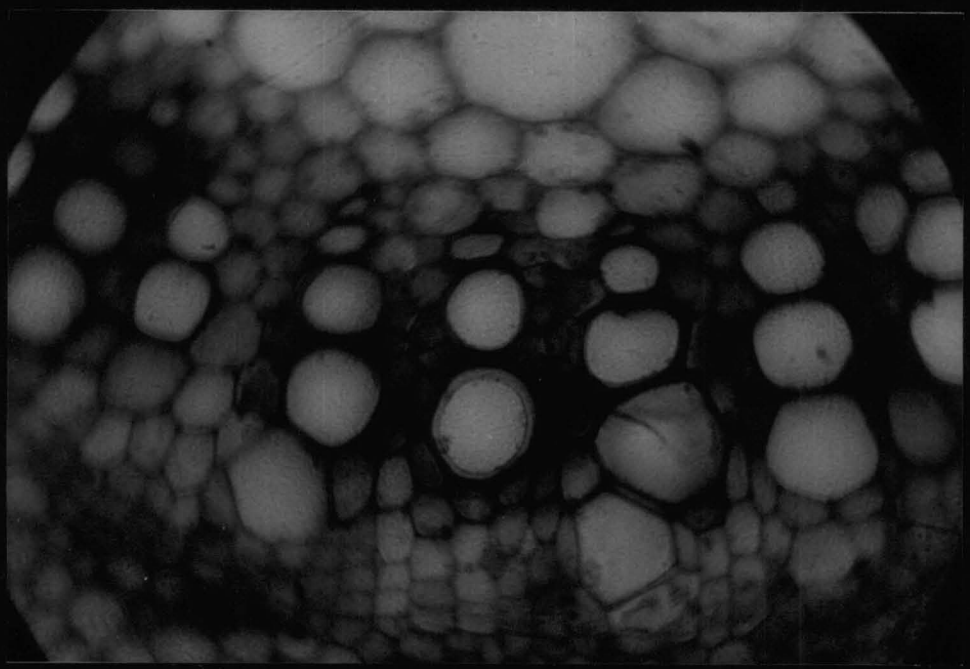


Plate XIII *Xylem vessel elements in stem T.S. (x 1260)*

c) Ocimum basilicum

d) Ocimum canum





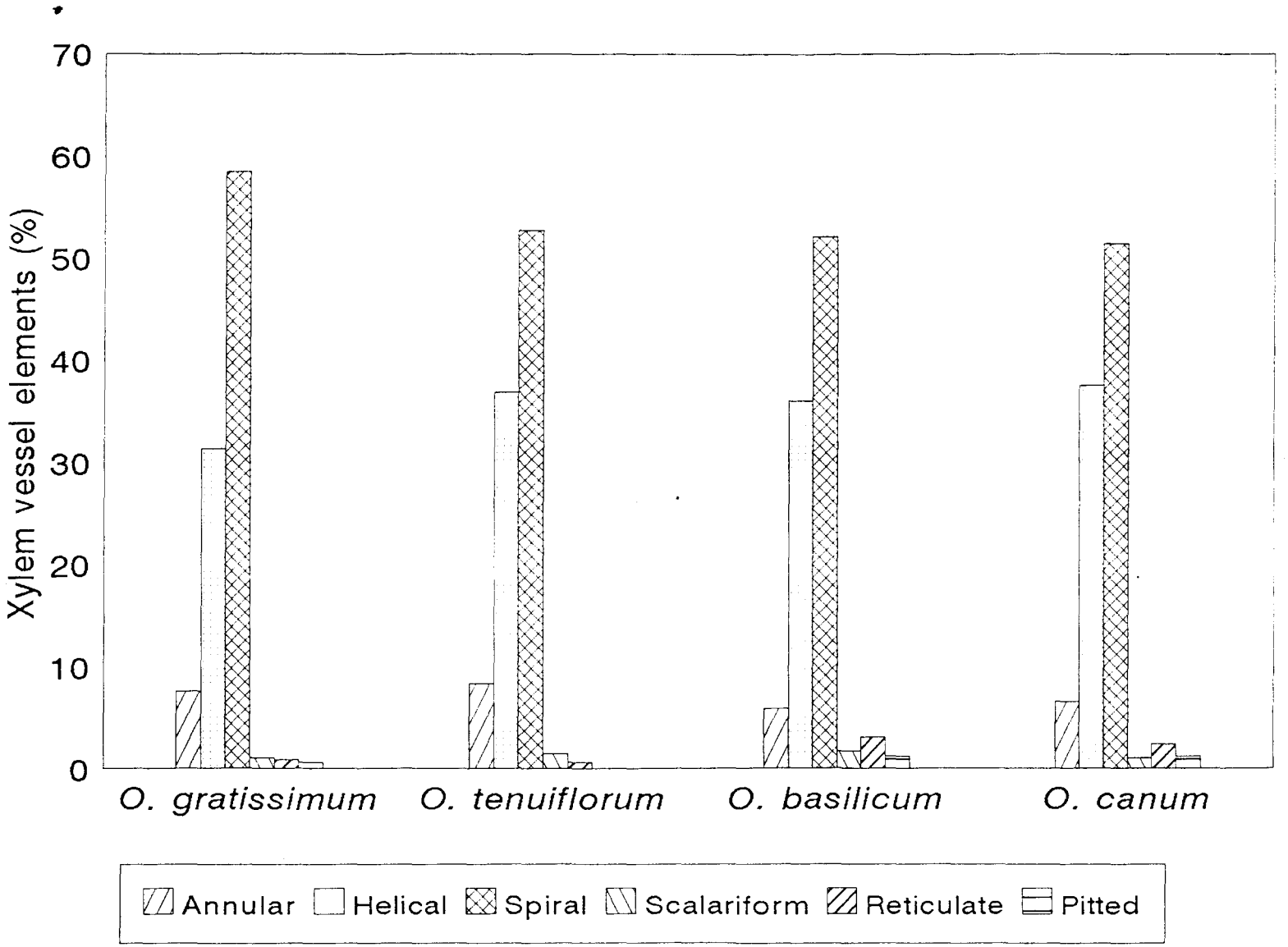


Fig.1. Proportion of *xylem vessel elements in four species of Ocimum*

Table 4 Percentage of different types of xylem vessel elements in four species of *Ocimum*

Species	Proportion of xylem vessel elements (%)					
	Annular	Helical	Spiral	Scalariform	*Reticulate*	*Pitted*
O. tenuiflorum	8.4	36.8	52.7	1.5	0.6	0
O. gratissimum	7.6	31.3	58.5	1.1	0.9	0.6
O. basilicum	5.9	36.0	52.1	1.7	3.1	1.2
O. canum	6.6	37.5	51.4	1.1	2.4	1.2

The mean diameter and length of different xylem vessel elements in different species are presented in Table 5, Fig.2 and Table 6 respectively.

Table 5 Mean diameter of different xylem vessel elements in different species of *Ocimum*

Vessel element	Mean diameter (μm)			
	O. tenuiflorum	*O. gratissimum*	*O. basilicum*	*O. canum*
Annular	16.4 ± 0.9	17.6 ± 0.7	20.4 ± 1.1	12.6 ± 0.7
Helical	16.8 ± 1.0	23.6 ± 0.9	18.0 ± 0.9	21.4 ± 0.2
Spiral	33.2 ± 0.6	34.8 ± 1.3	35.6 ± 0.9	26.8 ± 0.4
Scalariform	41.6 ± 0.9	34.4 ± 0.7	36.4 ± 1.3	28.8 ± 0.1
Reticulate	37.1 ± 0.8	34.8 ± 1.6	38.4 ± 1.9	28.8 ± 0.1
Pitted	-	-	39.1 ± 0.8	28.9 ± 0.3

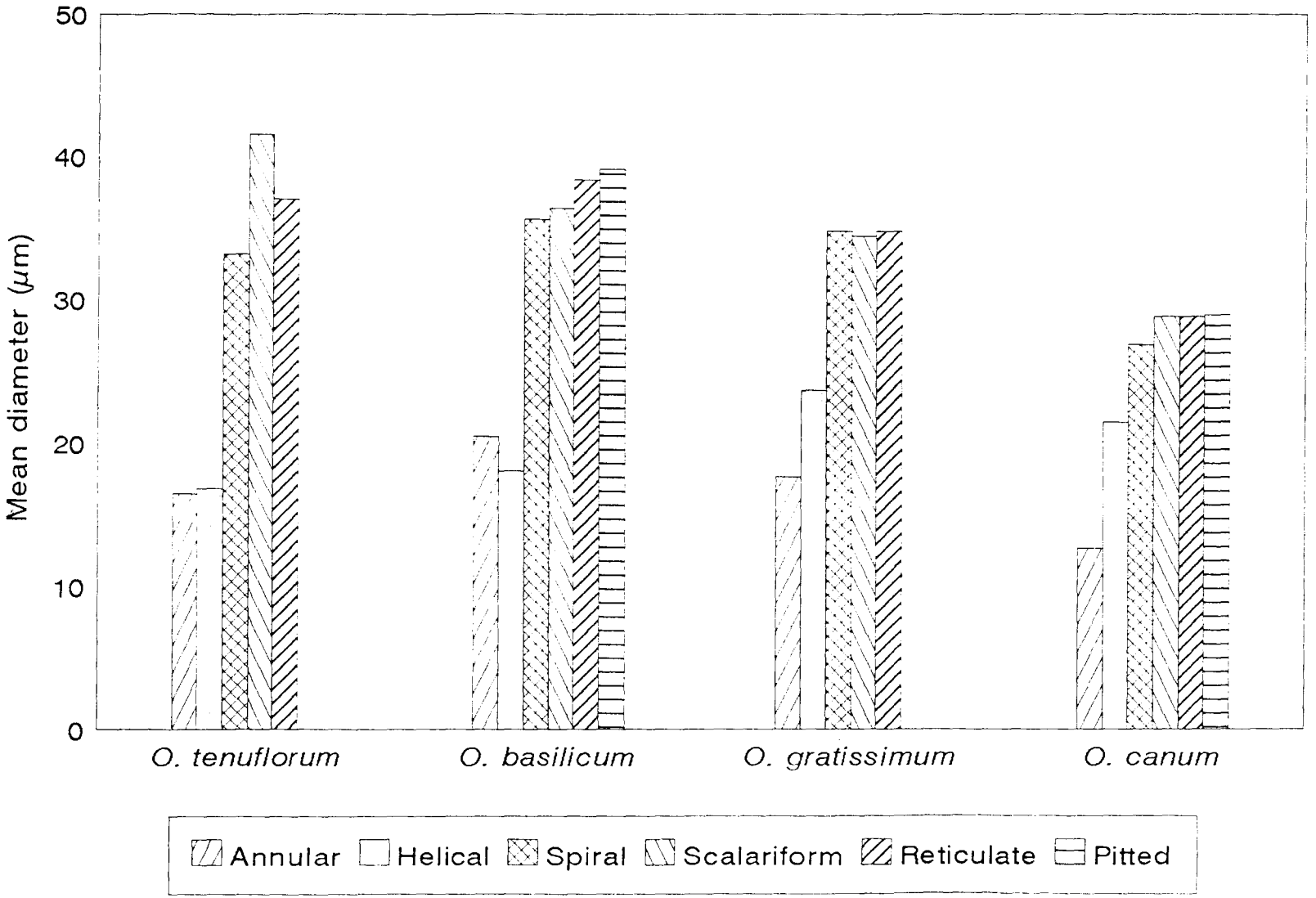


Fig.2. Mean diameter of xylem vessel elements in four species of *Ocimum*

Table 6 Mean length of different xylem vessel elements in different species of *Ocimum*

Vessel element	Mean length (μm)			
	O. tenuiflorum	*O. gratissimum*	*O. basilicum*	*O. canum*
Annular	-	-	-	-
Helical	-	-	-	-
Spiral	376.0±13.3	416.0±10.1	452.4±7.8	404.8±13.6
Scalariform	374.8±11.8	396.0±9.3	468.0±9.2	368.0±12.5
Reticulate	257.0±10.2	316.0±11.8	326.1±10.1	330.3±14.3
Pitted	-	-	328.3±9.8	329.2±11.6

The length of annular and helical vessel elements exceeded a microscopic field hence not taken.

Table 7 shows the L/B ratio different vessel elements in different species of *Ocimum*

Table 7 L/B ratio of different vessel elements in different species of *Ocimum*

Vessel element	L/B ratio			
	O. tenuiflorum	*O. gratissimum*	*O. basilicum*	*O. canum*
Annular	-	-	-	-
Helical	-	-	-	-
Spiral	11.3	11.9	12.7	15.1
Scalariform	9.0	11.5	12.8	13.1
Reticulate	6.9	9.1	8.5	11.5
Pitted	-	-	8.4	11.4

4.2.3 Leaf anatomy

Histologically *Ocimum* leaf showed three types of tissue systems: epidermis, mesophyll and vascular tissue(Plate XIV 'a' to 'd'). In all the four species of *Ocimum* under study, epidermis was composed of a single layer of cells both on adaxial and abaxial surfaces. Trichomes were present on both surfaces, more abundant in the region of veins on abaxial surface and along the leaf margins. Size and distribution of trichomes were slightly different in the four species and are shown in Table 8.

Table 8 Type and distribution of trichomes in different species of *Ocimum*

Species	Trichomes
O. tenuiflorum	2-3 celled medium sized trichomes all over the surface, slightly less on upper surface, more abundant on the veins and along the margins of the leaf.
O. gratissimum	Trichomes are less in number, but large in size, distributed all over the surfaces, plenty along the margins.
O. basilicum	Minute trichomes, mainly on veins, on both surfaces.
O. canum	A large number of long trichomes on both surfaces of the leaf

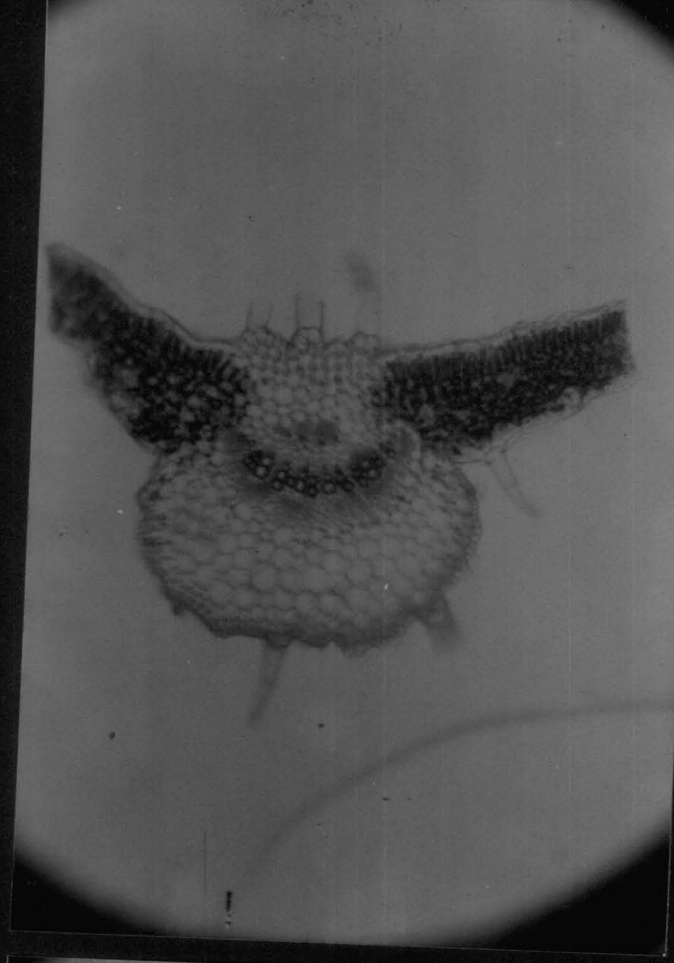
Glandular trichomes were also present on both surfaces of leaves of all species studied.

Hypodermis is absent in all the species. But in the region of midrib, the transverse section of leaves showed 1-2 layers of collenchyma, just below epidermis, on both surfaces of leaf. This feature was very prominent in *Ocimum gratissimum*.

Plate XIV T.S. of leaves of different species of Ocimum (x 230)

a) Ocimum tenuiflorum

b) Ocimum gratissimum



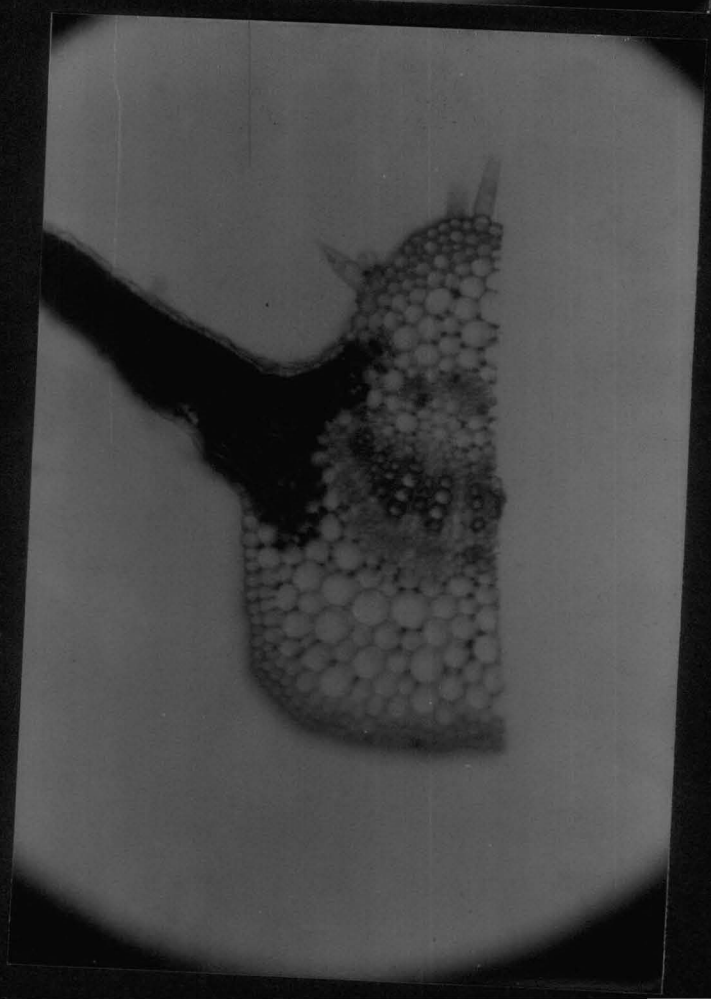
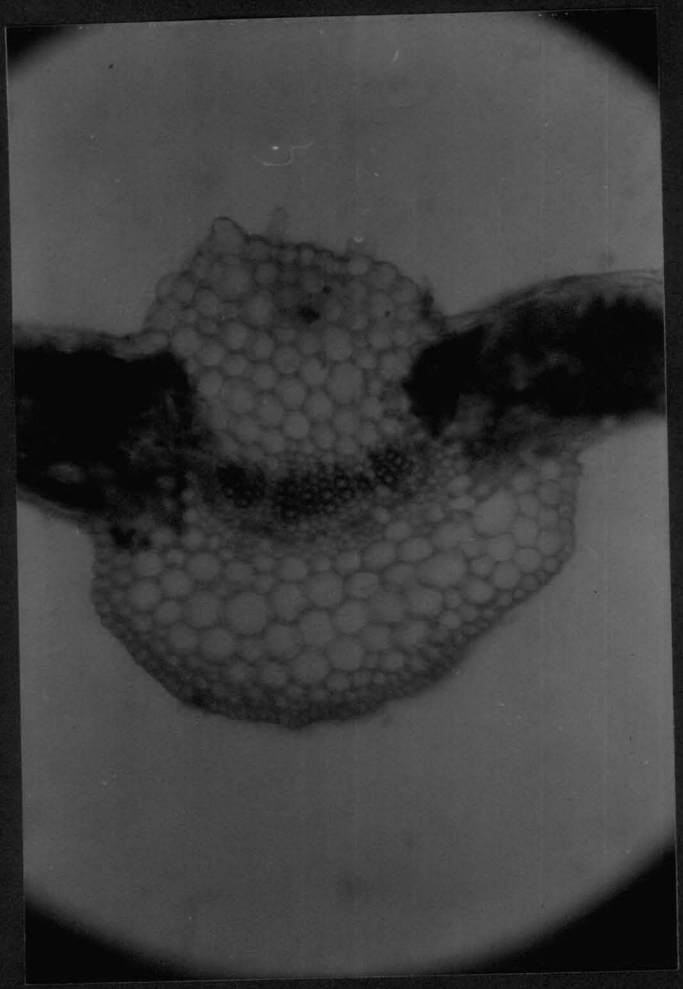
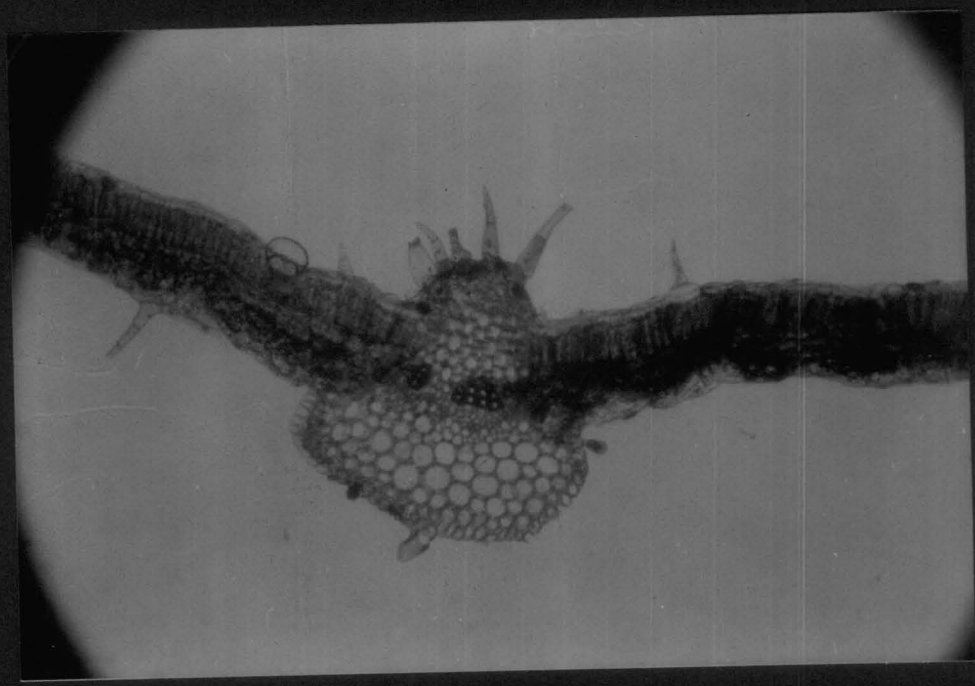


Plate XIV *T.S. of leaves of different species of Ocimum (x 280)*

c) Ocimum basilicum

d) Ocimum canum





Mesophyll tissue was found to be bifacial or dorsiventral ie., palisade parenchyma on one side of the leaf (adaxial surface) and the spongy parenchyma on the other (abaxial surface). The cells in the palisade tissue were elongated and arranged in rows. In cross section they appeared to be rod shaped. In spongy parenchyma tissue, cells were irregularly connected by their lobes. Vascular tissue consisted of xylem and phloem. Protoxylem was directed towards adaxial surface of leaf. Bundle sheath was absent in all the four species.

4.2.4 Stomata

Out of the four species of *Ocimum* selected for study, three species viz. *Ocimum tenuiflorum, Ocimum basilicum* and *Ocimum canum* had amphistomatic leaves (stomates are present on both surfaces of leaf). In *Ocimum gratissimum*, leaves were observed to be hypostomatic. In all the species stomatal count on lower surface was greater than that on upper surface. Stomates are diacytic. The stomatal size and stomatal indices in different *Ocimum* sp. are furnished in Table 9 and Fig.3. Stomatal Index (SI) is calculated as number of stomates per square millimetre area.

Table 9 Stomatal size and stomatal index in different species of *Ocimum*

Species	Stomatal size (μ m) L x B	Stomatal Index	
		Upper surface	Lower surface
O. tenuiflorum	22.5±2.2 x 18.3±2.2	132	199
O. gratissimum	29.2±1.9 x 20.8±1.9	2	251
O. basilicum	33.3±2.8 x 21.7±5.5	128	132
O. canum	30.4±3.4 x 20.0±1.8	101	132

4.2.5 Vein angle

The angle between midrib and third primary vein from base of leaf, observed in the four species, were within the range as given in Table 10 (Plate XV 'a' to 'd').

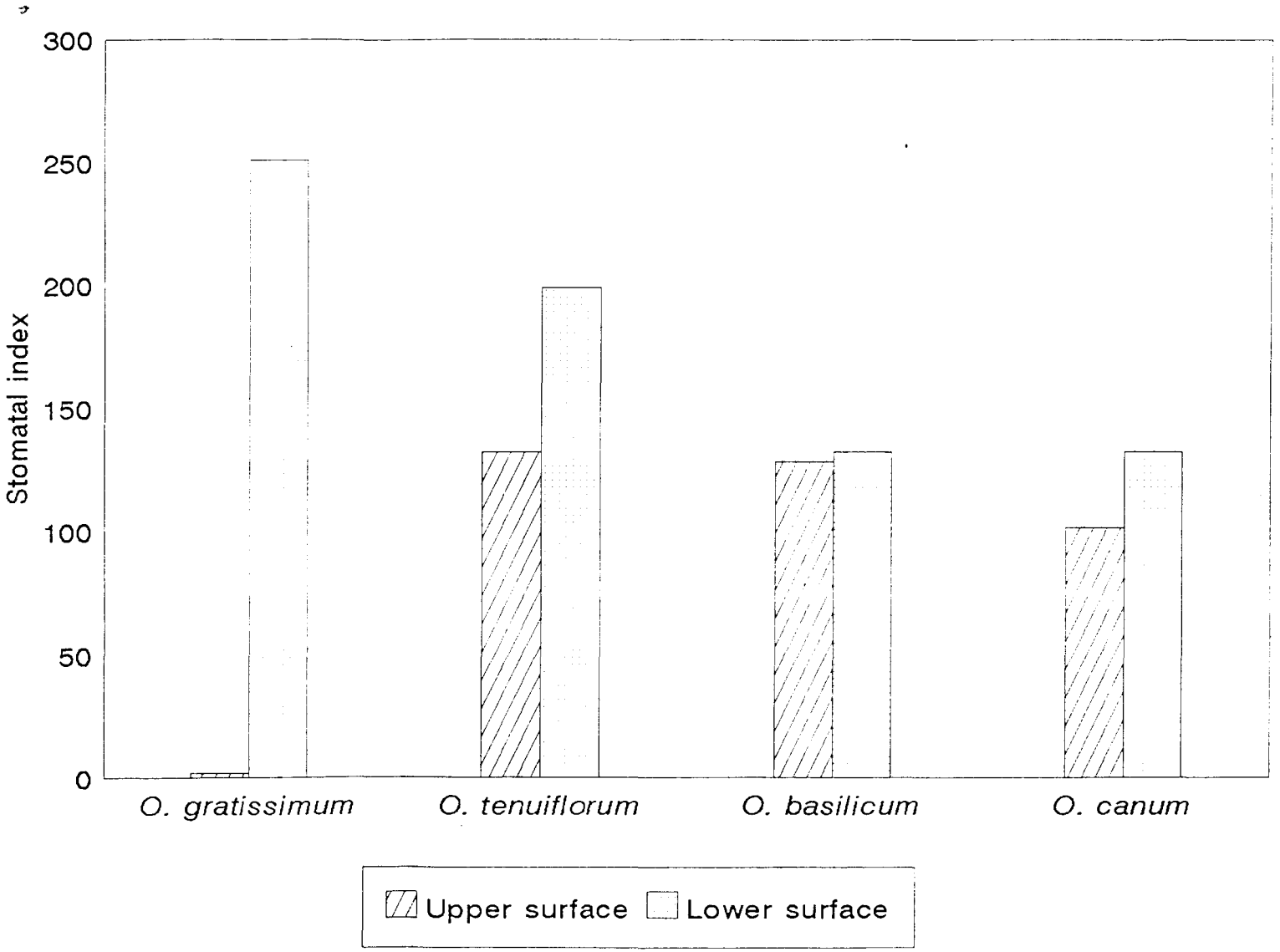


Fig.3. Stomatal index in four species of *Ocimum*

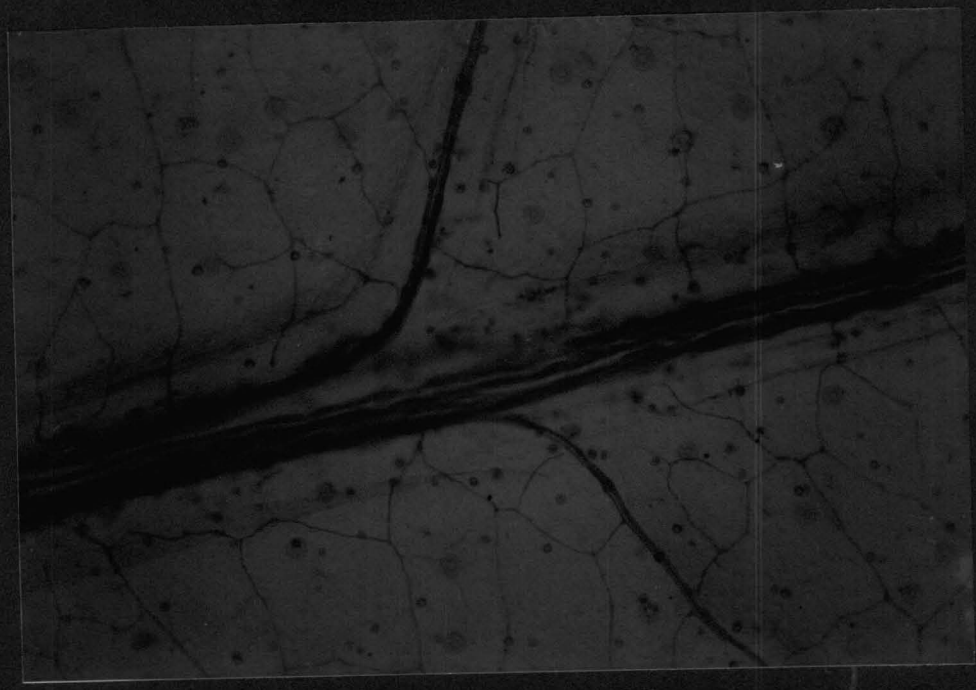
Plate XV Vein angle in different species of Ocimum (x 160)

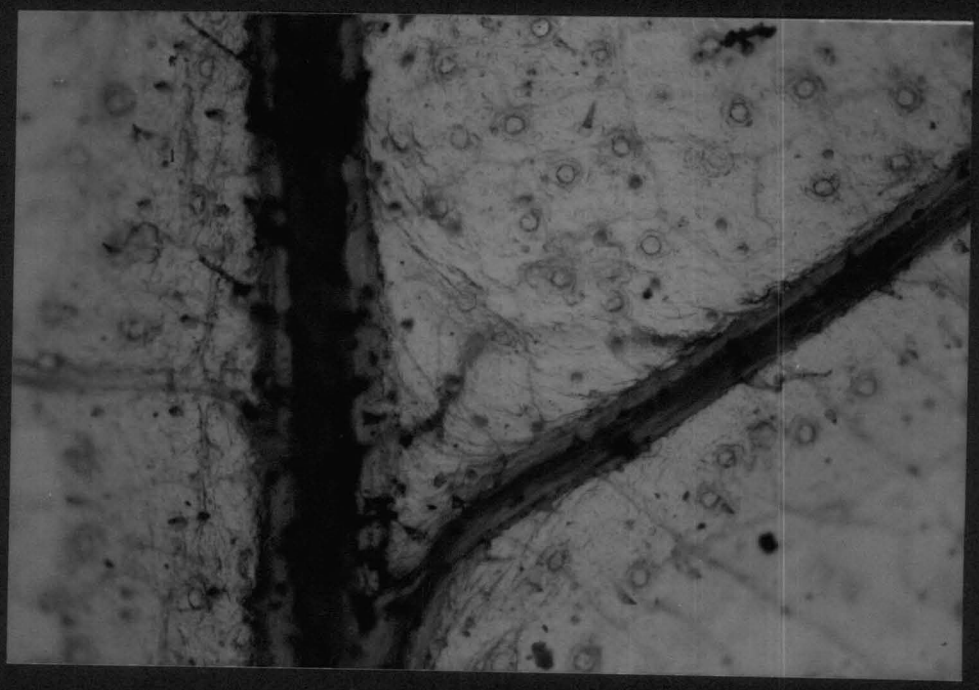
a) Ocimum tenuiflorum

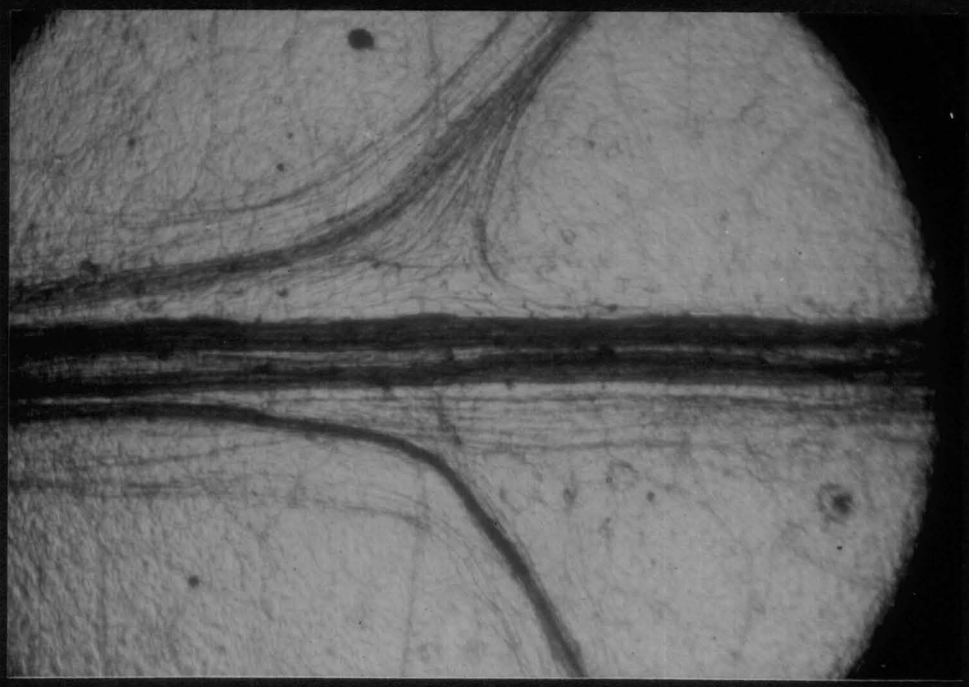
b) Ocimum gratissimum

c) Ocimum basilicum

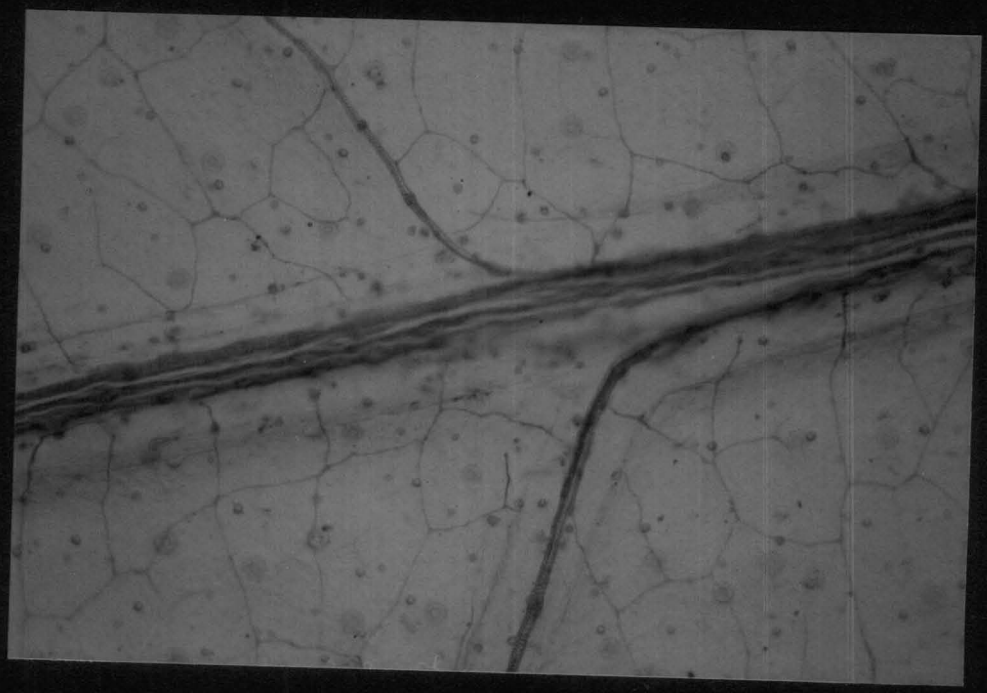
d) Ocimum canum







c



d

Table 10 Range of vein angle in different species of *Ocimum*

Species	Range of vein angle
O. tenuiflorum	55° - 72°
O. gratissimum	30° - 82°
O. basilicum	40° - 43°
O. canum	30° - 42°

4.3. Biochemical aspects

4.3.1. Essential oil yield

Essential oil from the leaves was extracted in two seasons - before monsoon showers and during monsoon showers. Slight variation in the oil yield was observed between species and also within a species between two seasons. Details of observations including volume and appearance of essential oil are given in Table 11.

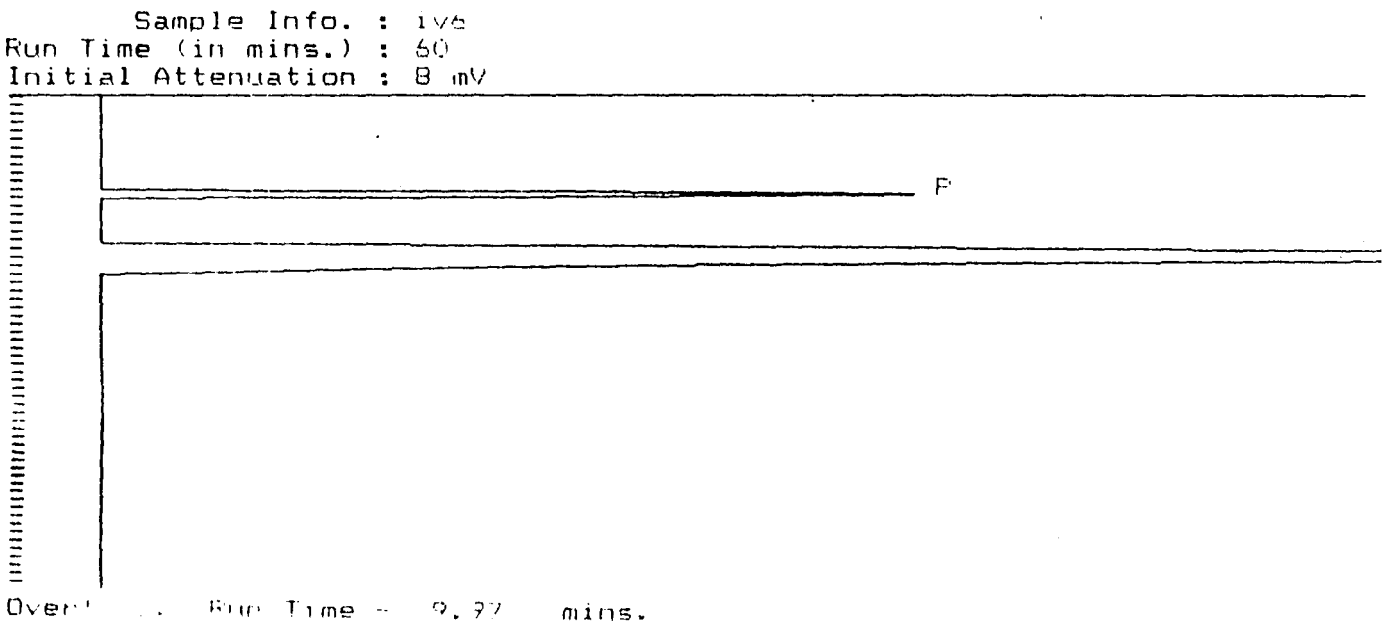
Table 11 Essential oil content in different species of *Ocimum*

Species / Character		*O. tenuiflourm*	*O. gratissimum*	*O. basilicum*	*O. canum*
Recovery per cent (ml/100 g)	before monsoon	0.17	0.17	0.17*	0.23
	during monsoon	0.15	0.35	0.30	0.47
Appearance of oil		Light and sparkling green	Pale green	cloudy with a pale greenish tinch	cloudy appearance
Temperature for distillation (°C)		75	80	100	100

[* In the case of *Ocimum basilicum* the oil collected in May showed two distinct layers - an upper cloudy area comprising one third of total volume of oil and a lower clear area comprising two third volume of oil collected. But the oil from the same species collected in July showed no such distinction of layers]

4.3.2. Constituents of essential oil

The chromatographs showed the retention time, per cent area and per cent height of the peaks of individual constituents (Fig.4 to 7).



Raw Area Normalized Report

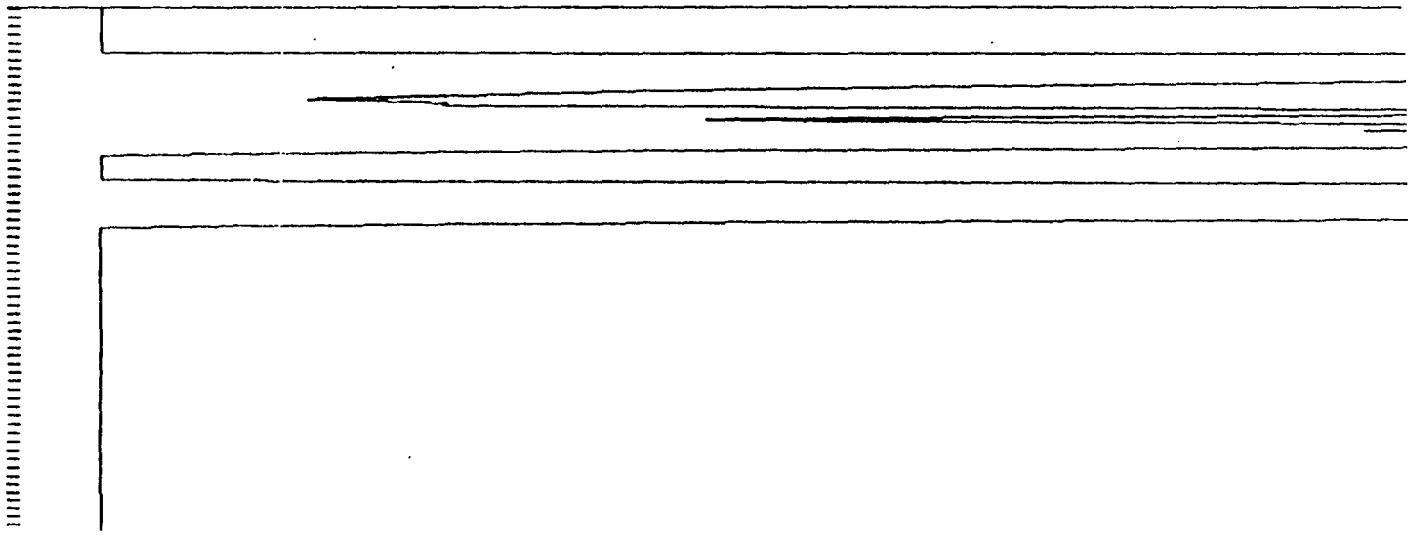
Mon Jun 06 05:58:31 1983

Sample Info : 1V6

No.	Ret time (min)	Height (uV)	Area (uV-s)	%Area	%Height
1	[illegible]	5004	33181	14.5491	32.4981
2	[illegible]	10398	194881	85.4509	67.5020
		15404	228062		

Fig.4 Chromatograph of essential oil of *O. tenuiflorum*

Sample Info. : iv6
Run Time (in mins.) : 60
Initial Attenuation : 8 mV



Over' .. Run Time - 10.28 mins.
0.00 3

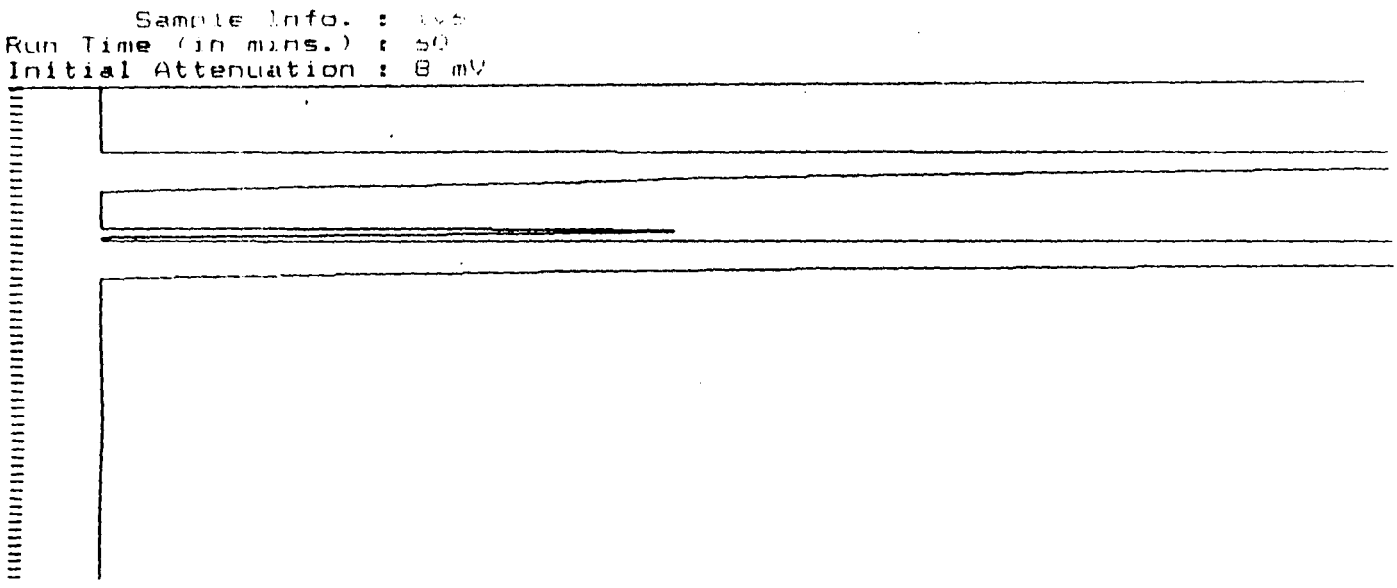
Raw Area Normalized Report

Mon Jun 06 06:38:01 1983

Sample Info. : iv6

No.	Ret Time (min)	Height (uV)	Area (uV-s)		%Area	%Height
1	[illegible]	34891	755478	V	27.9247	27.2099
[illegible]	[illegible]	12403	139375	V	5.1517	9.6725
3	[illegible]	9608	97913	V	3.6192	7.4928
4	2.53	13769	261313	V	9.6589	10.7378
5	3.53	57558	1451333		53.6455	44.8869
		128229	2705412			

Fig.5 Chromatograph of essential oil of *O. gratissimum*



Over' .. Run Time = 10.03 mins.

Raw Area Normalized Report

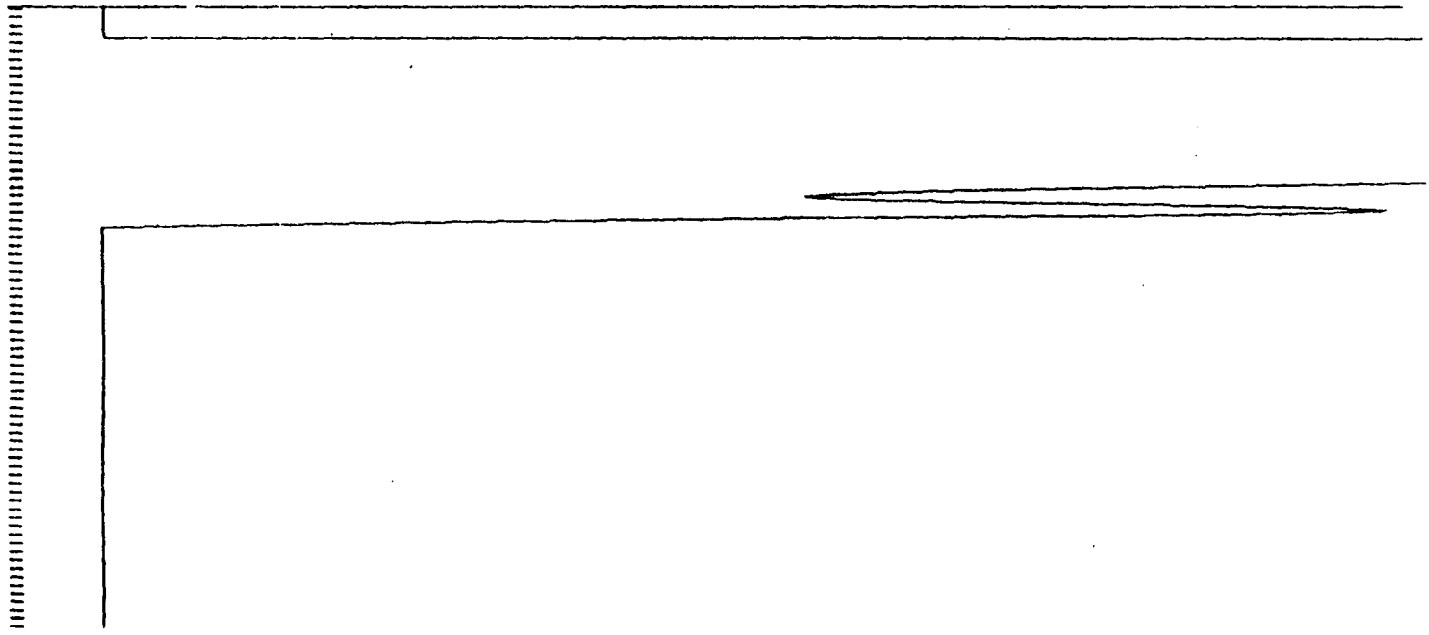
Mon Jun 06 05:45:33 1983

Sample Info : IV6

No.	Ret_time (min)	Height (uV)	Area (uV-s)		%Area	%Height
1	1.49	12372	311150		29.4751	23.7444
2	2.91	3527	24043	V	2.2776	6.7690
3	7.22	36206	720443	V	68.2473	69.4866
		52105	1055636			

Fig.6 Chromatograph of essential oil of *O. basilicum*

Sample Info. : iv6
Run Time (in mins.) : 60
Initial Attenuation : 8 mV



Over' .. Run Time - 12.18 mins.

Raw Area Normalized Report

Mon Jun 06 06:54:44 1983

Sample Info. : iv6

No.	Ret_time (min)	Height (uV)	Area (uV-s)		%Area	%Height
1	0.67	313015	1625819	V	14.9193	36.4731
2	0.85	102302	1332156	V	12.2245	11.9204
3	1.14	267709	4400495	V	40.3811	31.1940
4	1.98	75550	958289	V	8.7937	8.8032
5	2.29	94877	2484312	S	22.7973	11.0553
6	3.85	4754	96347	T	0.8841	0.5539
		858207	10897418			

Fig.7 Chromatograph of essential oil of *O. canum*

4.3.3. Protein banding pattern

Relative mobility - Rm values (Plate XIV) and the size of the protein bands formed in the four species are given in Tables 12 & 13.

Table 12 Rm values of protein bands in different species of *Ocimum*

Band No.	Rm value			
	O. tenuiflorum	*O. gratissimum*	*O. basilicum*	*O. canum*
1	0.9428	0.9238	0.9238	0.9143
2	1.0000	1.0000	0.9904	0.9809
3	1.0762	1.0670	1.0570	1.0285
4	-	-	1.0476	1.0952

Table 13 Thickness of protein bands in different species of *Ocimum* (cm)

Band No.	Thickness of band (cm)			
	O. tenuiflorum	*O. gratissimum*	*O. basilicum*	*O. canum*
1	0.4	0.5	0.4	0.5
2	0.3	0.5	0.4	0.3
3	0.4	0.5	0.3	0.4
4	-	-	0.2	0.2

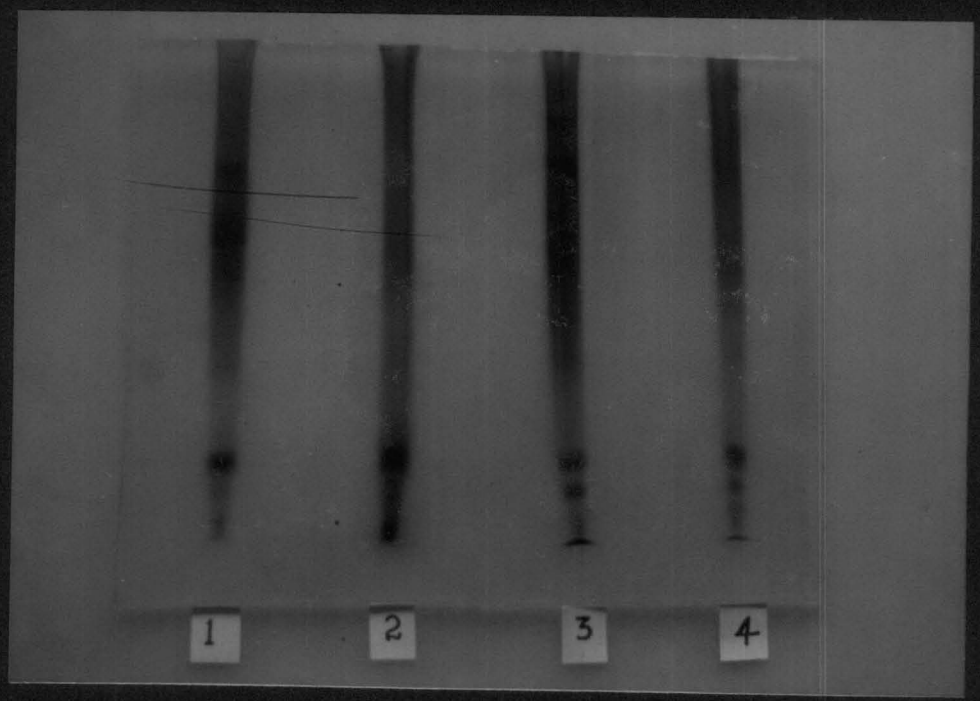
Plate XVI Protein banding pattern in four species of *Ocimum*

1. *Ocimum tenuiflorum*

2. *Ocimum gratissimum*

3. *Ocimum basilicum*

4. *Ocimum canum*


1
2
3
4

4.3.4. Chlorophyll estimation

The chlorophyll contents in the leaves of the four species expressed in milligram per unit weight of fresh leaf blade (Fig.8) and milligram per unit leaf area (Fig.9) are given in Table 14 and 15 respectively.

Table 14 Chlorophyll content per unit weight of leaves of different species of *Ocimum*

Species	Chlorophyll content (mg/g)			
	Chl.a	Chl. b	Chl.a+b	a/b
O.tenuiflorum	1.2488	4.8842	6.1300	0.2560
O.gratissimum	1.6220	2.2470	3.8700	0.7220
O.basilicum	1.0956	1.3071	2.4000	0.8380
O.canum	1.1641	1.5976	2.7600	0.7280

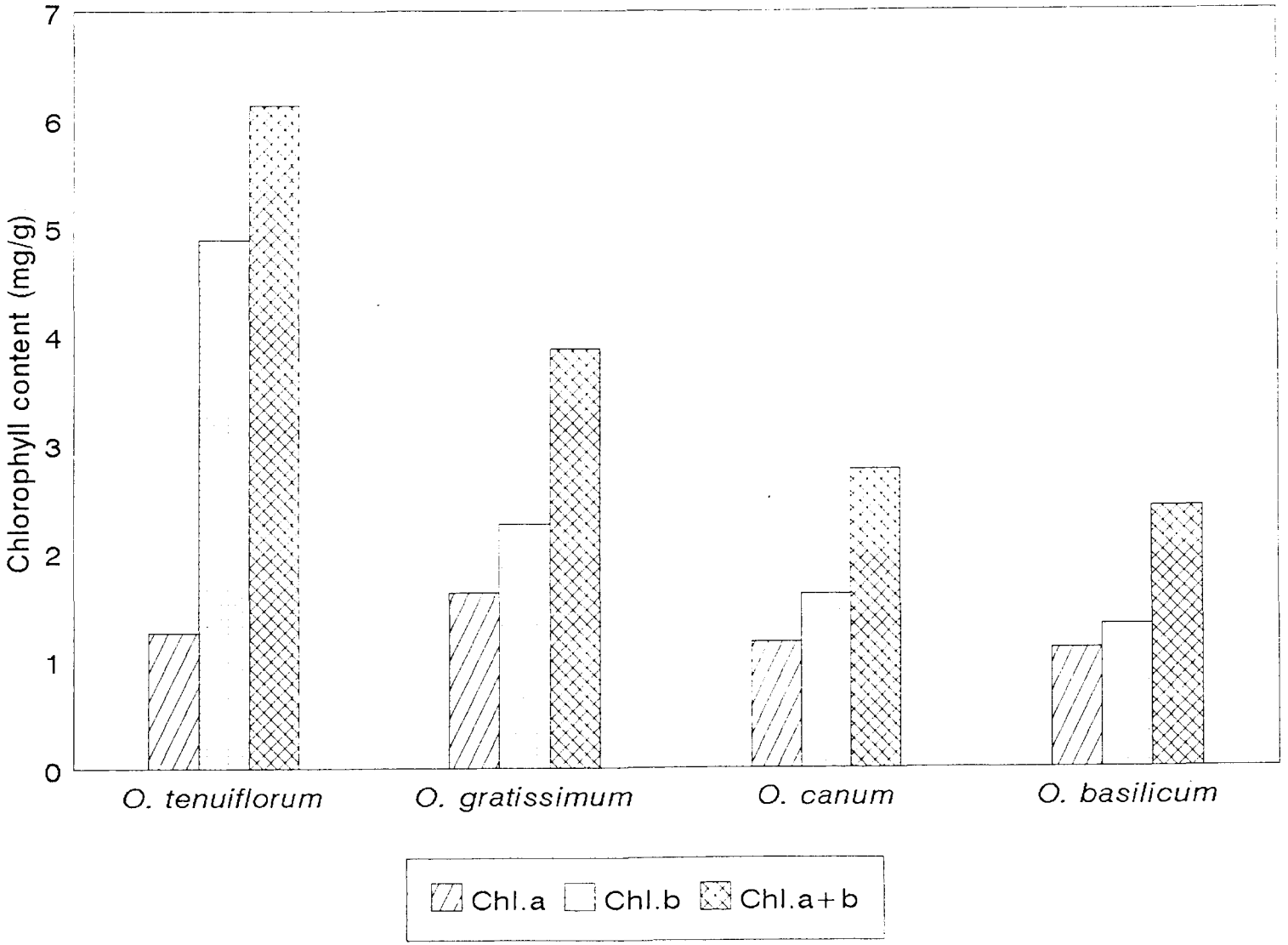


Fig.8. Chlorophyll content per unit weight of leaves of different species of Ocimum

Table 15 Chlorophyll content per unit area of the leaves of different species of *Ocimum*

Species	Chlorophyll content (mg/cm^2)			
	Chl.a	Chl. b	Chl.a+b	a/b
O.tenuiflorum	0.0165	0.0645	0.0809	0.2556
O.gratissimum	0.0217	0.0301	0.0520	0.7219
O.basilicum	0.0195	0.0233	0.0428	0.8370
O.canum	0.0186	0.0256	0.0442	0.7270

The chlorophyll index expressed as grams per square meter of ground area in different species of *Ocimum* is presented in Table 16 and Fig.10.

Table 16 Chlorophyll Index (CI) in different species of *Ocimum*

Species	C.I.
O. tenuiflorum	0.5762
O. gratissimum	0.7378
O. basilicum	0.2521
O. canum	0.1821

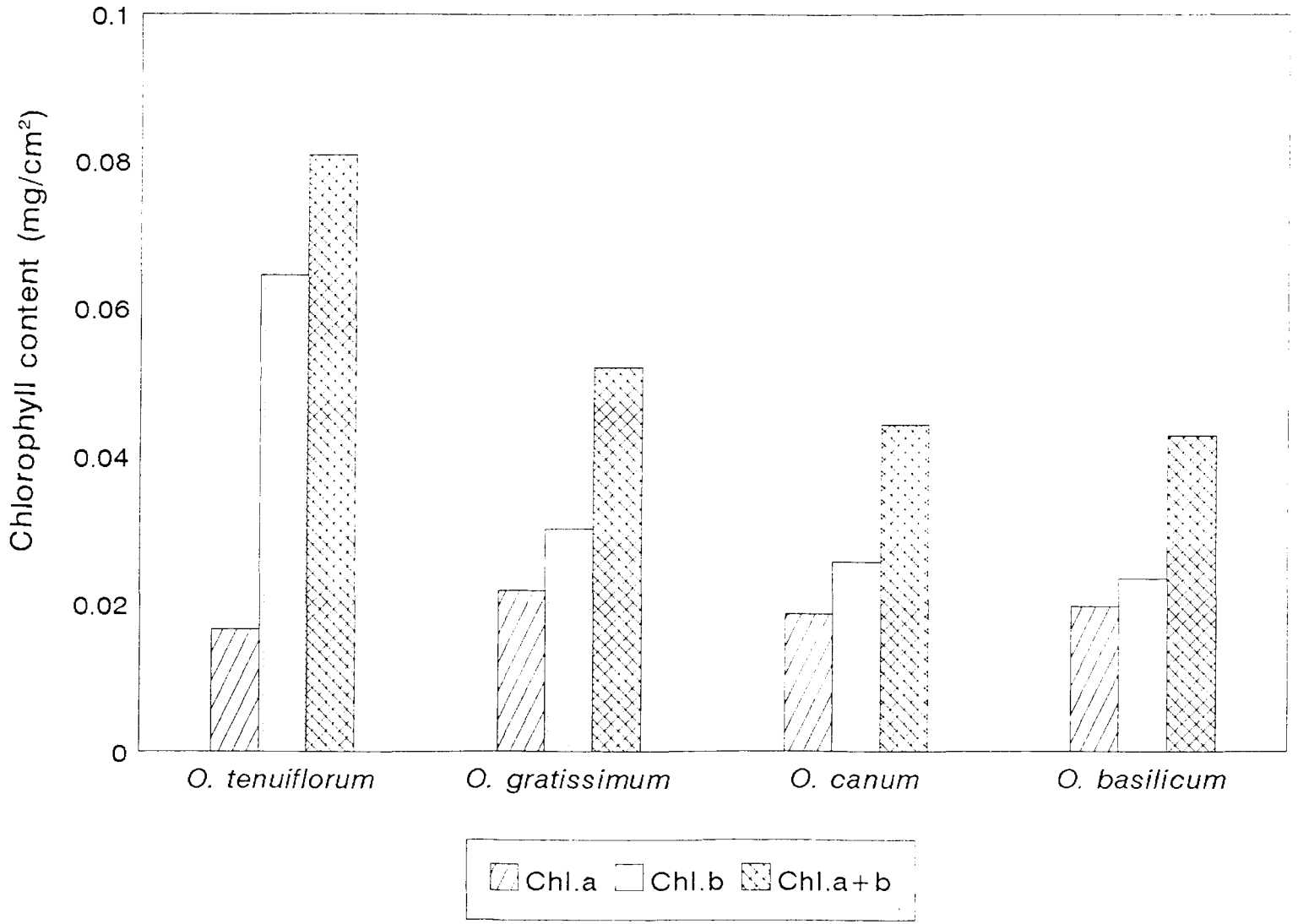


Fig.9. Chlorophyll content per unit area of leaves of four species of Ocimum

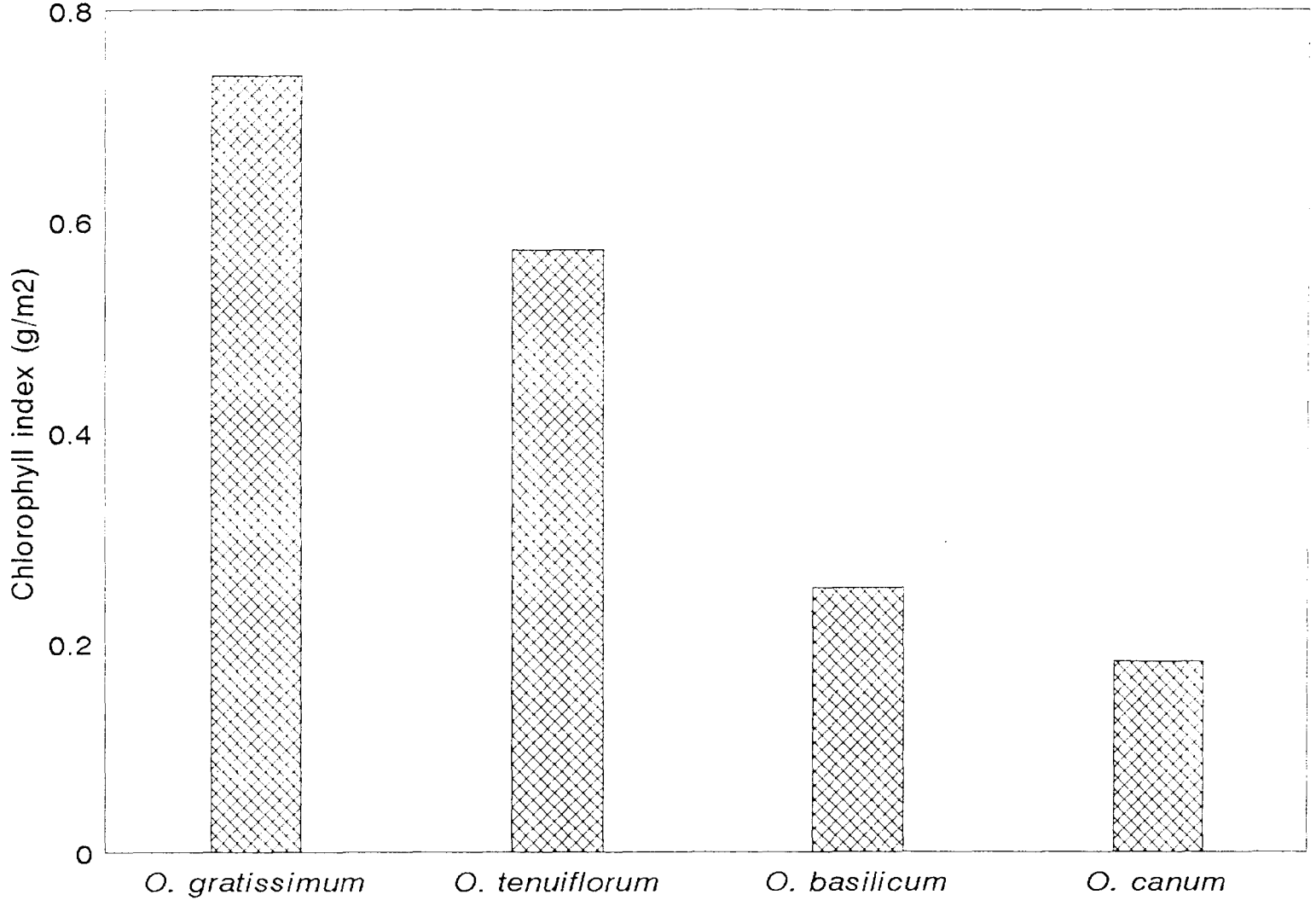


Fig.10. Chlorophyll Index (CI) in four species of *Ocimum*

Discussion

DISCUSSION

The four different species of *Ocimum*, viz., *O. tenuiflorum*, *O. gratissimum*, *O. basilicum* and *O. canum* were compared on the basis of morphological and anatomical features and protein banding pattern.

5.1 Morphological aspects

All the four species studied showed general labiatae characters. But they can be identified by specific morphological characteristics (Table 1).

O. tenuiflorum could be distinguished by the presence of ovate leaves with acute apex, crenate margin (undulate in mature leaves), flowers with pale violet petals violet filaments bearing yellow anthers and yellow pollen grains and violet style and brown subglobose seeds which are slightly mucilagenous when wetted.

O. gratissimum, a perennial shrub, could be identified by the presence of ovate leaves with acuminate apex, crenate margin and long petiole and long inflorescence with 20-24 floral whorls. The flowers are characterised with white petals, stamens having white filaments and yellow anthers producing creamy white pollen grains. Seeds are large brown subglobose and slightly mucilagenous when wetted.

O. basilicum, a herbaceous annual was found to be quite different from other species due to the presence of glabrous leaves, with small petiole, acuminate apex and serrulate margin, large persistant bracts at the base of floral whorls, flowers with white petals, white filaments, carrying yellow anthers and white pollen grains and large black ellipsoid seeds which are mucilagenous when wetted.

The distinguishing features of *O. canum* were found to be the presence of glabrous leaves with acuminate apex and serrulate margin, bracts with long hairs on the margin, green inflorescence axis, flowers with white petals, pale yellow anthers having white pollen grains borne on pale violet filaments and black small ellipsoid seeds with mucilage when wetted.

Pushpangadhan *et al.* (1993) had described the different species of *Ocimum* and had pointed out that *O. canum*, a herbaceous annual, is having a peculiar aroma for the leaves by which it can be identified.

The four species described above could thus be identified based on the following diagnostic features.

1. habit of the plant
2. leaf shape

3. L/W ratio of leaf
4. leaf margin
5. leaf apex
6. leaf base
7. colour of inflorescence axis
8. nature of bracts
9. floral characters like
    (a) features of calyx
    (b) colour of petal
    (c) colour of filament
    (d) colour of anther
    (e) colour of pollengrain
    (f) colour of style
10. size, shape and nature of seeds.

Eventhough the four species differed in many characters, similarities could also be observed. *O. gratissimum* and *O. tenuiflorum* resembled in leaf shape, L/W ratio, nature of leaf base and leaf margin. At the same time *O. basilicum* and *O. canum* were observed to be similar in the above characters (Table 1). In the colour of petal and inflorescence axis *O. tenuiflorum* and *O. basilicum* were found to be identical. Similarity was found

in the colour of style among *O. tenuiflorum, O. basilicum* and *O. canum*. *O. tenuiflorum* and *O. canum* were related in filament colour.

The evaluation of morphological features revealed that *O. canum* and *O. basilicum* are herbaceous annuals where as *O. tenuiflorum* and *O. gratissimum* are woody plants. Annual/biennial habit, reduced size and herbaceous nature are considered as advanced characters (Smith, 1967). Sporne, 1954, had discussed the statistical correlations between floral and vegetative characters in dicotyledons and according to him woody habit and perennial nature are reliable indicators of primitiveness. Hence it can be concluded that the two species, *O. canum* and *O. basilicum* are more evolved than the other two - *O. gratissimum* and *O. tenuiflorum*.

5.2 Anatomical aspects

5.2.1 Stem anatomy

A comparison of the stem anatomy of the four species revealed that though there was uniformity in the fundamental structure, there was variation in the size of epidermal cells, density of trichomes, nature of cortical cells, nature of xylem vessels and size of pith (Table 2).

In all the four species, epidermis was composed of almost rectangular cells with cuticular layer. *O. gratissimum* was observed to

have the largest epidermal cells and *O. tenuiflorum* the smallest (Table 2). The difference was quantitative rather than qualitative.

Ample variability was observed in trichome density and characters. Trichomes were rare in *O. basilicum* less in *O. gratissimum* and numerous in *O. tenuiflorum* and *O. canum*. The trichomes were found to be uniseriate filiform in all the species. However, they differed in the number of cells per trichome and number of foot cells(Table 2). Short stalked multicelled glandular trichomes were observed in all the species examined.

Cortex was found to be heterogenous in all the four species. Transverse sections of the stem showed the presence of mechanical tissue. Collenchyma was prominently seen in the corners of the quadrangular stem in all the species under study. In the case of *O. gratissimum* unlike in other species, several layers of collenchyma could be observed. This may be because it is a perennial woody shrub. Extrastelar secondary thickening was initiated in the third internodal region, in *O. gratissimum* only, possibly because of its perennial woody nature.

Pericycle was found to be ill defined in *O. tenuiflorum* and *O. gratissimum* and absent in *O. canum*. But discontinuous patches of pericycle, 3-4 layers thick, were observed in *O. basilicum* which will serve as anatomical identification mark of *O. basilicum*. Secondary thickening had

already started in the third node of all the species and hence cambial ring present in all of them. Xylem vessels were found to be almost circular in Transverse section in *O. canum* and *O. basilicum*. The vessels looked like tracheids in *O. gratissimum* and *O. tenuiflorum* (Table 2).

Comparatively large and prominent pith was present in *O. basilicum*.

Metcalfe (1968) had stated that anatomy of vegetative parts of flowering plants are useful in the identification of fragmentary materials and as an aid in establishing inter relationships of taxa at and above species level.

The anatomical evidences for identification of the four species can be summarised as follows:-

O. canum	:	absence of pericycle presence of xylem vessels which are circular in T.S.
O. basilicum	:	Presence of discontinuous patches of pericycle
	:	Presence of xylem vessels which are circular in T.S.
	:	Presence of prominent pith

O. gratissimum : Presence of 5-6 layers of collenchyma in the corners of cortical regions.

: Angular xylem vessels

: Early extra stelar secondary thickening

O. tenuiflorum : Presence of 3-4 layers of collenchyma in the corners of stem

: Presence of xylem vessels which are angular in T.S.

Anatomical evidences also support the fact that *O. basilicum* and *O. canum* are advanced when compared to *O. gratissimum* and *tenuiflorum*. Presence of several layers of collenchyma and angular xylem vessels in *O. gratissimum* and *O. tenuiflorum* and early extrastelar secondary thickening in *O. gratissimum* are indicators of the primitiveness (Sporne, 1954).

5.2.2 xylem vessel elements

Xylem vessel elements are considered to be valuable features in phylogeny and classification and hence they were examined for the sake of comparison.

The vessel elements in all the four species were devoid of tails. A wide range of forms of vessel elements including short to large, narrow to wide etc.

were common in different species of *Ocimum*. The pattern of thickening of vessel elements was uniform in all the species (Table 3) with the only exception that pitted vessel elements were absent in *O. tenuiflorum* and *O. gratissimum*. Annualr, helical, spiral, scalariform and reticulate types were observed in all the four species.

Among the different types, spiral vessel elements occurred with maximum frequency in all the species (Table 4).

Mean diameter of vessel elements varied with the species and also with the type of vessel element. Annular and helical vessel elements were extremely small (less than 25μm) in all the species (Table 5). Spiral, scalariform and reticulate could be grouped as small vessel elements (25-50 μ m). This grouping was based in the classification of vessel elements proposed by CSTCS (1939).

Mean length of vessel elements also varied with the type and species (Table 6). Annular and helical ones were very long and exceeded one microscopic field and hence could not be measured accurately. Spiral and scalariform vessel element in all the four species could be classified as medium sized (350-800 μ m) and reticulate vessel elements as moderately short (250-350 μ m) according to CSTCS classification (1937).

Spiral and scalariform vessels were longer and wider in all the species. Among the four species, *O. canum* was having the longest spiral and scalariform vessel elements and *O. basilicum* was having the widest spiral and scalariform vessel elements.

The L/B ratio of spiral and scalariform vessel elements varied with species (Table 7). The L/B ratio was the highest for *O. canum* in the case of scalariform and spiral vessel elements.

The trends of evolution of vessel elements have been firmly established through comparative anatomical studies on fossils and extant plants. Hence the evolutionary trends from tracheids to vessel elements is the most reliable tool in the study of phylogeny. This is true because this trend is both unidirectional and irreversible (Radford *et al.*, 1974). The vessel elements which are tracheid like are the most primitive types. Change in appearance of vessel elements from angular to circular in Transverse Section is considered to be advanced. Among the four species studied vessel elements of *O. canum* and *O. basilicum* were circular in Transverse section and *O. tenuiflorm* and *O. gratissimum* were angular. The xylem vessels appeared to have evolved in the series annular, helical, scalariform, reticulate and pitted (Scagel *et al.*, 1965). Among the four species compared *O. basilicum* and *O. canum* were having pitted vessels also. The presence of pitted xylem vessel elements and circular appearance of

xylem vessels in Transverse Section points to the fact that *O. basilicum* and *O. canum* are more evolved than the other two species.

5.2.3 Leaf anatomy

Irrespective of the species, epidermis was uniseriate. There was no remarkable difference in the nature of epidermal cells of abaxial and adaxial surfaces. Trichomes were observed in the adaxial and abaxial surfaces and they varied in size and distribution (Table 8). Trichomes were fewer in *O. gratissimum* leaves when compared to other species collenchymatous layer near the veins were found to be very prominent in *O. gratissimum*. There the leaves were hypostomatic also (Table 9). The highest stomatal index was recorded on the lower surface of *O. gratissimum* among the four species. Crystals were absent in all the species.

5.2.4 Vein angle

Table 10 shows the vein angle in different *ocimum* spp. As evident from the Table 10 it can't be considered as a criteria for identification of the species. Gupta and Bambie (1978b) had also reported that vein angle did not have much significance in the identification of different species in the genus *Ocimum*.

5.3 Biochemical aspects

5.3.1 Essential oil yield

Marked difference was observed in the oil yield of *Ocimum basilicum* during May and June (Table 11). Substantial increase in oil content was observed during monsoon except for *Ocimum tenuiflorum*. Oil obtained during May showed two distinct layers. But no such layer could be observed in the oil obtained during the month of July. The oil content of the species varied from 0.15 per cent in *O. tenuiflorum* to 0.47 per cent in *O. canum* on fresh weight basis. It is interesting to note that oil recovery was the highest in *O. basilicum* before rainy season and *O. canum* during rainy season. Clear sparkling oil was obtained for *O. tenuiflorum* and *O. gratissimum*. But the oil obtained from *O. basilicum* and *O. canum* were cloudy even after passing through anhydrous sodium sulphate. The essential oil yield in *Ocimum* spp. may be influenced by several factors like environment, stage of growth, harvest time, parts used etc. Charles *et al.* (1990).

The retention time for all the four samples were in the range of 0.63-3.85 minutes. In Neucon 5700, geraniol and related compounds have a retention time in the range of 0.43 to 5.30 in similar analytical conditions.

In *O. tenuiflorum*, the per cent area of individual peaks (Fig.4) indicated the presence of a component in a concentration of 85.4 per cent. Brophy *et al.* (1993) reported that the essential oil of *O. tenuiflorum* contained 87% methyl chavicol.

In *O. gratissimum* the concentration of a component was 53.6 per cent (Fig.5). Demissew (1993) reported that *O. gratissimum* contained 57.4 per cent eugenol.

In *O. basilicum* the concentration of major components obtained in the chromatograph (Fig.6) were 68.3 and 29.4 per cent respectively. In *O. canum* (Fig.7) the concentration of major components were 40.4 and 22.8 per cent respectively.

5.3.2 Protein banding pattern

Three bands were obtained in *O. tenuiflorum* and *O. gratissimum*, the Rm values of which were similar (Table 12). In *O. basilicum* and *O. canum* four bands were formed. The Rm values of three of these bands were almost similar to the other two species *O. gratissimum* and *O. tenuiflorum*. Hence it may be concluded that there is similarity in the protein banding pattern of the four species with the exception that *O. canum* and *O. basilicum* possessed an additional band. The Rm values and number of bands are expected to be

markers of species difference. Dickerson (1972) had reported that proteins are valuable taxonomic characters as they exhibit conservatism in evolution. Protein banding pattern of the four species of Ocimum points to the fact that the four species are phylogenetically related and two of them are more advanced from the evolutionary point of view.

5.3.3 Chlorophyll estimation

Variation was observed in the contents of chlorophyll a, b and total chlorophyll of four species of *Ocimum*. The chlorophyll a/b ratio was the highest in *O. basilicum* and the lowest in *O. tenuiflorum* (Table 14 and 15).

From Table 16 it is clear that chlorophyll index was the highest in *O. gratissimum* which is a perennial shrub and the lowest in *O. canum* which is a herbaceous annual. Chlorophyll index is a good substitute for Leaf Area Index (Sestak *et al.*, 1971).

Based on the variations in morphological features, anatomical features and protein banding pattern a key for identification is proposed.

Key for identification of *Ocimum* spp.

1a Perennial shrub with leaf base obtuse, leaf margin crenate, L/W ratio of leaves 3:2, pericycle ill defined, xylem vessels angular in T.S., anthers yellow, seeds dark brown, subglobose and slightly mucilagenous and with three protein bands in electrophoresis.

2a leaf apex acute, 3-4 layers of collenchyma in the cortex, inflorescence axis purple, bracts small and hairy, calyx with lower lip longer, petals pale violet, style videt pollen grains creamy yellow and seeds small

..... *O. tenuiflorum.*

2b leaf apex acuminate, 5-6 layers of collenchyma in the cortex towards the corners of stem, inflorescence axis green, bracts large and hairy, calyx with upper lip longer, petals white with greenish tinch, style white, pollen grains creamy white and seeds large ..... *O. gratissimum.*

1b Annual herb with leaf base acute, leaf margin serrulate, L/W ratio 2:1, cortex with 3-4 layers of collenchyma towards the corners of stem, xylem vessels circular in T.S., anthers pale yellow; pollen grains white, seeds black ellipsoidal and densely mucilagenous, with four protein bands in electrophoresis.

3a stem with discontinuous patches of pericycle, inflorescence axis purple, bracts very large and hairy, sepals green with purple tinch and slightly hairy, petals white with purple stripes on lower lip, style white with violet tinch at the base and seeds large ..... *O. basilicum.*

3b stem with pericycle absent, inflorescence axis green, sepals palegreen with long hairs, petals white, style paleviolet and seeds small ..... *O. canum.*

Summary

SUMMARY

Investigations were undertaken in the Department of Genetics and Plant Breeding during 1994-95 to evaluate the morphological and anatomical features, protein banding pattern and essential oil content of the four different species of *Ocimum* viz., *O. tenuiflorum, O. gratissimum, O. basilicum* and *O. canum.*

The salient findings could be summarised as follows:

The four different species of Ocimum used for the present study showed general labiatae characters. But variations were observed in morphological features like the habit of the plant, nature of leaf base, leaf margin, leaf apex, bracts, colour of inflorescence axis, nature of calyx, colour of petals, anthers, pollen grains, style, size shape and nature of seeds etc.

The anatomical studies revealed that *O. gratissimum* was having several layers of collenchyma in the cortical region in the corners of the stem and extra stelar secondary thickening in the third internodal region. Pericycle, 3-4 layers thick and in discontinuous patches, was a characteristic anatomical features of *O. basilicum.* Pericycle was found to be totally absent in *O. canum* but ill defined in *O. tenuiflorum* and *O. gratissimum.* Xylem vessels were observed to be circular in *O. basilicum* and *O. canum* where as they were angular in *O. gratissimum* and *O. tenuiflorum.*

Diameter and length of vessel elements varied with the species and also with the type of vessel element. Pitted vessel elements were observed only in *O. basilicum* and *O. canum*.

Biochemical studies revealed that oil content and quality, chlorophyll content and chlorophyll index varied with the species.

The electrophoretic studies showed that there is similarity in the banding pattern of the four species of *Ocimum*. But one additional band was found in *O. basilicum* and *O. canum*.

A key has been proposed for the identification of the species. Morphological and anatomical features of the four species studied revealed that *O. tenuiflorum* and *O. gratissimum* are related and *O. basilicum* and *O. canum* are related. The protein banding pattern obtained also supports this view. Among the four species observed, *O. basilicum* and *O. canum* appears to be more advanced from evolutionary point of view since they are herbaceous and possess xylem vessels circular in T.S., pitted xylem vessel elements and one additional protein band in electrophoresis.

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EVALUATION OF MORPHO-ANATOMICAL VARIATIONS IN *Ocimum* Spp.

BY

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ABSTRACT OF A THESIS

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ABSTRACT

A comparative evaluation of the morphological and anatomical features the protein banding pattern of the four different species of *Ocimum* viz., *O. tenuiflorum, O. gratissimum, O. basilicum* and *O. canum* was carried out in the Department of Plant Breeding and Genetics during 1994-95 to find out the evolutionary relationships existing among the species and to prepare a key for their identification.

Ample variability was observed among the four species of *Ocimum* for morphological and anatomical features as well as biochemical aspects. Based on these observations a key for identification of the species is proposed.

From the morphological and anatomical observations made during the study it appears that *O. gratissimum* and *O. tenuiflorum* are phylogenetically related and so also *O. basilicum* and *O. canum*. The protein banding pattern of the four species further confirms this view.

From the herbaceous nature, presence of pitted xylem vessel elements and vessels which are circular in Transverse Section it seems that *O. basilicum* and *O. canum* are more evolved than *O. tenuiflorum* and *O. gratissimum.*