SEETHALAKSHMI RAMASWAMI COLLEGE (AUTONOMOUS)

ACCREDITED AT 'A' GRADE (3rd CYCLE) BY NAAC
AFFILIATED TO BHARATHIDASAN UNIVERSITY
TIRUCHIRAPPALLI – 620 002

LAB MANUAL
III B.Sc., Botany

PRACTICAL PAPER –III

MORPHOLOGY, TAXONOMY, ECONOMIC BOTANY, BOTANICAL TECHNIQUES AND GENERAL MICROBIOLOGY

Mrs. T. Radhamani
Associate Professor and Head

PREFACE

The scientific frame of mind is necessary to observe and examine the different facets of scientific facts. The laboratory training prepares futuristic in the subject botany. With the increasing dependence on laboratory training, there is a need for basic instructions all together in one place. So, the laboratory manual has been compiled, considering the nature of training imparted and demands of the syllabi. In Morphology and Taxonomy, illustrations have been designed in such a way, so that salient features are clearly displayed. In Botanical techniques, principles and usage of compound and other microscopes, colorimeter as well as cytological techniques are discussed. In general microbiology, various laboratory methods presented in lucid and systematic manner for culturing of bacteria & fungi and study of soil, water & food microbes. Keeping in mind problems faced by students do's and don'ts while microbial experiments also enumerated. Diagrams and flow charts help to make learners easier and more interesting. I am sure this manual will be of great help to students.

I am highly indebted to our managing trustee, for his valuable support and also to Dr.(Mrs) Kanaka Bhashyam, Principal, Seethalakshmi Ramswami College for her fruitful guidance and timely help during the preparation of this manual. I sincerely thank DBT Star College Scheme, New Delhi for the financial assistance in strengthening the life science at UG level.

Mrs. T. Radhamani
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PRACTICAL PAPER- III

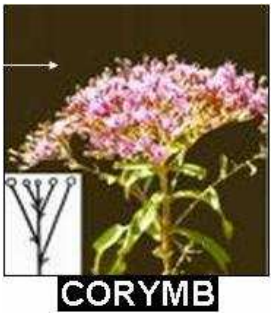
MORPHOLOGY, TAXOMONY, ECONOMIC BOTANY, BOTANICAL TECHNIQUES AND GENERAL MICROBIOLOGY

CONTENTS

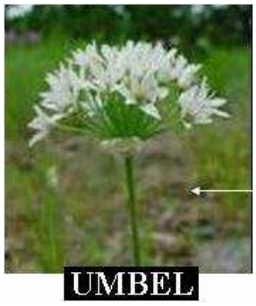

SPIKE


SPIKELET

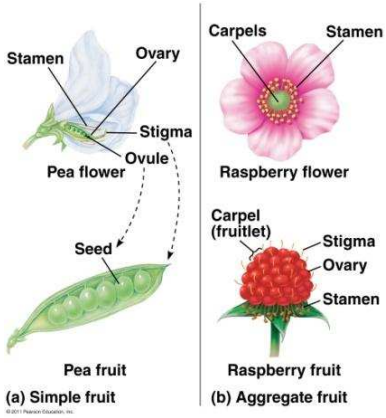

CATKIN

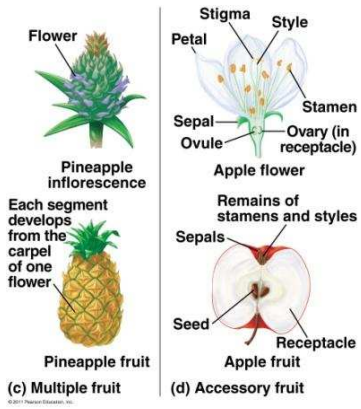

CORYMB


SPADIX


UMBEL

MORPHOLOGY

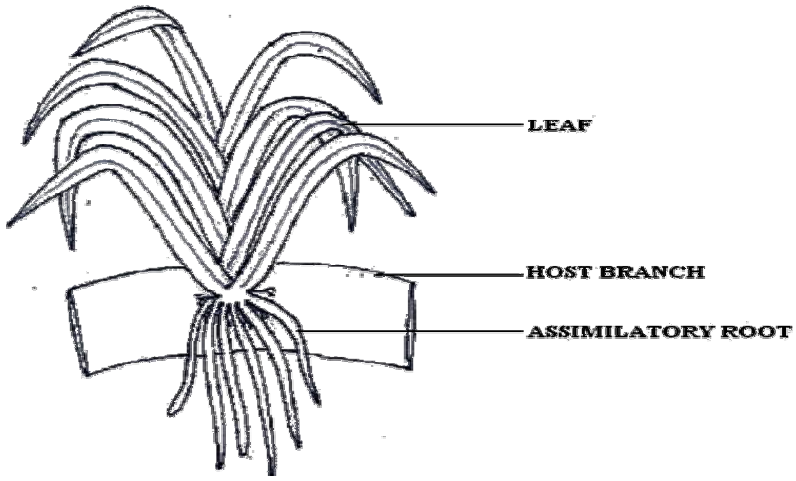

Stamen
Ovary
Stigma
Ovule
Pea flower
Seed
Pea fruit
(a) Simple fruit
Carpels
Stamen
Raspberry flower
Carpel (fruitlet)
Stigma
Ovary
Stamen
Raspberry fruit
(b) Aggregate fruit


Flower
Pineapple inflorescence
Each segment develops from the carpel of one flower
Pineapple fruit
(c) Multiple fruit
Stigma
Style
Petal
Stamen
Sepal
Ovule
Ovary (in receptacle)
Apple flower
Remains of stamens and styles
Sepals
Seed
Receptacle
Apple fruit
(d) Accessory fruit

ROOT MODIFICACTIONS

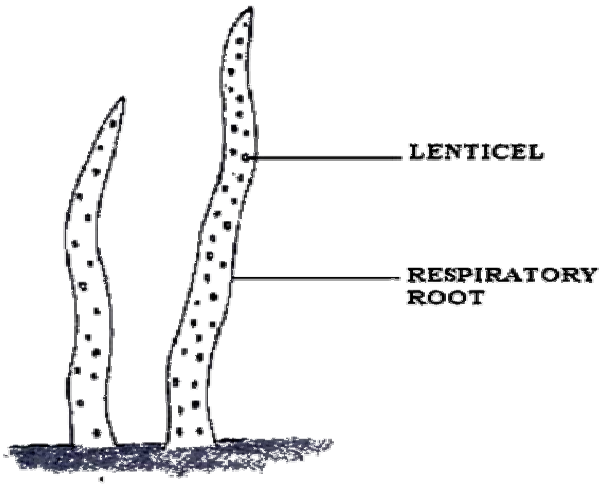
ASSIMILATORY ROOT – Eg. *VANDA*

- ***Vanda*** is an epiphyte which produces numerous aerial roots.
- These aerial roots hang freely in air and are dull white when dry and become green when wetted with water.
- This is due to the presence of chlorophyllous tissue called velamen which is seen below the epiblema.
- With the help of chlorophyll, these roots carry out the assimilatory function and hence these roots are called as assimilatory roots.



PNEUMATOPHORE – Eg. *AVICENNIA*

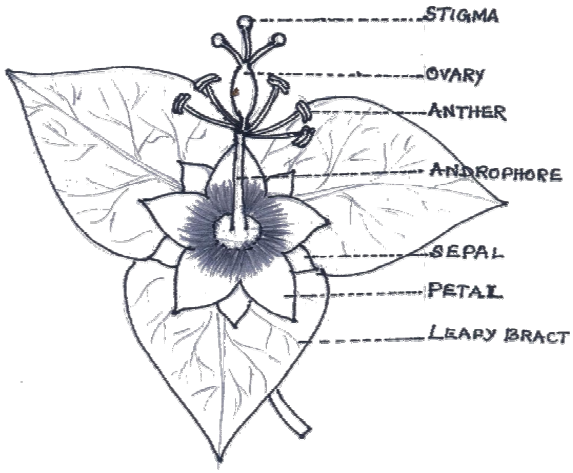
- The mangrove plant ***Avicennia*** grows in salt water logged soil.
- Numerous vertical apogeotrophic roots developed from the normal lateral root system.
- These roots possess small pores called lenticels.
- Gaseous exchange occurs through these pores.
- Since these roots help in respiration, so they are called respiratory roots or pneumatophores.



TORUS MODIFICACTION

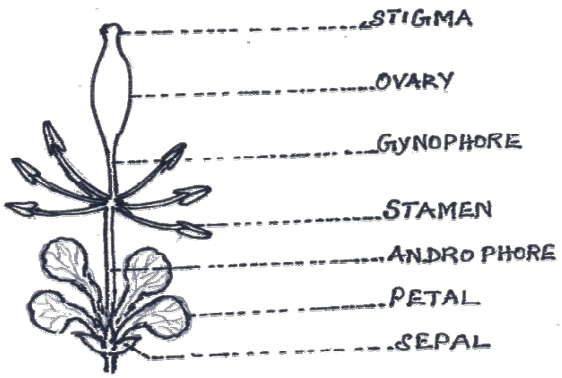
ANDROPHORE –Eg. *PASSIFLORA*

- In ***Passiflora***, the torus is elongated between corolla and androecium.
- This elongated torus is called androphore.



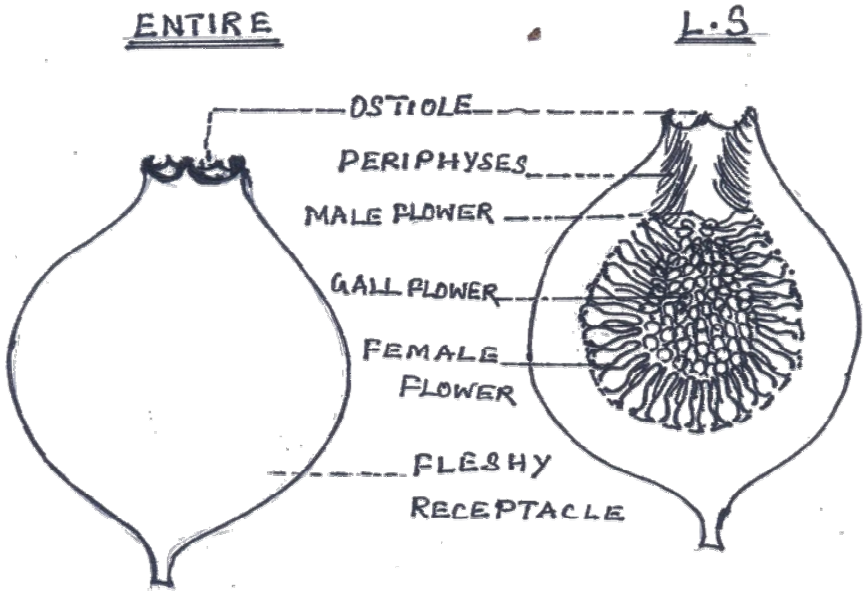
GYNANDROPHORE- Eg .*GYNANDROPSIS*

- In *Gynandropsis*, the torus is not only elongated between corolla and androecium.
- But also elongated between androecium and gynoecium.So it is called as gynandrophore.



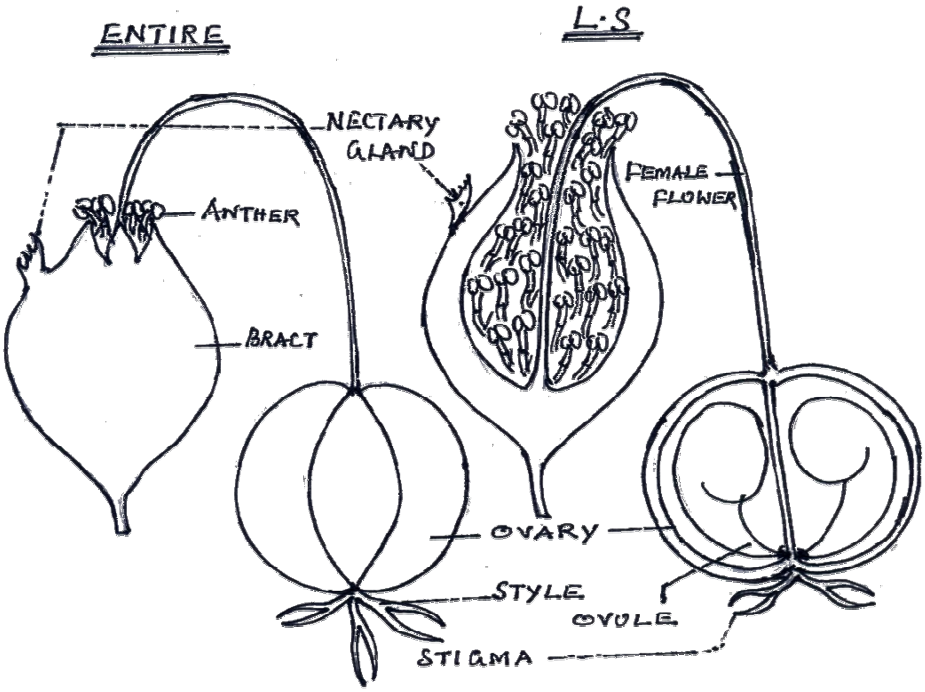
SPECIAL TYPES OF INFLORESCENCE
HYPANTHODIUM – Eg. *FICUS*

- The inflorescence consists of a thick fleshy receptacle.
- The receptacle becomes hollowed out to form a central cavity which
- encloses numerous unisexual sessile flowers.
- A small hole called ostiole is seen at the apex.
- Numerous hairs called periphyses are seen near the ostiole.
- This type of inflorescence is characteristic of the family Moraceae.



CYATHIUM – Eg. *EUPHORBIA HETEROPHYLLA*

- The inflorescence consists of five involucral bracts which are united to form an involucral cup.
- This cup encloses a solitary central pedicellate achlamydeous female flower surrounded by five scopioid cymes of male flowers.
- An extra floral nectary gland is present at the rim of the cup.
- The inflorescence is very much condensed looking like a single flower.
- This type is the characteristic of the family Euphorbiaceae.



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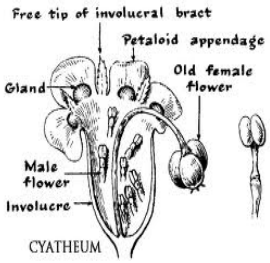
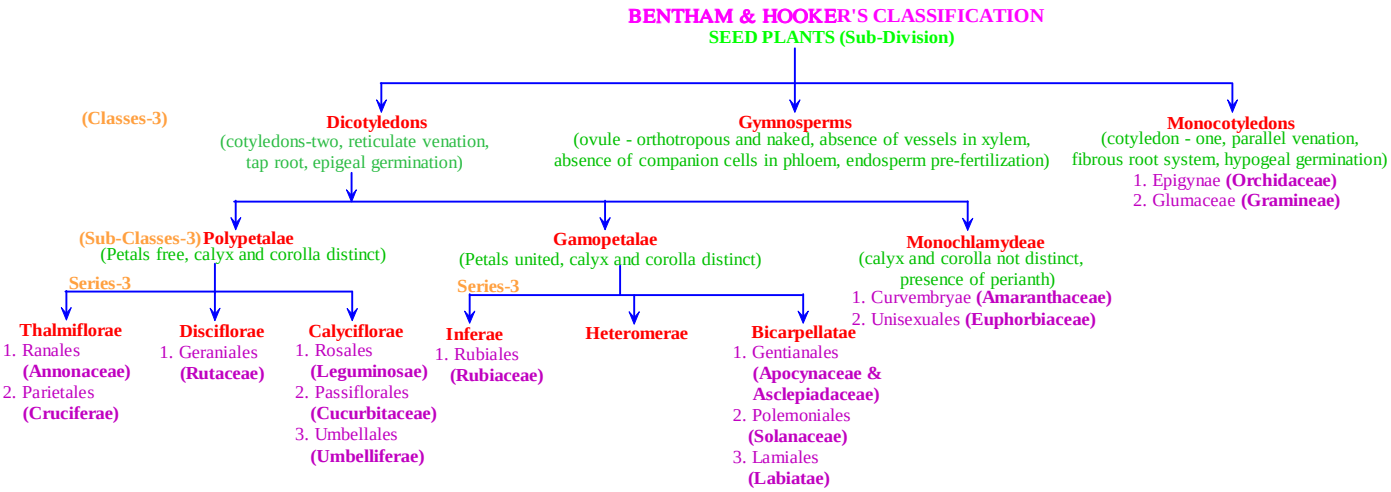


TAXONOMY AND ECONOMIC BOTANY


BENTHAM & HOOKER'S CLASSIFICATION
SEED PLANTS (Sub-Division)
(Classes-3)
Dicotyledons
(cotyledons-two, reticulate venation, tap root, epigeal germination)
Gymnosperms
(ovule - orthotropous and naked, absence of vessels in xylem, absence of companion cells in phloem, endosperm pre-fertilization)
Monocotyledons
(cotyledon - one, parallel venation, fibrous root system, hypogeal germination)
1. Epigynae (Orchidaceae)
2. Glumaceae (Gramineae)
(Sub-Classes-3)
Polypetalae
(Petals free, calyx and corolla distinct)
Gamopetalae
(Petals united, calyx and corolla distinct)
Monochlamydeae
(calyx and corolla not distinct, presence of perianth)
1. Curvembryae (Amaranthaceae)
2. Unisexuales (Euphorbiaceae)
Series-3
Series-3
Thalmiflorae
1. Ranales (Annonaceae)
2. Parietales (Cruciferae)
Disciflorae
1. Geraniales (Rutaceae)
Calyciflorae
1. Rosales (Leguminosae)
2. Passiflorales (Cucurbitaceae)
3. Umbellales (Umbelliferae)
Inferae
1. Rubiales (Rubiaceae)
Heteromerae
Bicarpellatae
1. Gentianales (Apocynaceae & Asclepiadaceae)
2. Polemoniales (Solanaceae)
3. Lamiales (Labiatae)

FAMILY: ANNONACEAE

ANONA SQUAMOSA, LINN

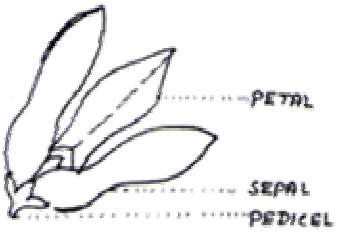
CHARACTERISTICS

- Simple, alternate, bifarious, exstipulate leaves.
- Leaf opposed fascicle.
- Bisexual, actinomorphic, trimerous, hemicyclic, hypogynous flower.
- 3 small free sepals, valvate.
- 3 petals, free, spoon shaped, sepaloid, valvate.
- Many free stamens & carpels spirally arranged on conical torus.
- Many free stamens with short filaments and dithecous, dorsifixed, extrorse, hooded anthers.
- Many carpels, apocarpous, superior ovary.
- Each carpel unilocular with one ovule on basal placentation Aggregate fruit.

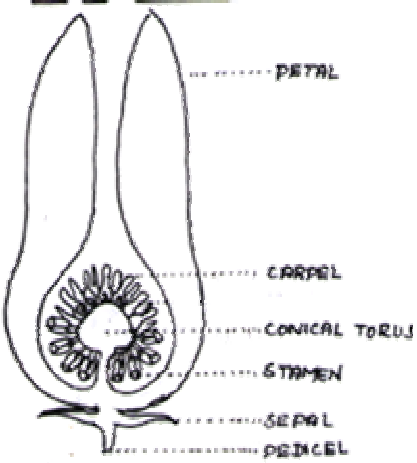
FAMILY : ANNONACEAE

ANNONA SQUAMOSA LINN

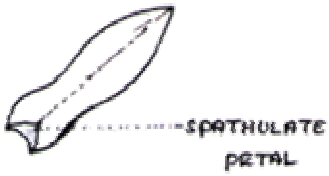
ENTIRE FLOWER



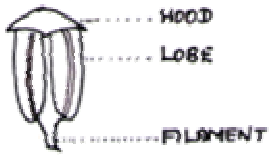
L.S OF FLOWER



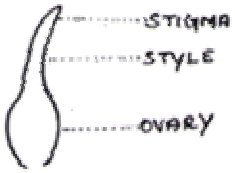
PETAL



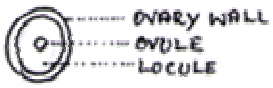
STAMEN



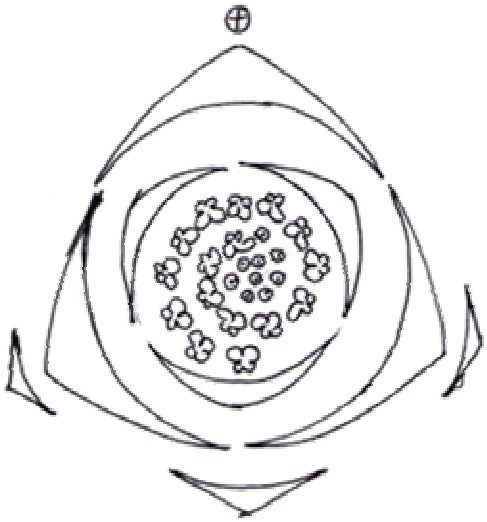
CARPEL



T.S OF OVARY



FLORAL DIAGRAM



FLORAL FORMULA

$Br.\ Btl, \female\oplus, K_3.C_3.A_x.\underline{G_x}$

FAMILY: CRUCIFERAE

BRASSICA JUNCEA, HK & F.

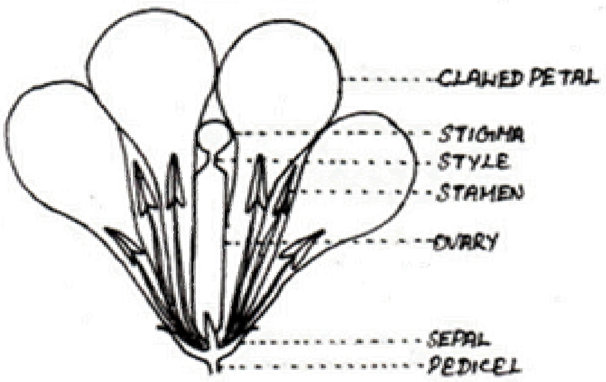
CHARACTERISTICS

- Simple, alternate, bifarious, lyrate, exstipulate leaves with pungent smell.
- Terminal corymb.
- Bisexual, actinomorphic, trimerous, cyclic, hypogynous flower.
- 4 .4-sepals, free, anteroposteriorly placed, imbricate aestivation.
- 4-petals, free, clawed, cruciform, valvate.
- 6-stamens, free, tetradynamous, outer 2 short & inner 4 long with stout filaments and dithecous, introrse anthers.
- Bicarpellary, syncarpous, unilocular, superior ovary becomes bilocular due to the formation of false septum.
- Ovules on parietal placentation.
- Capitate stigma.
- Siliqua fruit.

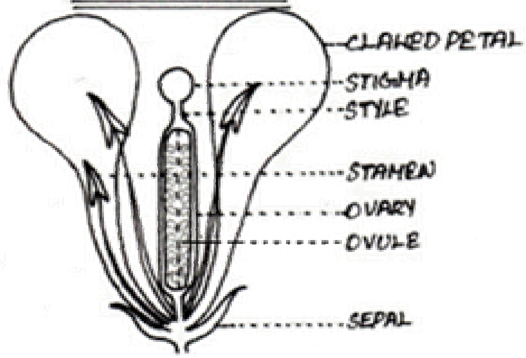
FAMILY : CRUCIFERAE

BRASSICA JUNCEA

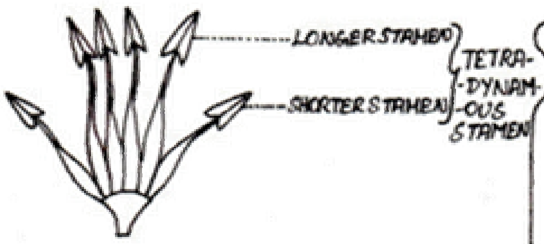
ENTIRE FLOWER



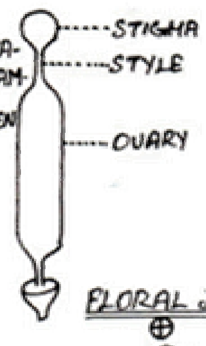
L.S OF FLOWER



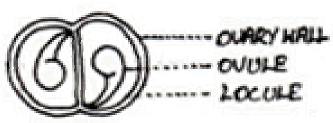
ANDROECIUM



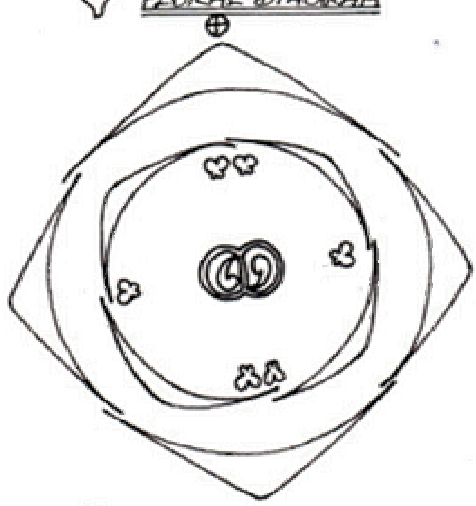
GYNOECIUM



C.S OF OVARY



FLORAL DIAGRAM



FLORAL FORMULA : EBr, EBrl, ⚥, $K_{(2+2)}$, C_4, A_{2+4}, $\underline{G}_{(2)}$

FAMILY: RUTACEAE

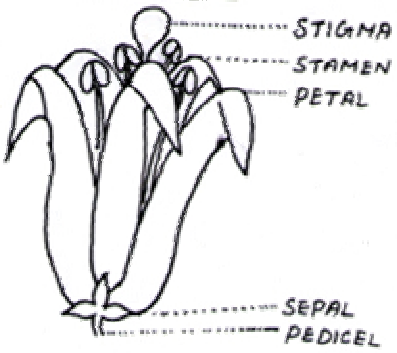
MURRAYA EXOTICA, LINN.

CHARACTERISTICS

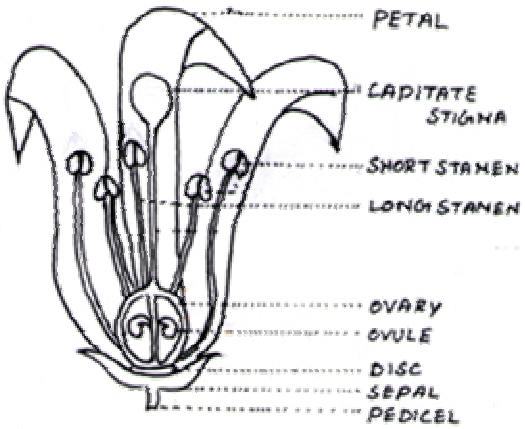
- Pinnately compound, exstipulate, aromatic gland dotted leaves with intramarginal reticulate venation.
- Axillary and terminal cymose clusters.
- Bisexual, actinomorphic, pentamerous, pentacyclic, hypogynous flowers.
- 5-petals, free, quincuncial aestivation.
- 5-petals, free twisted.
- 10 stamens, free, obdiplostemony, outer 5 long antipetalous stamens and inner 5 short antisepalous stamens.
- Filaments stout, anthers are dithecous, introrse.
- Bicarpellary, syncarpous, bilocular, superior ovary with ovules in each locule on axile placentation.
- Style simple, stigma capitate.
- Presence of a hypogynous disc.
- Berry fruit.

Family: Rutaceae
Murraya exotica

ENTIRE FLOWER



L.S OF FLOWER



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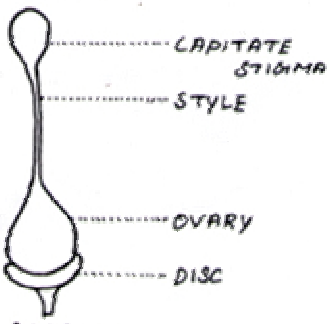
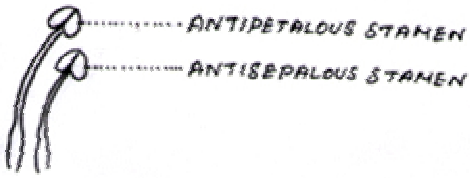
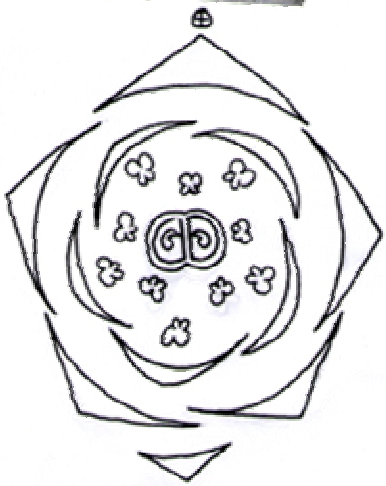


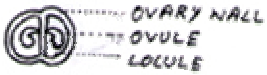
ANDROECIUM



FLORAL DIAGRAM



OVARY OF C.S



F.F : $Br\overline{EBr}l, \bar{\varphi}, \oplus, K_{(5)}, C_5, A_{5+5}, \underline{G}_{(2)}$

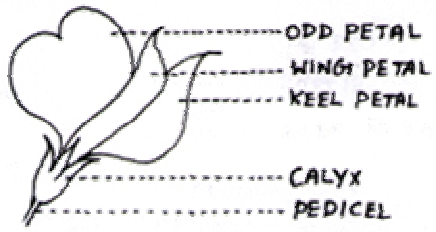
FAMILY: LEGUMINOSAE
SUB- FAMILY: PAPILIONACEAE
CROTALARIA STRIATA, D.C.

CHARACTERISTICS

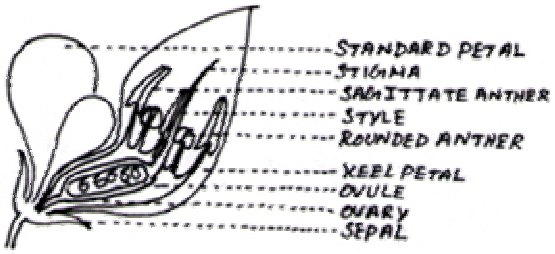
- Pulvinate, alternate, trifoliately compound leaves.
- Terminal long raceme.
- Bisexual, zygomorphic, pentamerous, hypogynous flowers.
- 5-petals, united, valvate.
- 5-petals, irregular papilionaceous corolla with a large standard petal, 2 wing petals and 2 keel petals which united to form a boat like structure enclosing the essential organs, descendingly imbricate aestivation.
- 10 stamens-monadelphous, 5 long filaments with arrow shaped anthers & 5 short filaments with rounded anthers, dimorphic introrse.
- Monocarpellary, unilocular, superior ovary with ovules on marginal placentation.
- Style feathery ending in a minute stigma.
- Legume fruit.

FAMILY : LEGUMINOSAE
SUB FAMILY : PAPILIONACEAE
CROTALARIA STRIATA

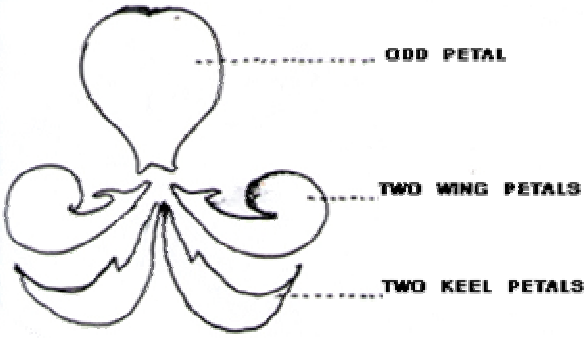
ENTIRE FLOWER



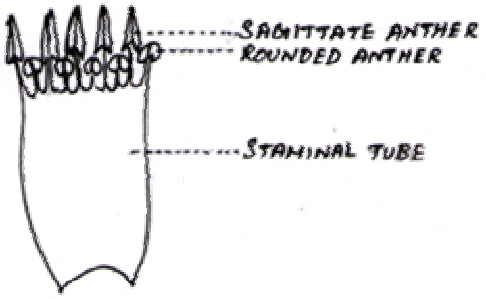
FLOWER L.S



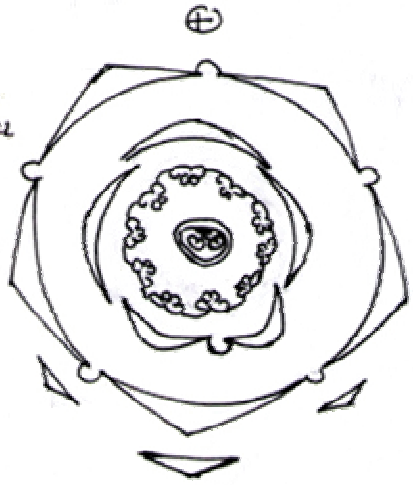
PAPILIONACEOUS COROLLA



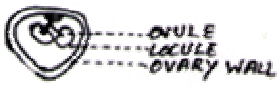
ANDROECIUM



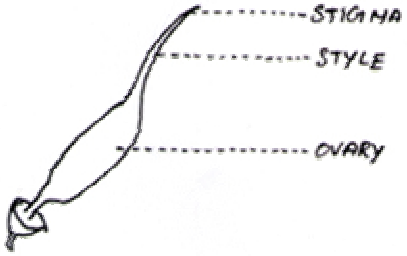
FLORAL DIAGRAM



OVARY C.S



GYNOECIUM



E.F : Br, Brl, ♂, ♀, K(5), C_5, A(∞), $\underline{G}_1$

FAMILY:LEGUMINOSAE
SUB- FAMILY:CAESALPINEAE
CASSIA AURICULATA, LINN.

CHARACTERISTICS

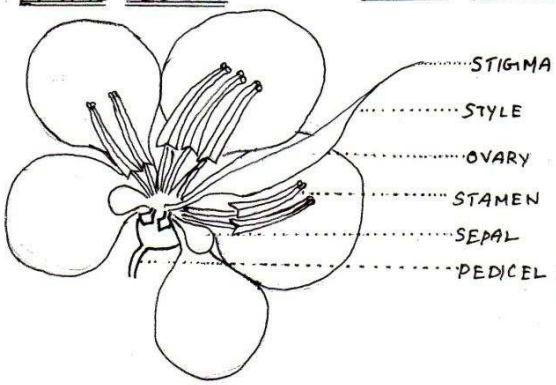
- Alternate, pulvinate, paripinnately compound stipulate (auriculaceous) leaves with small oblong leaf lets.
- Terminal corymbs.
- Bisexual, zygomorphic, pentamerous, perigynous flowers.
- 5 sepals, free, odd sepal anterior, imbricate.
- 5 petals, clawed, free, odd petal posterior ascendingly imbricate aestivation.
- 10 stamens, 7 fertile and 3 posterior staminode, anthers are dithecous, introrse with porous dehiscence.
- Monocarpellary, unilocular, half inferior ovary with ovules on marginal placentation, style-simple, ending in a minute stigma.
- Legume fruit.

FAMILY : LEGUMINOSAE

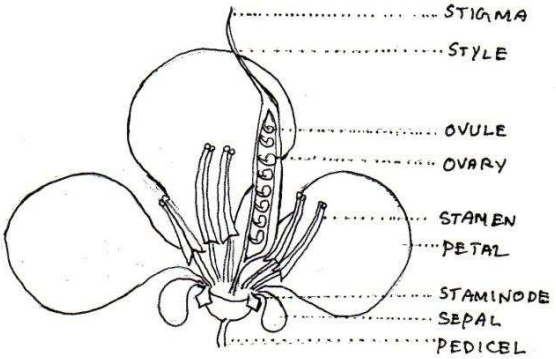
SUBFAMILY: CAESALPINEAE

CASSIA AURICULATA

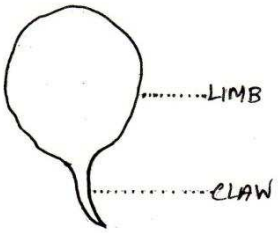
ENTIRE FLOWER



L.S OF FLOWER



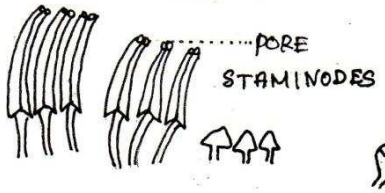
CLAWED PETAL



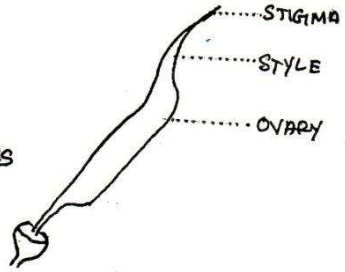
ANDROECIUM

3 LONGER STAMENS

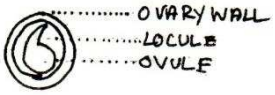
4 MEDIAN SHORTER STAMENS



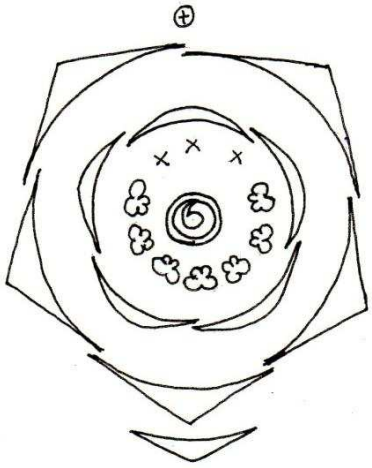
GYNOECIUM



T.S OF OVARY



FLORAL DIAGRAM



E.P : Br, EBrl, ♂, ♀, K5, C5, A3+4+3, G1

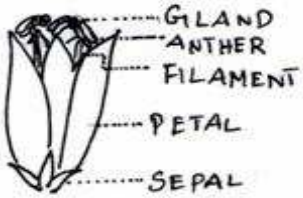
FAMILY: LEGUMINOSAE
SUB- FAMILY: MIMOSEAE
PROSOPIS SPICIGERA, LINN.

CHARACTERISTICS

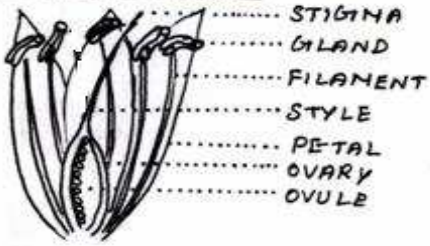
- Bipinnately compound, stipulate leaves with small oblong leaf lets.
- Pendulous spike.
- Bisexual, sessile, actinomorphic, pentamerous, cyclic, hypogynous flowers.
- 5-sepals, united, valvate.
- 5-petals, free, valvate.
- 10 stamens free, dithecous, introrse, anthers with glandular connective.
- Monocarpellary, unilocular, superior ovary with hairy outgrowth, ovules on marginal placentation.
- Style-simple, stigma minute.
- Legume fruit.

FAMILY: LEGUMINOSAE
SUB-FAMILY: MIMOSEAE
PROSOPIS SPICIGERA

ENTIRE FLOWER

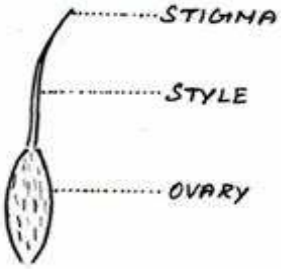


L.S OF FLOWER

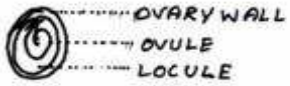


STAMENS

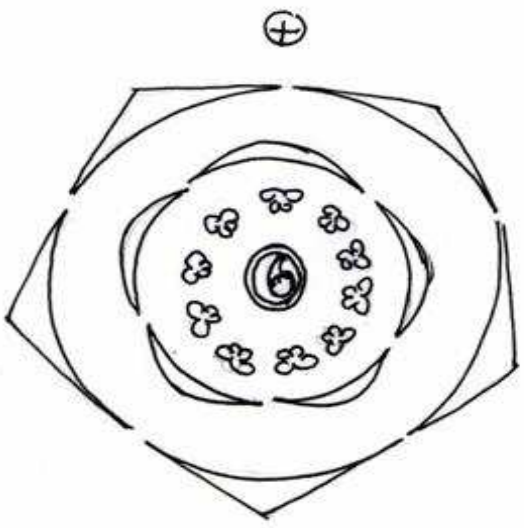
GYNOECIUM



T.S OF OVARY



FLORAL DIAGRAM



F.F : $EBr, EBrl, \varphi, \oplus, K_5, C_5, A_{10}, \underline{G}_1$

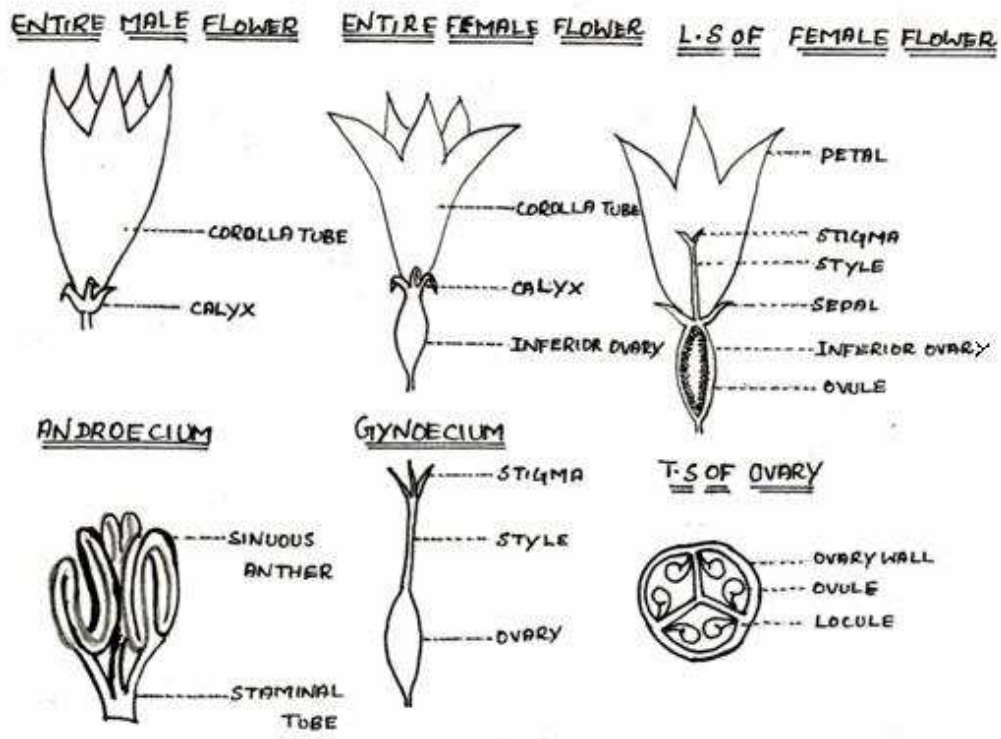
FAMILY:CUCURBITACEAE
CEPHALANDRA INDICA, NAUD.

CHARACTERISTICS

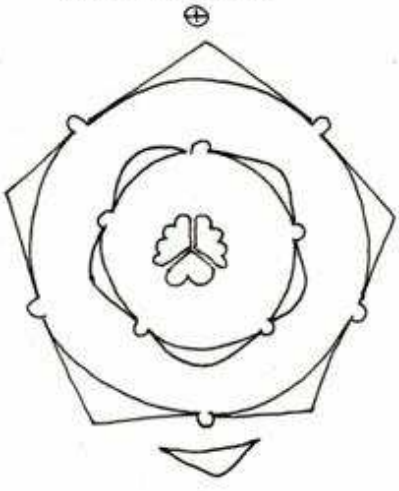
- A herbaceous, dioecious, tendril climber.
- Simple, exstipulate, alternate, palmately lobed leaves with palmately reticulate venation.
- Unisexual, regular, incomplete pentamerous, tetracyclic epigynous flower.
- 5-sepals, united, valvate.
- 5-petals, united, valvate.
- Male flowers: 5 stamens forming synandrium, filamentous column with sinuous anthers fused extrorse anthers at the top.
- Female flowers: Tricarpellary syncarpous trilocular, inferior ovary with ovules on parietal placentation.
- Style - simple, stigma trifid.
- Pepo fruit.

FAMILY : CUCURBITACEAE

CEPHALANDRA INDICA NAUD

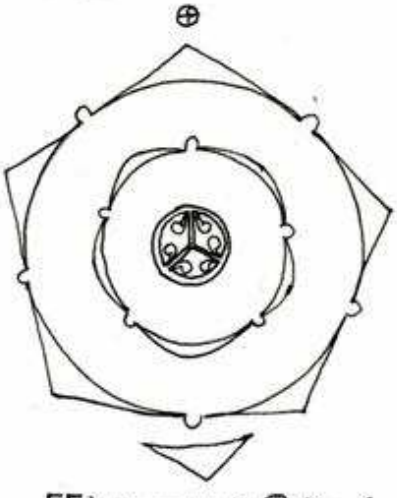


FLORAL DIAGRAM



F.F : Br, ebr, ♂, ⊕, $K_{(5)}$, $C_{(5)}$, $P_{(2+2+1)}$

FLORAL DIAGRAM



F.F : Br, ebr, ♀, ⊕, $K_{(5)}$, $C_{(5)}$, $\bar{G}_{(3)}$

FAMILY: APIACEAE (UMBELLIFERAE)
CENTELLA ASIATICA, URB

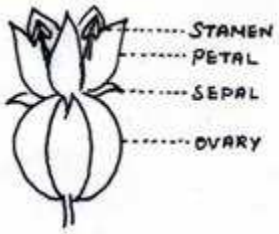
CHARACTERISTICS

- Prostrate herb, rooted at the nodes with long inter nodes.
- Long petiolate, exstipulate, simple, reniform, dentate leaves with palmately reticulate venation.
- Axillary umbellate cymes.
- Bisexual, regular, pentamerous, tetracyclic, epigynous flower.
- 5 small, sepals, valvate.
- 5 petals, free, imbricate.
- 5 stamens, petaloid, dithecous introrse anthers.
- Bicarpellary, syncarpous, bilocular, inferior ovary with pendulous ovule in each locule on axile placentation.
- 2 styles with basal stylapodium ending in capitate stigma.
- Cremocarp fruit.

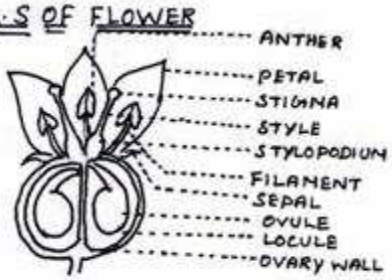
FAMILY - UMBELLIFERAE

CENTELLA ASIATICA

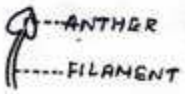
ENTIRE FLOWER



L.S OF FLOWER

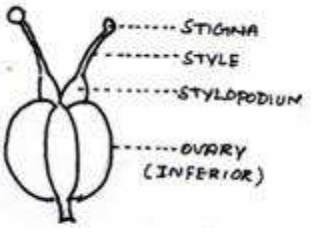


STAMEN

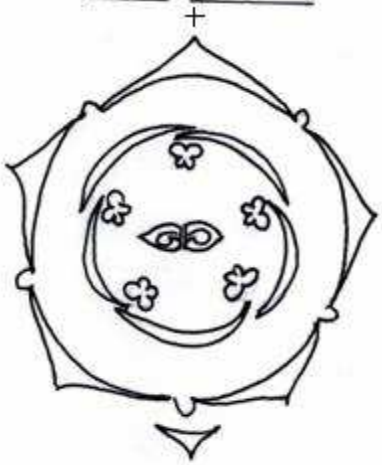


T.S OF OVARY

GYNOECIUM



FLORAL DIAGRAM



E.F - Br, EBr, ♀, ⊕, K_5, C_5, A_5, $G_{(2)}$

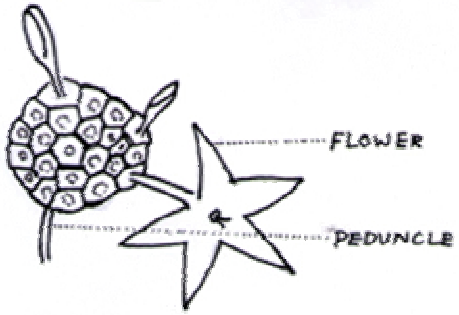
FAMILY: RUBIACEAE
MORINDA TINCTORIA, ROXB.

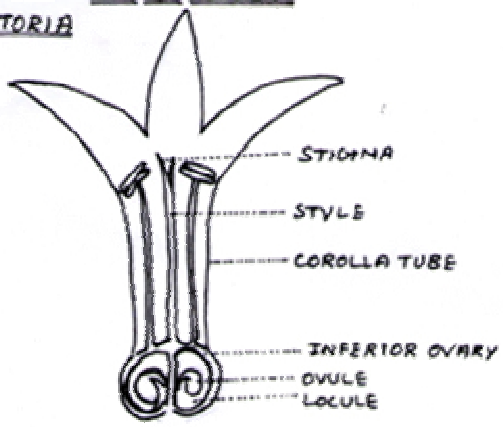
CHARACTERISTICS

- Aerial, erect, quadrangular, solid stem.
- Simple, opposite decussate, elliptic, stipulate (interpetiolar stipule) leaves.
- Leaf opposed or axillary head inflorescence.
- Sessile, bisexual, incomplete, pentamerous, epigynous flower.
- Calyx reduced into broken ring.
- 5 petals gamapetalous, twisted aestivation.
- 5 stamens, epipetalous, dithecous, introrse anthers.
- Bicarpellary, syncarpous, bilocular, inferior ovary with ovules on axile placentation, style simple, stigma bifid.
- Multiple fruit.

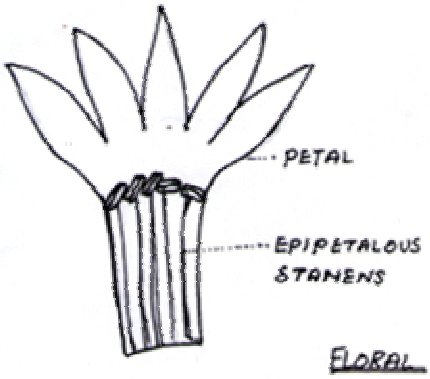
FAMILY : RUBIACEAE L.S OF FLOWER
MORINDA TINCTORIA

ENTIRE FLOWER

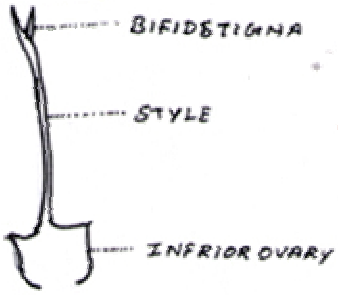




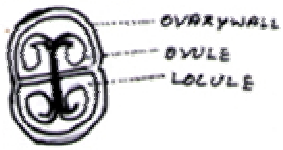
COROLLA CUT OPEN



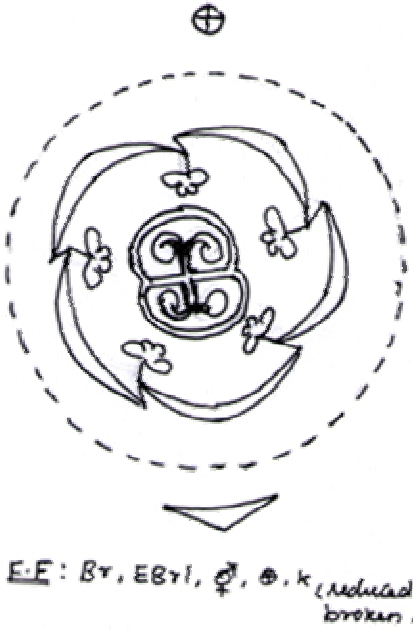
GYNOECIUM



T.S OF OVARY



FLORAL DIAGRAM



E.F : Br, EBrl, ♀, ⊕, K (reduced into broken ring), $\overline{(5)}$, A_5, $\underline{G}_{(2)}$

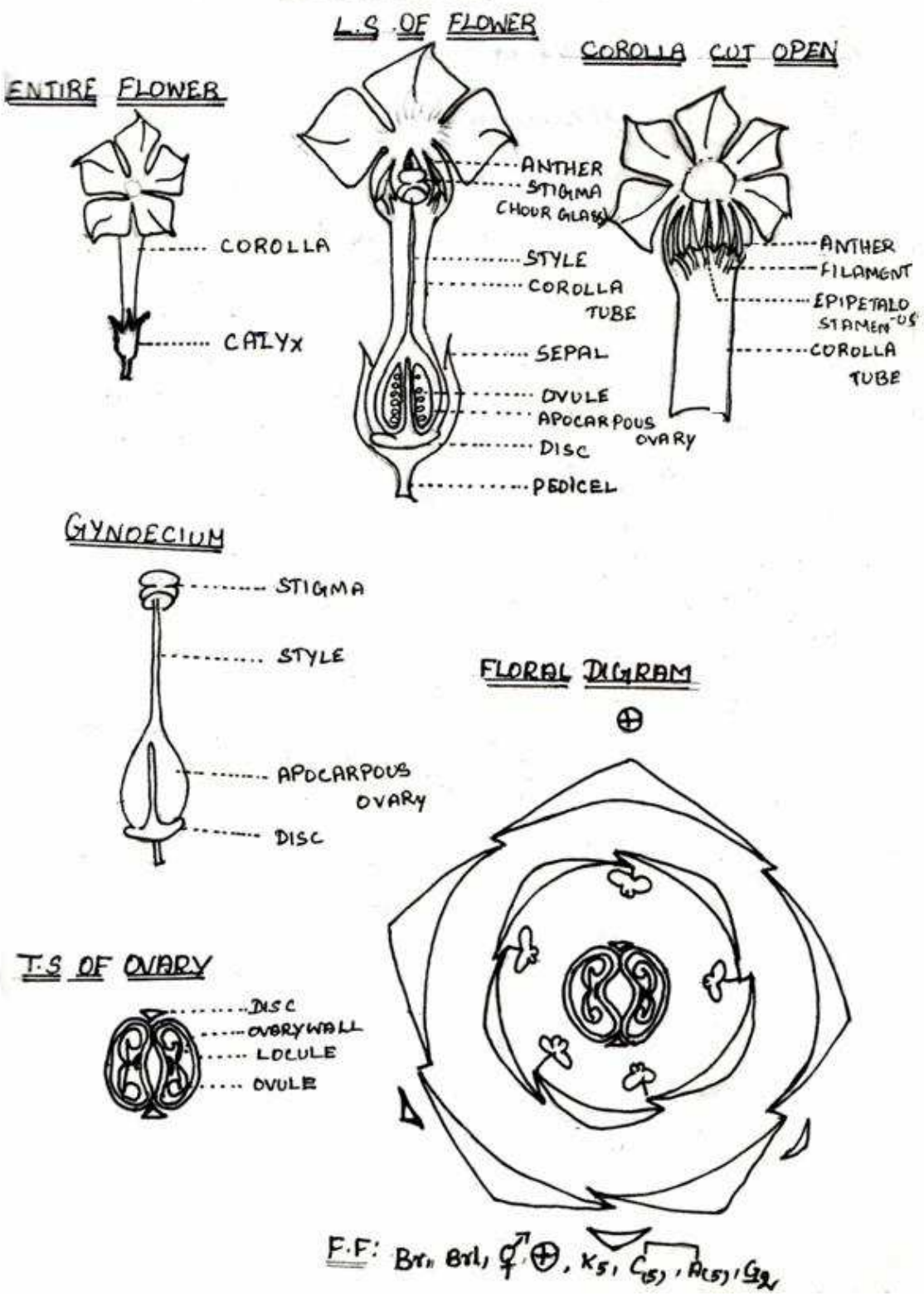
FAMILY: APOCYNACEAE
VINCA ROSEA, R.BR.

CHARACTERISTICS

- Presence of milky latex.
- Simple, opposite decussate, exstipulate leaves.
- Axillary paired cyme.
- Bisexual, actinomorphic, pentamerous, hypogynous flowers.
- 5 sepals, gamosepalous, imbricate.
- 5 petals, gamopetalous, hypocrateriform corolla, twisted. Corolline corona is present.
- 5 epipetalous stamens with dithecous, sagittate, introrse anthers
- Bicarpellary, apocarpous, superior ovary with ovules on marginal placentation.
- Style simple ending in hour glass shaped stigma.
- Fruit - Double follicle.

FAMILY : APOCYNACEAE

VINCA ROSEA



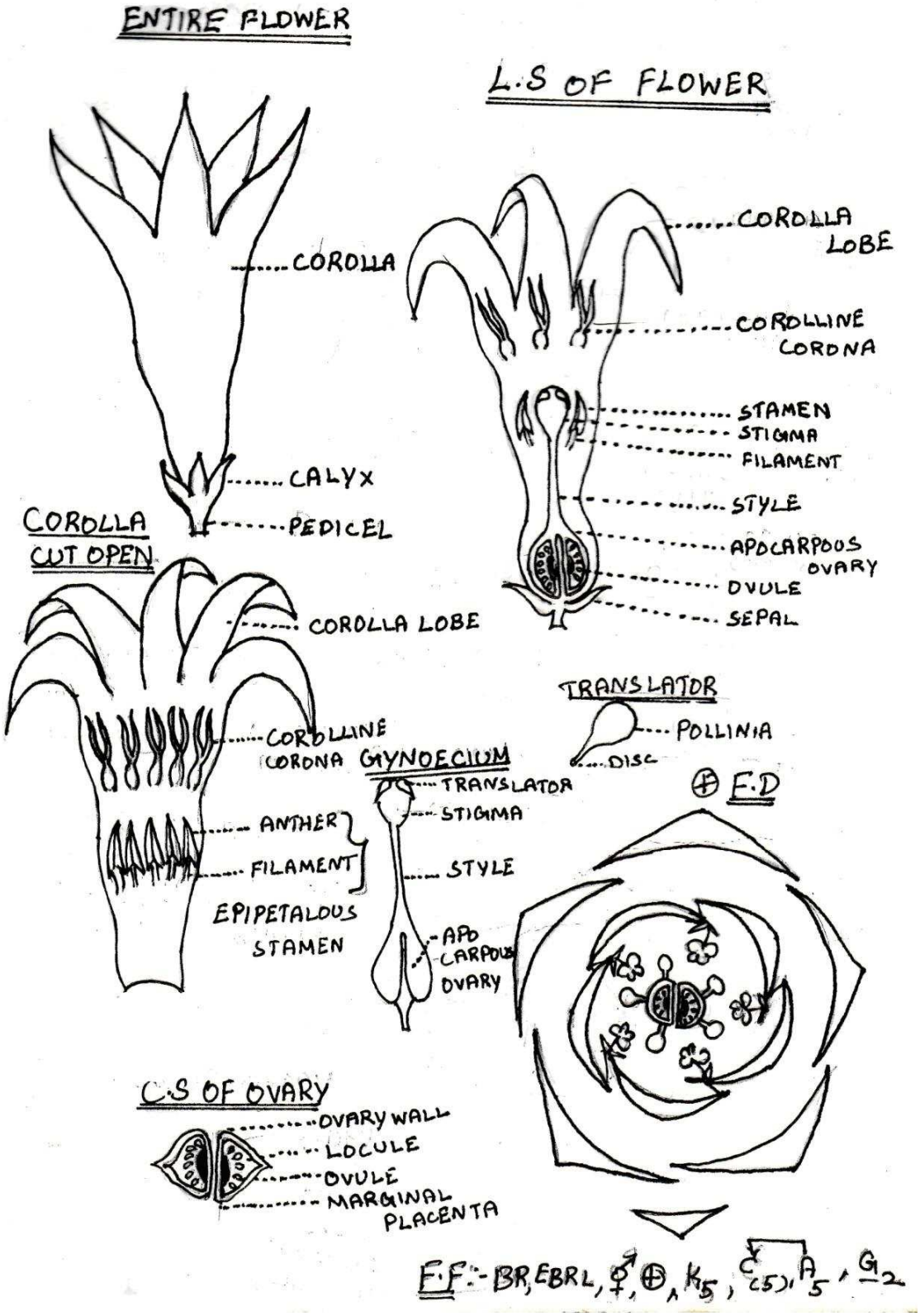
FAMILY: ASCLEPIADACEAE
CRYPTOSTEGIA GRANDIFLORA, R.Br.

CHARACTERISTICS

- Stem: Presence of milky latex.
- Leaves: Simple, opposite decussate leaves.
- Inflorescence: Flowers in cymose clusters.
- Flowers: Bisexual, actinomorphic, pentamerous, tetracyclic, hypogynous flowers.
- Calyx: 5 sepals, gamosepalous, imbricate aestivation.
- Corolla: 5 petals, gamopetalous, twisted aestivation with corolline corona.
- Androecium: 5 stamens, free, epipetalous with dithecous, basifixed, introrse anthers. The pollengrains are collected in spoon shaped translators which are adhering to the stigma.
- Gynoecium: Bicarpellary apocarpous, superior ovary, each carpel unilocular with many ovules on marginal placentation. Two styles united ending in a pentagonal stigma.
- Fruit: Double follicle.

FAMILY: ASCLEPIADACEAE

CRYPTOSTEGIA GLANDULIFERA

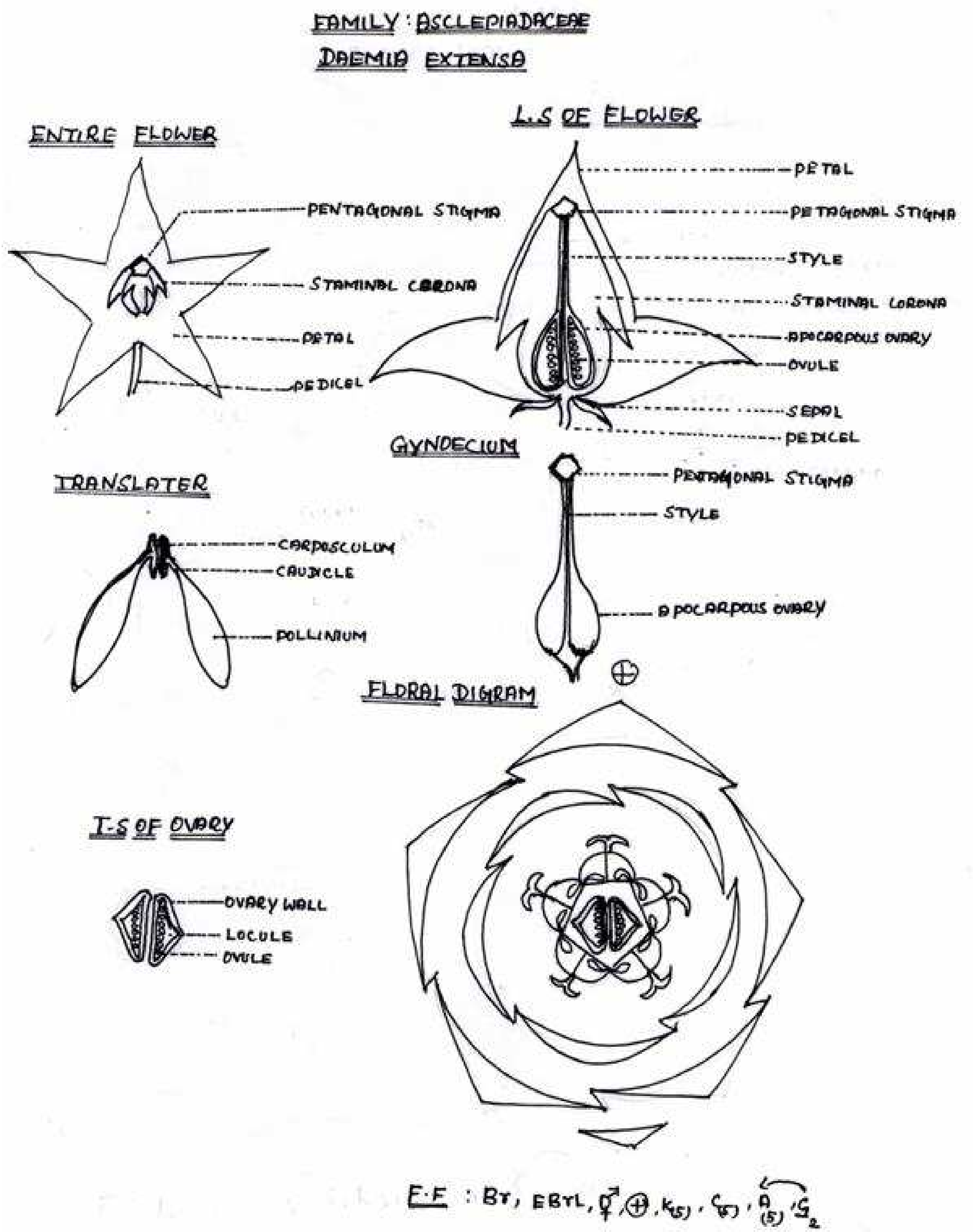


FAMILY: ASCLEPIADACEAE

DAEMIA EXTENSA, R.BR.

CHARACTERISTICS

- Presence of milky latex.
- Simple, opposite decussate exstipulate, cordate leaves.
- Axillary umbellate cymes.
- Bisexual, actinomorphic, pentamerous, hypogynous flower.
- 5 sepals, gamosepalous, valvate aestivation.
- 5 petals, gamopetalous, rotate, sepaloid, twisted aestivation.
- 5 stamens, dithecous attached to the stigma to form gynostegium, filaments form ornamental structures called staminal corona. Presence of 5 translators, each derived from two anther lobes of adjacent anthers. Each translator consists of a disc - corpusculum, 2 retinacula and 2 pollinia.
- Bicarpellary, apocarpous, superior ovary with ovules on marginal placentation.
- 2 Styles united ending in a pentagonal stigma.
- Double follicle fruit.


FAMILY : ASCLEPIADACEAE
DAEMIA EXTENSA
ENTIRE FLOWER
PENTAGONAL STIGMA
STAMINAL CORONA
PETAL
PEDICEL
L.S OF FLOWER
PETAL
PETAGONAL STIGMA
STYLE
STAMINAL CORONA
APOCARPOUS OVARY
OVULE
SEPAL
PEDICEL
GYNOECIUM
PENTAGONAL STIGMA
STYLE
APOCARPOUS OVARY
TRANSLATER
CARPOSCULUM
CAUDICLE
POLLINIUM
FLORAL DIGRAM
T.S OF OVARY
OVARY WALL
LOCULE
OVULE
F.F : Br, Ebrl,

FAMILY: SOLANACEAE
SOLANUM TORVUM, SWARTZ.

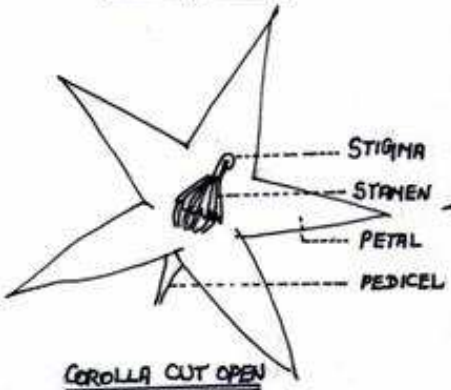
CHARACTERISTICS

- Pubescent, prickly stem.
- Simple, alternate, exstipulate, lobed leaves showing adnation.
- Extra axillary, cymose clusters.
- Bisexual, slightly zygomorphic, pentamerous, hypogynous flower.
- 5 sepals, gamosepalous valvate.
- 5 petals, gamopetalous, rotate, plicate, twisted aestivation.
- 5 epipetalous stamens with dithecous, sagittate, connate anthers with porous dehiscence
- Bicarpellary , syncarpous, bilocular ,superior ovary with many ovules in each locule on swollen, axile placentation.
- Carpels are obliquely placed.
- Style simple , stigma capitate.
- Berry fruit.

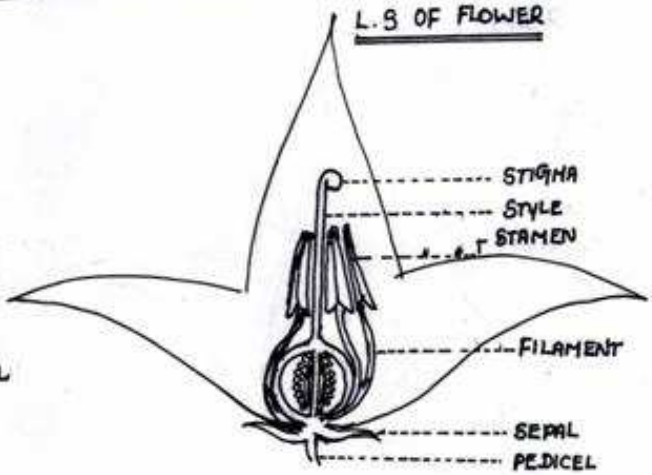
FAMILY : SOLANACEAE

SOLANUM TONVUM SWARTZ

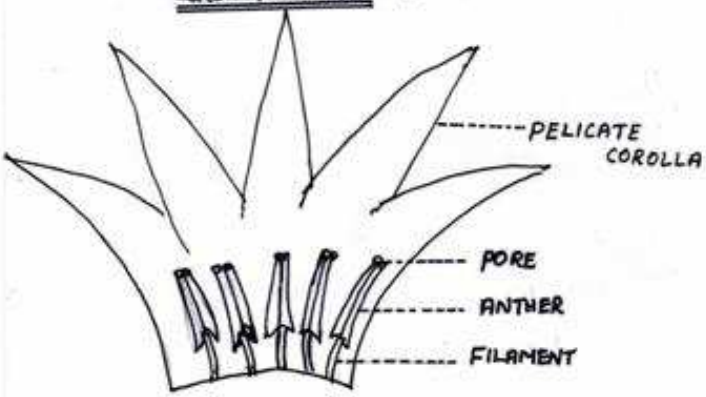
ENTIRE FLOWER



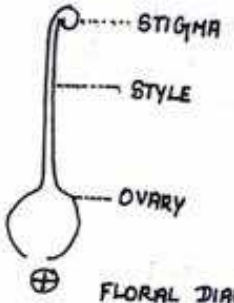
L.S OF FLOWER



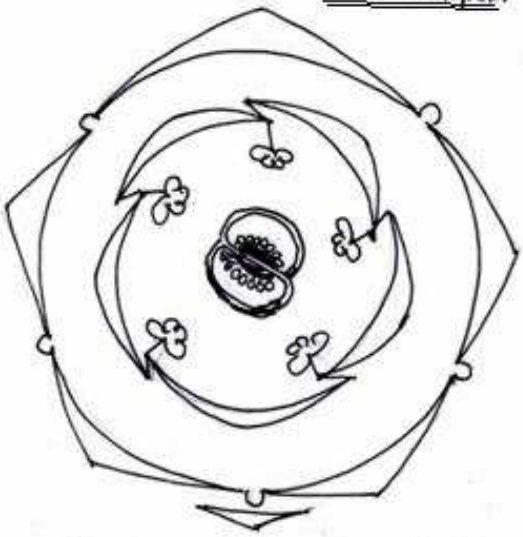
COROLLA CUT OPEN



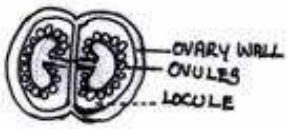
GYNOECIUM



FLORAL DIAGRAM



T.S OF OVARY



FLORAL FORMULA :

Br. Ebr. $\sigma^{\uparrow}$, $\oplus$, K_5, $\overline{C_{(5)}}$, A_5, $\underline{G_{(2)}}$

FAMILY: LABIATAE

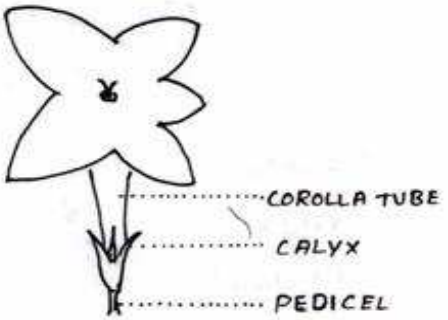
HYPTIS SUAVEOLENS, URB.

CHARACTERISTICS

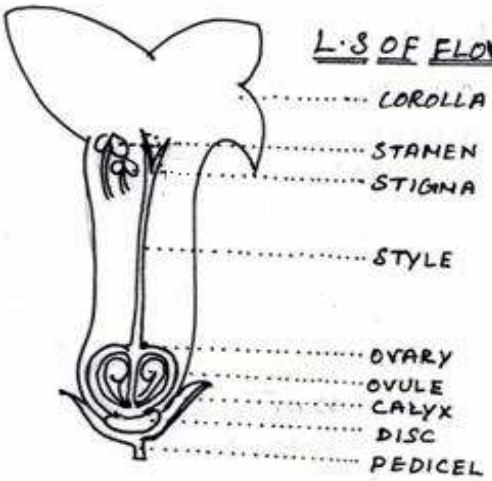
- Aromatic, quadrangular, herbaceous hairy stem.
- Simple, opposite, decussate, exstipulate leaves.
- Axillary cymose clusters.
- Bisexual, zygomorphic, pentamerous, hypogynous flower.
- 5 sepals, gamosepalous valvate, hairy with 5-15 veins
- 5 petals, gamopetalous, irregular, bilabiatae with upper limb of 2 petals and lower limb of 3 petals, imbricate aestivation.
- 4 epipetalous, didynamous stamens, dithecous, divergent, introrse with longitudinal dehiscence.
- Bicarpellary, syncarpous, bilocular, superior ovary becomes tetralocular due to the formation of false septum.
- One ovule in each locule on axile placentation.
- Gynobasic style, bifid stigma.
- A group of 4 nutlets.

FAMILY: LABIATAE
HYPTIS SUAVEOLENS

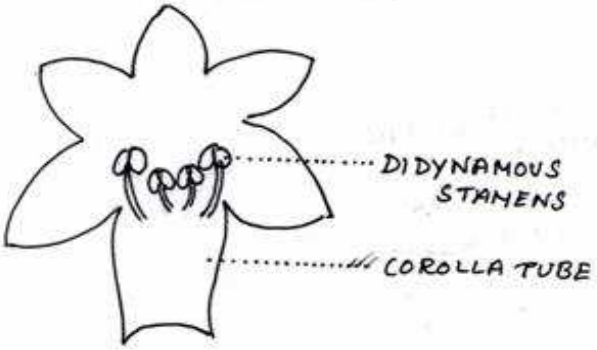
ENTIRE FLOWER



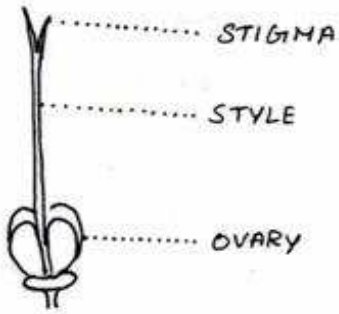
L.S OF FLOWER



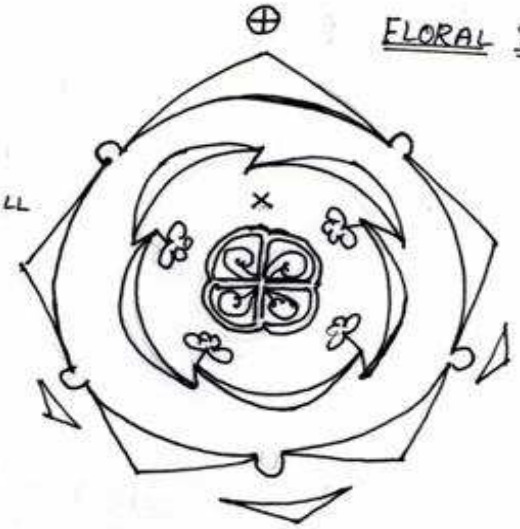
COROLLA CUT OPEN



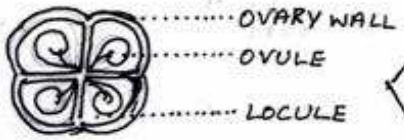
GYNOECIUM



FLORAL DIAGRAM



C.S OF OVARY



E.E : $Br,\ Bal,\ \varphi,\ K_5,\ \overline{C_{(2+3)}},\ A_{(4)},\ \underline{G}_{(2)}$

FAMILY: AMARANTHACEAE

ACHYRANTHES ASPERA, LINN.

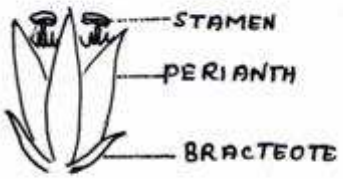
CHARACTERISTICS

- Herbaceous, hairy stem.
- Simple, opposite decussate, exstipulate leaves.
- Long terminal spike.
- Sessile, bracteate, bisexual, monochlamydeous, pentamerous, hypogynous flower.
- Perianth with 5 tepals, polyphyllous, imbricate.
- 10 monadelphous with 5 fertile antiphyllous stamens, alternate with 5 fimbricate staminodes.
- Anthers are dithecous, versatile and introrse.
- Bicarpellary, syncarpous, superior unilocular ovary, one ovule with long curved funicle on basal placentation.
- Fruit - urticle.

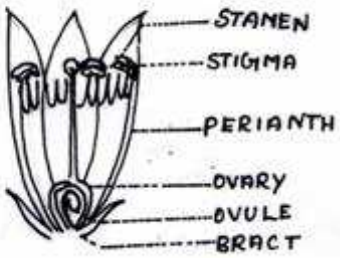
FAMILY: AMARANTHACEAE

ACHYRANTHES ASPERA

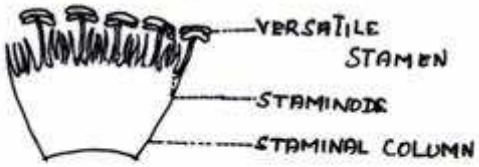
ENTIRE FLOWER



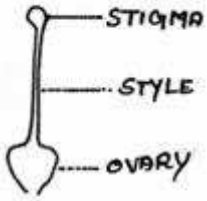
L.S OF FLOWER



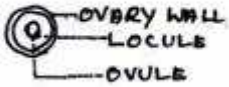
ANDROECIUM



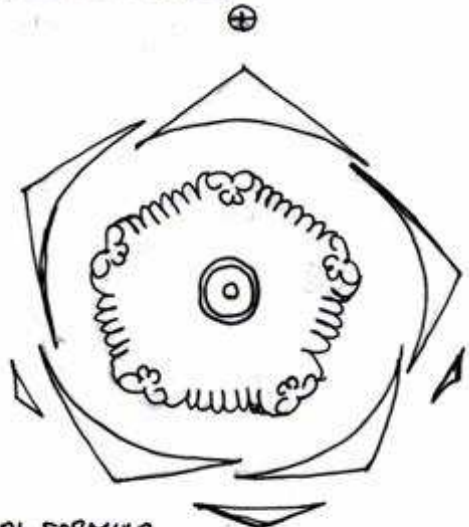
GYNOECIUM



T.S OF OVARY



FLORAL DIGRAM



FLORAL FORMULA

$Br, Br1, ♀, ⊕, P_5, A_{(5+5)}, G_{(3)}$

FAMILY: EUPHORBIACEAE
CROTON SPARCIFLOROUS, MORONG.

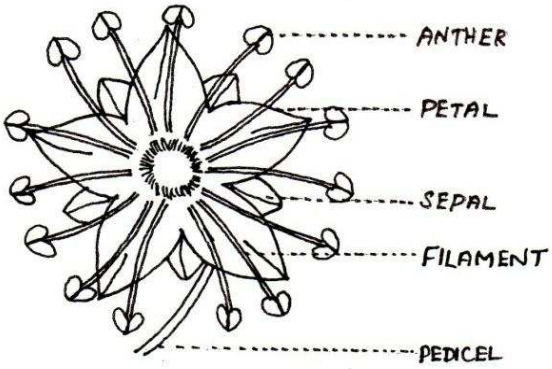
CHARACTERISTICS

- Annual herb.
- Hairy stem with milky latex.
- Simple, alternate, variegated, exstipulate leaves.
- Terminal raceme.
- Unisexual, monoecious flowers.
- Male flower: pedicellate, dichlamydeous, pentamerous calyx 5 sepals, free, valvate. Corolla 5 petals, free, valvate with orange coloured nectar gland at the base of each petal, valvate. Anderoecium: many stamens with dithecous ,extrorse anthers.
- Female flower: monochlamydeous, hypogynous. Perianth - 5 tepals, polyphyllous, valvate. tricarpellary, syncarpous, trilocular, superior ovary with one ovule in each locule on axile placentation. 3 styles, each ending with bifid stigma.

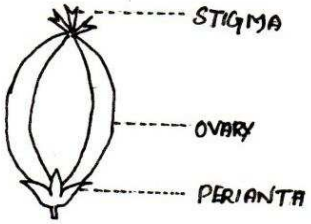
FAMILY : EUPHORBIACEAE

CROTON SPARSIFLORUS , MORONG.,

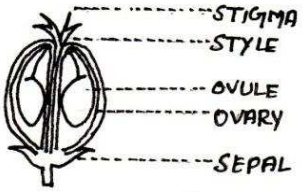
MALE FLOWER



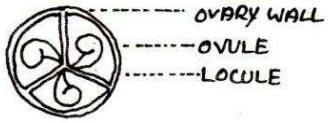
FEMALE FLOWER



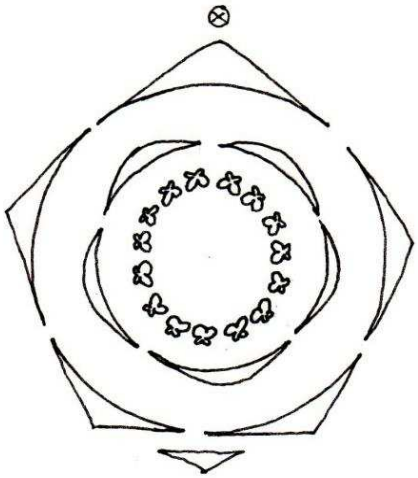
L.S OF FEMALE FLOWER



C.S OF OVARY

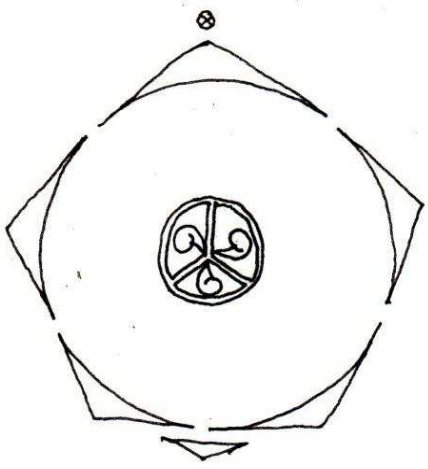


FLORAL DIAGRAM FOR ♂ FLOWER



F.F : Br, EBrl, ♂, ⊕, K_5, C_5, A_∞

FLORAL DIAGRAM FOR ♀ FLOWER



F.F : Br, EBrl, ⊕, ♀, P_5, $G_{(3)}$

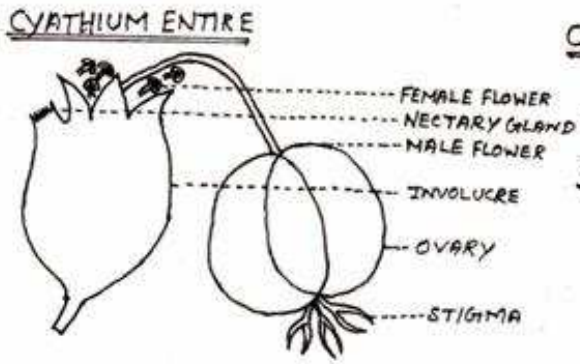
FAMILY: EUPHORBIACEAE
EUPHORBIA HETEROPHYLLA, LINN.

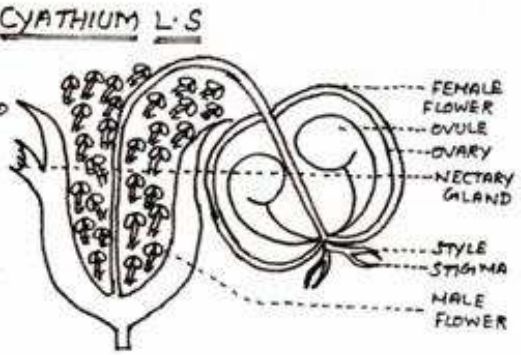
CHARACTERISTICS

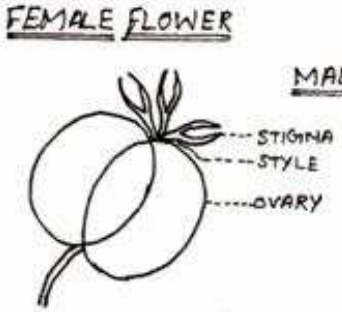
- Presence of milky latex.
- Simple alternate spiral exstipulate lobed heterophyllous leaves.
- Terminal clusters of cyathium.
- Cyathium is a special type of inflorescence consisting of 5 involucral bracts which are united to form a cup enclosing a central female flower surrounded by 5 scorpioid cymes of male flowers. An extra floral nectary gland is present at the rim of the cup.
- Pedicellate unisexual monoecious achlamydeous hypogynous flowers.
- Perianth absent.
- Male flower with single stamen-dithecous, divergent extrorse anther.
- Female flower with tricarpellary, syncarpous, trilocular superior ovary with ovule in each locule on axile placentation.
- 3 styles each ending in a bifid stigma

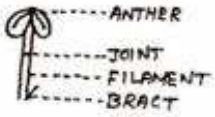
FAMILY : EUPHORBIACEAE

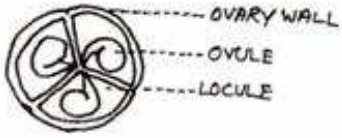
EUPHORBIA HETEROPHYLLA, LINN.



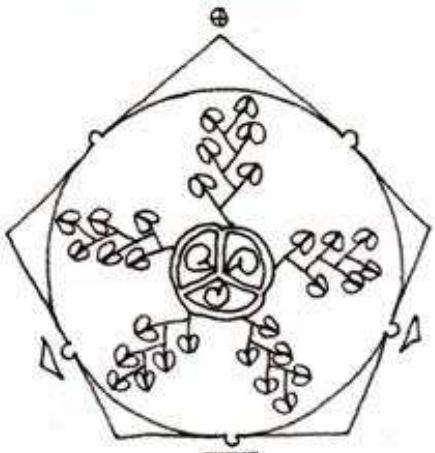








FLORAL DIAGRAM



F.F : ♂ FLOWER - Br, Brl, ♂, ⊕, P_0, A,

♀ FLOWER - Br, Brl, ♀, ⊕, P_0, $\underline{G_{(3)}}$

FAMILY: POACEAE (GRAMINEAE)

CHLORIS BARBATA, SWARTZ.

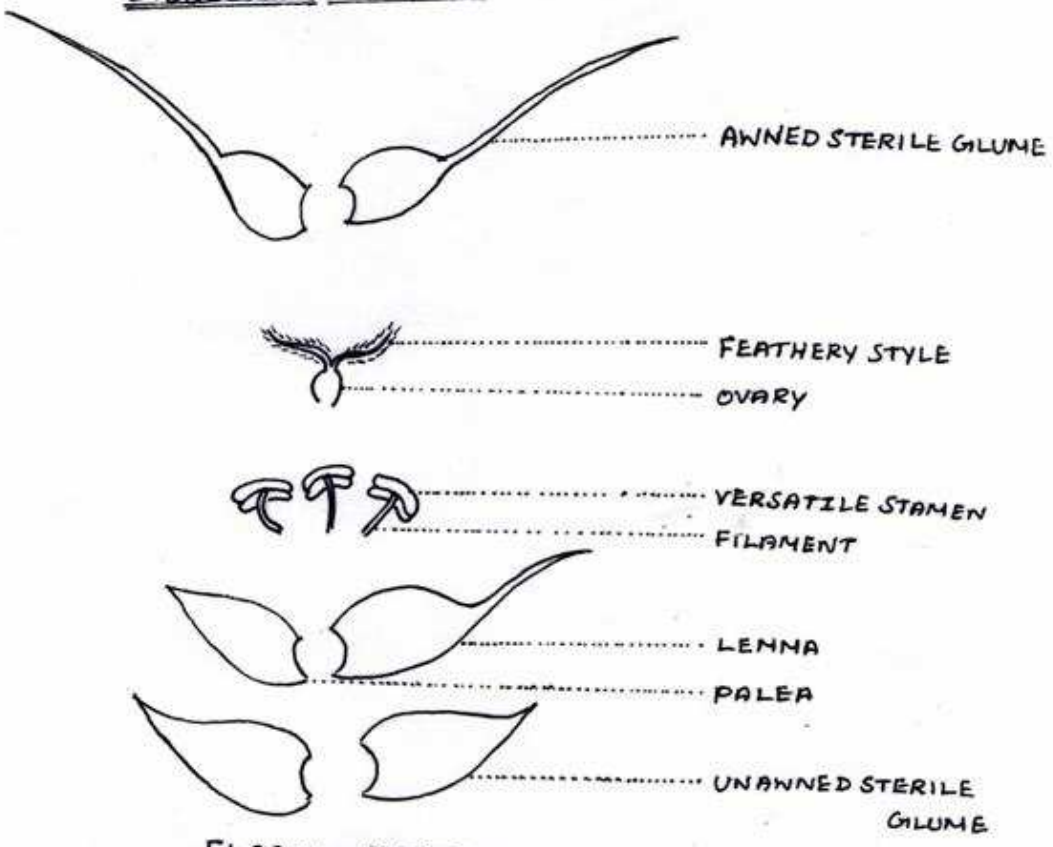
CHARACTERISTICS

- Perennial herb.
- Herbaceous, fistular, culm with long internodes.
- Simple linear leaves with parallel venation.
- Compound spike.
- Each spikelet possesses 2 sterile basal glumes, awned palea & lemma, 3stamens, gynoecium and upper a pair of sterile empty awned glumes attached to rachilla.
- Flower: Bracteate, bisexual, incomplete, trimerous, achlamydeous and hypogynous.
- Lodicules absent.
- 3 stamens with dithecous, introrse, versatile anther.
- Tricarpellary, syncarpous, unilocular superior ovary with ovule on basal placentation.
- 2 feathery styles, each ending in stigma.

FAMILY : GRAMINEAE

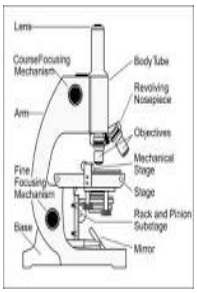
CHLORIS BARBATA SW.

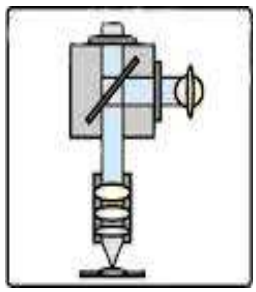
DISSECTED SPIKELET

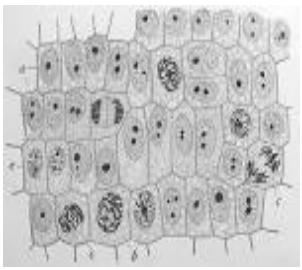


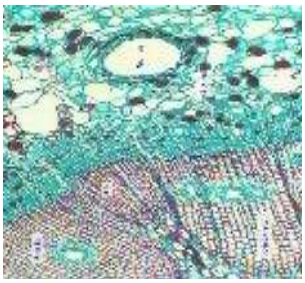
FLORAL DIAGRAM

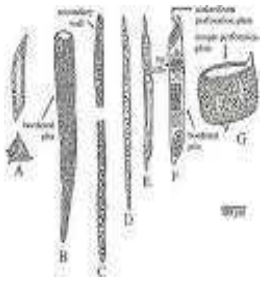
F.F: Br, Brl, ♂, ♀, P(0), A_3, $\underline{G}_{(3)}$

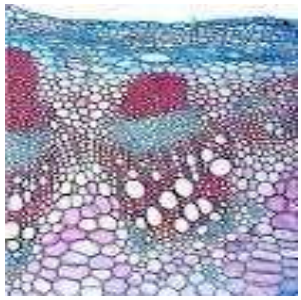




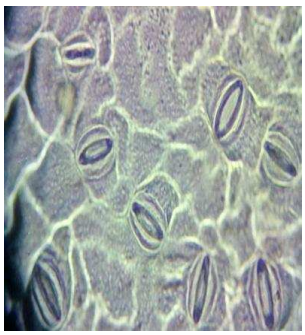


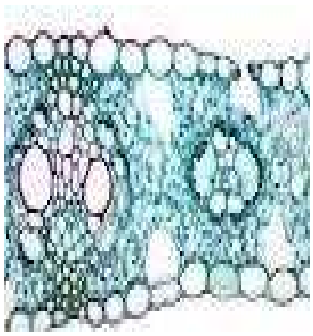


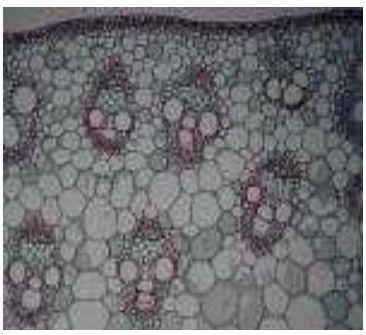


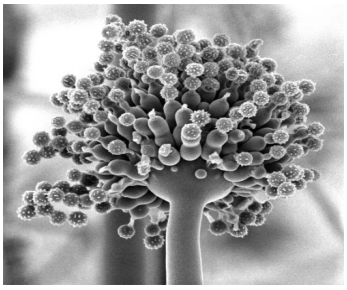


BOTANICAL TECHNIQUES









COMPOUND MICROSCOPE

The compound microscope is provided with the following parts.

STAND

There is a horse-shoe shaped foot which is mounted an inclinable body which is composed three parts.

Mirror holder,

The stage,

Tube-carrier

The stage is furnished with two clips. The tube carrier is provided with coarse and fine adjustment with the aid of which the tube can be move up and down over a distance of 2 to 3 inches respectively.

TUBE

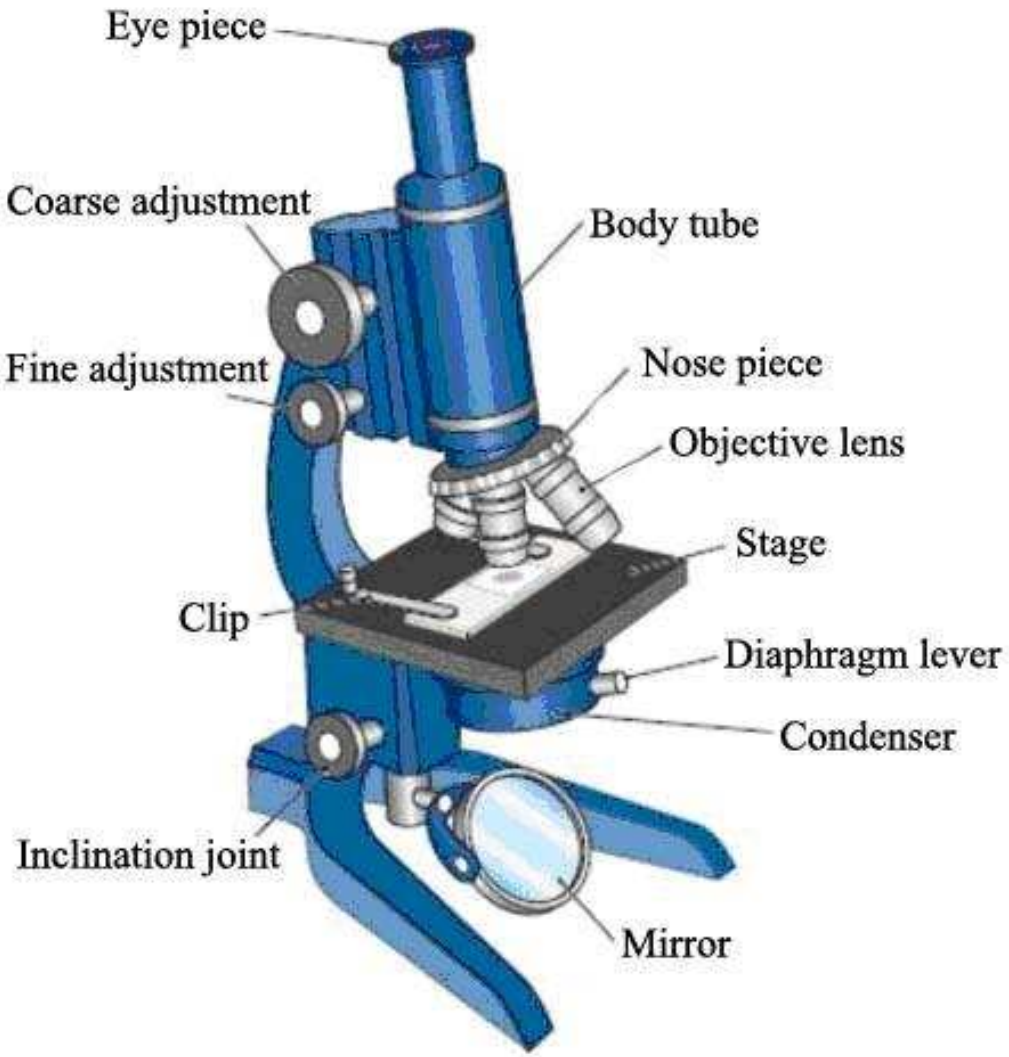
The tube is of fixed length usually 160 mm and is smaller diameter at the upper end in order to fit the ocular or eyepiece. The lower end carries the three lens turret called triple nose piece which rotates on a simple core bearing.

OBJECTIVES

It is usual to provide the microscope with achromatic objectives.

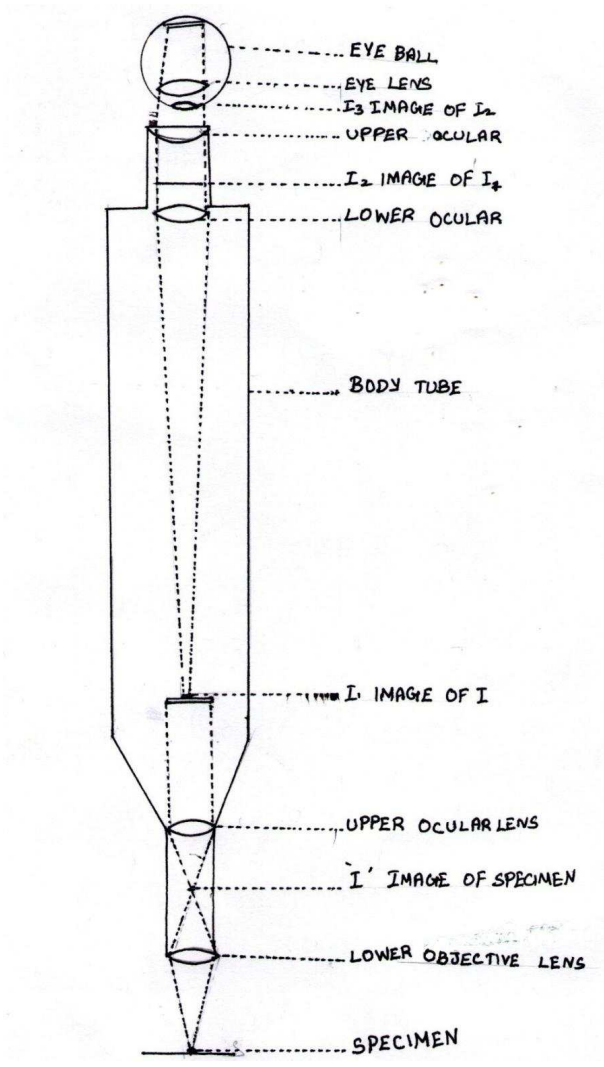
ILLIMINATING SYSTEM

No special lamp is required.


Eye piece
Coarse adjustment
Body tube
Fine adjustment
Nose piece
Objective lens
Stage
Clip
Diaphragm lever
Condenser
Inclination joint
Mirror

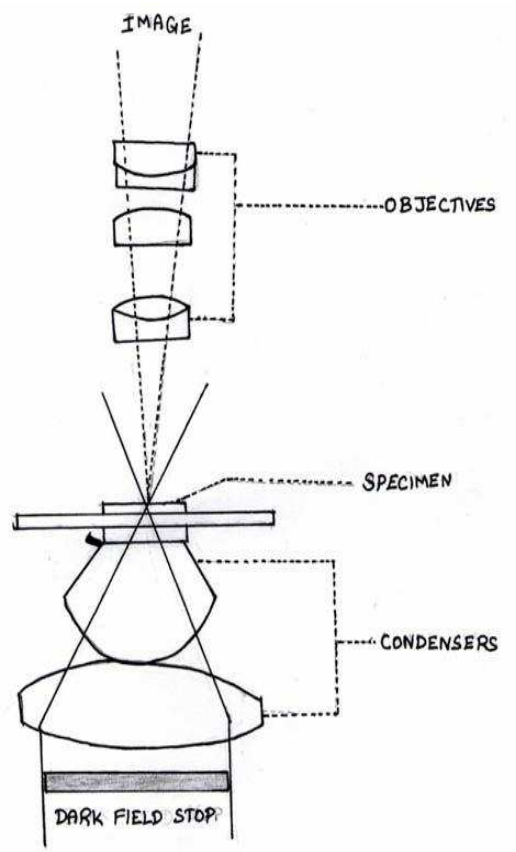
FORMATION OF IMAGE IN COMPOUND MICROSCOPE

- The objective lens of a compound microscope forms enlarged image of object ‘o’ at I. The first lens of the I_1 at I_2.
- The second lens of ocular produces a small image of diameter about 1mm of I_2 at I_3.
- This image is called ‘Ramsden’s disc’.
- The lens of the eye casts an image of Ramsden’s disc over the whole surface of retina at I_4.
- Image now fills the eye.



DARK FIELD MICROSCOPE

- In Dark field microscope, specimens are brightly illuminated under dark black back ground.
- Here, a special type of condenser lens is used.
- An opaque disc present at the under surface of the condenser which prevents the entry of light rays at the central part.
- The specimen is illuminated only with the oblique peripheral rays from the condenser.
- The refracted (scattered) light or reflected (bent) light from the specimen forms an image.
- **Uses:** Dark field microscope used to observe live and unstained organisms.
- Mobility of very small bacteria can be studied.



PHASE CONTRAST MICROSCOPE

Principle

- When light rays pass through the specimen , they undergo different phase changes
- due to different refractive indices and thickness of the cell contents.
- If the direct and diffracted rays are of the same frequency combined together in a same phase
- (the crest or trough of both light waves coincide), the amplitude or brightness will increased doubly. This is called **coincidence** and the subject looks very bright.
- When the rays are in out of phase, the amplitude or brightness decreased. This is called as **interference**. Both these phenomena (**interference and coincidence**) are used in phase contrast microscope.

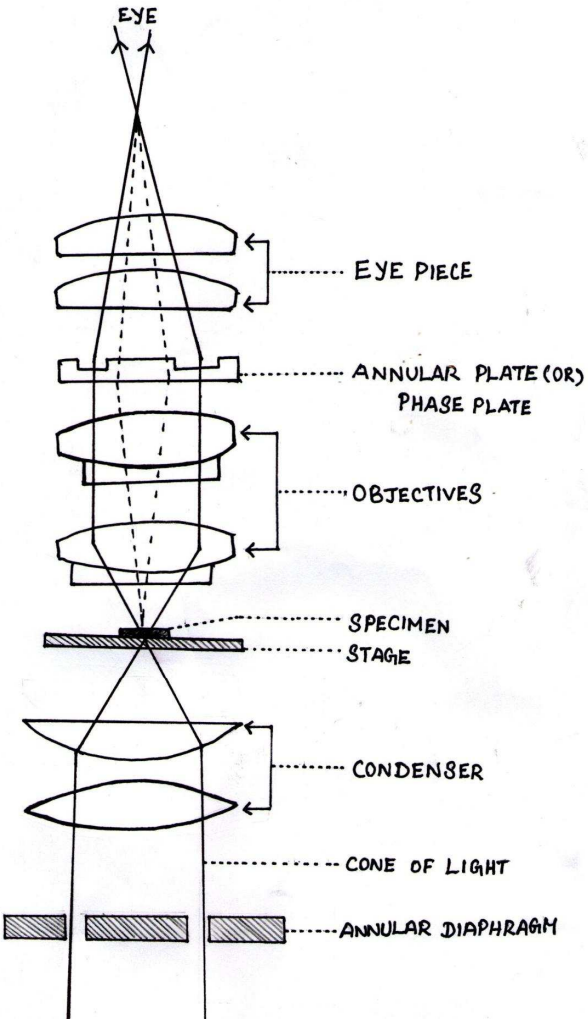
Working procedure

- A hollow cone of light travels through the condenser and enter in to the specimen. Some rays are diffracted (reduced ¼ wavelength) and some are direct rays.
- Direct rays pass through grooves of annular plate and the wavelength is enhanced ¼ **(1 ¼ wavelength).** It forms background of the image.
- The diffracted ray travel through the thicker part of annular plate and the wavelength is **¾.**
- These two rays are joined together with high amplitude and then pass through ocular lens which forms image.

- Based on the image formation, configuration and properties of the phase ring, there are two types.
- **Dark phase or positive contrast microscope**- image is dark and back ground is bright.
- **Bright phase or negative phase contrast microscope**- image is bright than the surroundings.

Applications

- It is used for studying live unstained specimens.
- Microbial mobility.
- Cell division.
- Shape, bands and contents of chromosomes.



FLUORESCENT MICROSCOPE

Principle

- It is based on the phenomenon of **fluorescence.**
- Certain chemicals absorb short wave length light (Ex**. UV rays 290-490 nm**), after short time(**less than 10^{-5} seconds**) visible light of long wave length is **reemitted**. It is called **fluorescence**.
- The substance which emits fluorescence is known as **fluorophore.**
- The specimens are coated with fluorescent dye (Ex. **Fluorescein isothiocyanate**, **Auramine O, Acridine orange** etc.) and illuminated by blue light or UV rays(short wave length with more energy).
- After few seconds specimens emit long visible green light.

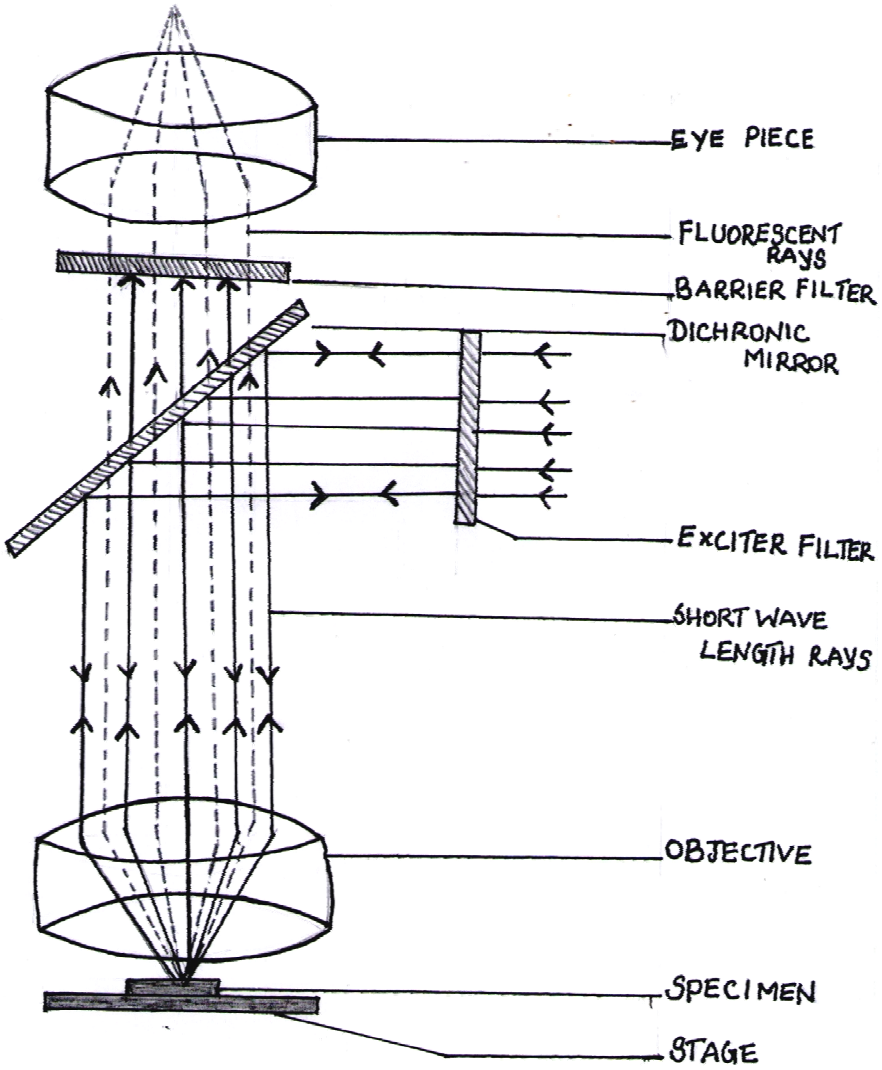
Working procedure

- Lamp source produce UV rays to **exciter filter.**
- The exciter filter passes the UV rays on the **diachronic mirror**.
- The mirror reflects shorter rays to the objective condenser which focuses into the **fluorescent coated specimen**.
- The specimen emits **longer visible rays**. This ray again reaches the mirror.
- The **diachronic mirror** absorbs shorter waves and transmits only longer ones.
- The **barrier filter** absorbs all shorter waves which is harmful to eye.

- The long fluorescent light to the ocular lens.
- Thus the final fluorescent image with black background is formed.

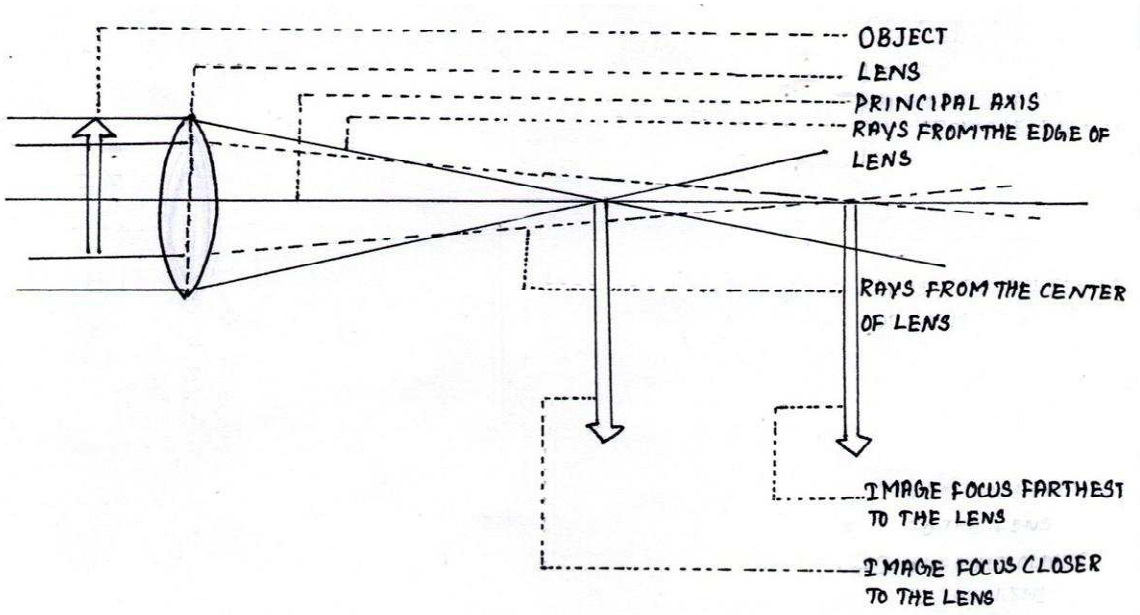
Applications

- It is used to study the biological materials like **DNA, RNA, proteins**, etc.
- It helps to identify the **pattern, structures and aberration of chromosomes**.
- **Detection of cell components** with labelled antibodies.



SPHERICAL ABERRATION

When a wide parallel beam of monochromatic light, parallel to the principal axis, is incident on the lens, the paraxial rays, after refraction, are brought to focus farthest and the marginal rays are brought to focus closer to lens.



Defects

- There is no sharp image.
- The image is blurred and distorted.

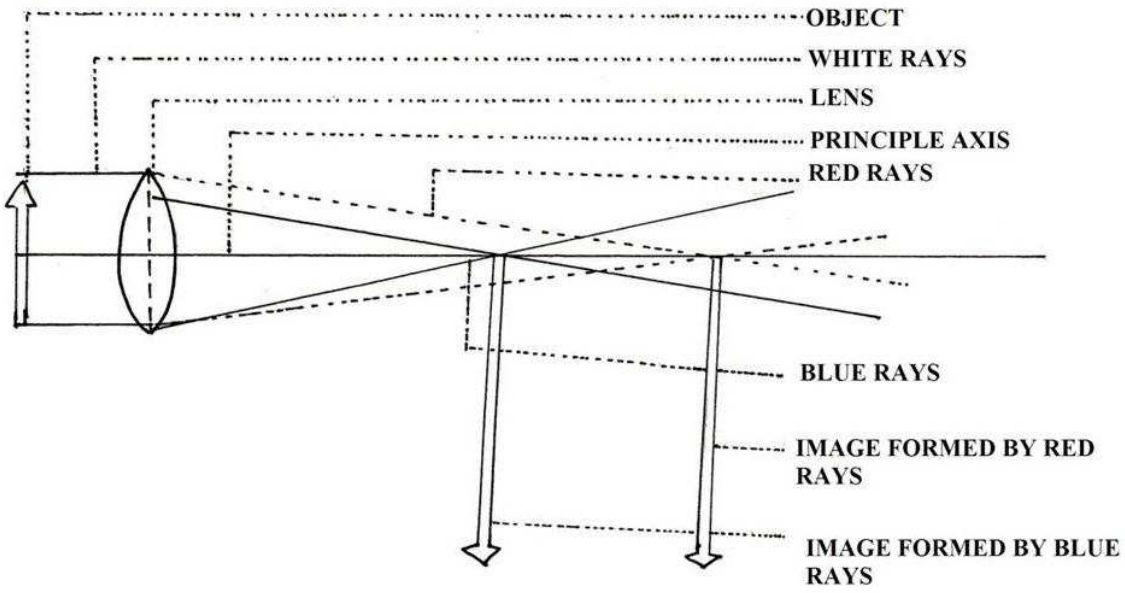
Corrections

By using

- Diaphragms or stops in front of the condenser.
- Lenses of large focal length.
- Compound lens system, combination of concave and convex lens.
- Two Plano convex lenses.

CHROMATIC ABERRATIONS

When the object is illuminated by white light, the formation of image is in different colours at different positions &blurred. This optical defect image is called chromatic aberration.



This is due to

- Variations in the wavelength of light. (For example, the focal lengths of red and violet light are different.
- The longer wavelength of red light comes to a focus farther from the lens than the violet light.

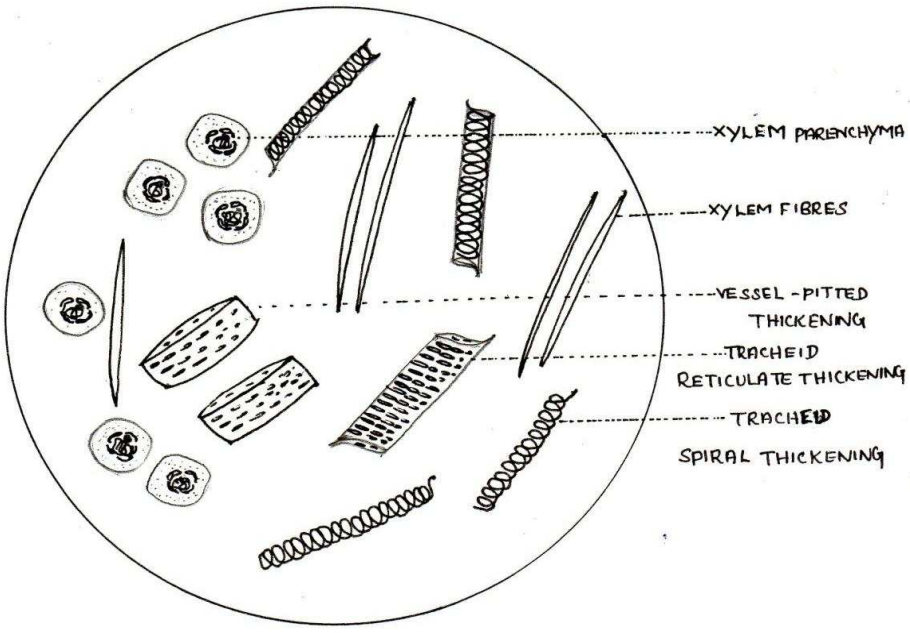
Corrections

- Using achromatic or apochromatic objectives.
- Using two convex lenses with specific distance.

MACERATION

SCHULTZ'S METHOD

- This is a technique by which stem or other organ is treated with chemicals which dissolve middle lamella and allow the cells to become separated from one another.
- Cut the longitudinal sections of cucurbita stem and put it in a test tube.
- Pour approximately 5 ml of concentrated nitric acid and add a pinch of potassium chlorate, boil it for a few minutes until the materials become soft.
- Pour the soften material into a petridish, wash it into water.
- Tease out the material with the help of a needle.
- Stain the material in safranin and mount in glycerine.
- The xylem elements are seen as separate cells.
- The tracheids and vessels show different thickenings like spiral, annular, scalariform, reticulate and pitted.
- Measure the length and breadth of various tissues by micrometer.



CALCULATION FOR USING MICROMETRY TECHNIQUE.

MEASUREMENT

One division of stage micrometer = 10µ

__ divisions of ocular coincide with 100 divisions of stage micrometer.

100 divisions of stage micrometer = 10 × 100 = 1000µ

Therefore, 1 division of ocular = 1000 ÷ --- = ---- µ.

Length of the spiral tracheid = --- divisions of the ocular

Breadth of the spiral tracheid = --- divisions of the ocular

Length of the vessel = --- divisions of the ocular

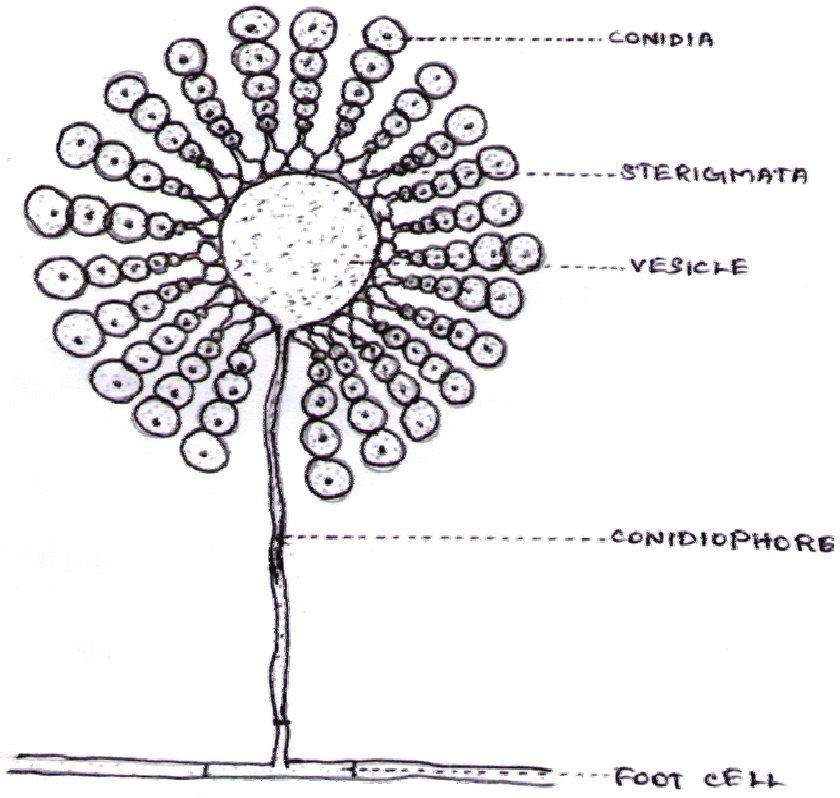
Breadth of the vessel = --- divisions of the ocular

Length of the fibre = --- divisions of the ocular

Breadth of the fibre = --- divisions of the ocular

WHOLE MOUNT OF FUNGI

- The Micro organisms from infected portions of bread are removed with the help of needle.
- They are teased out and stained with safranin.
- It is mounted in glycerine and examined under the microscope.
- The micro organism in the spoilt materials is identified as Aspergillus.



CAMERA LUCIDA

- This technique is useful in studying the epidermal tissues of leaf.
- The epidermal study will help us to gather information regarding
- The nature of epidermal cells.
- The structure of stoma.
- Frequency of stomata both in upper and lower epidermal layer.
- Structure of epidermal appendages if any replica preparation can be done by any one of the following method.

REPLICA PREPARATION

I. Cellulose acetate method

- Take fresh leaves
- Keep the surface dry
- Add a few drops of acetone on a small portion of dry surface of the leaf.
- Place a square bit of cellulose acetate paper, where acetone dissolves the acetate which settles against the surface
- After a minute peel of hardened acetate film.
- Mount the peel on a clean glass slide with the impression side facing the cover slip
- Place the cover slip over the peel
- Seal the material by using cello tape at the edges of the cover slip.

II. Nail polish method

- Fresh leaves are taken.
- The surface is made dry.
- A thin uniform coating of nail polish is applied on the surface of leaf.
- It is dried; the cast is peeled off with forceps.
- The peel is mounted on the clean glass slide with the impression side facing the cover slip.
- The cover slip is placed over the peel.
- The peel is sealed by using cello tape at the edge of cover slip.

STAINING PROCEDURE FOR HAND SECTIONS- DOUBLE STAINING

- The sections taken from the material which were already fixed in FAA are washed in water for a number of times to remove dirt particles adhering to the tissue.
- Transfer the sections to 1% safranin for 30 minutes.
- Again wash the sections in distilled water for a few minutes.
- Then transfer the sections in the following series of alcohol.

70% alcohol+ picric acid [To differentiate the tissue and acting like mordant]

↓

80% alcohol [2 minutes]

↓

90% alcohol[2 minutes]

↓

Absolute alcohol I [5 minutes]

↓

Absolute alcohol II [5 minutes]

↓

Stain in fast green for 30 sec to 2 minutes(counter stain)

↓

Absolute alcohol [5 minutes]

↓

Xylol : Alcohol 1:1 [5 minutes]

↓

Xylol I [5 minutes]

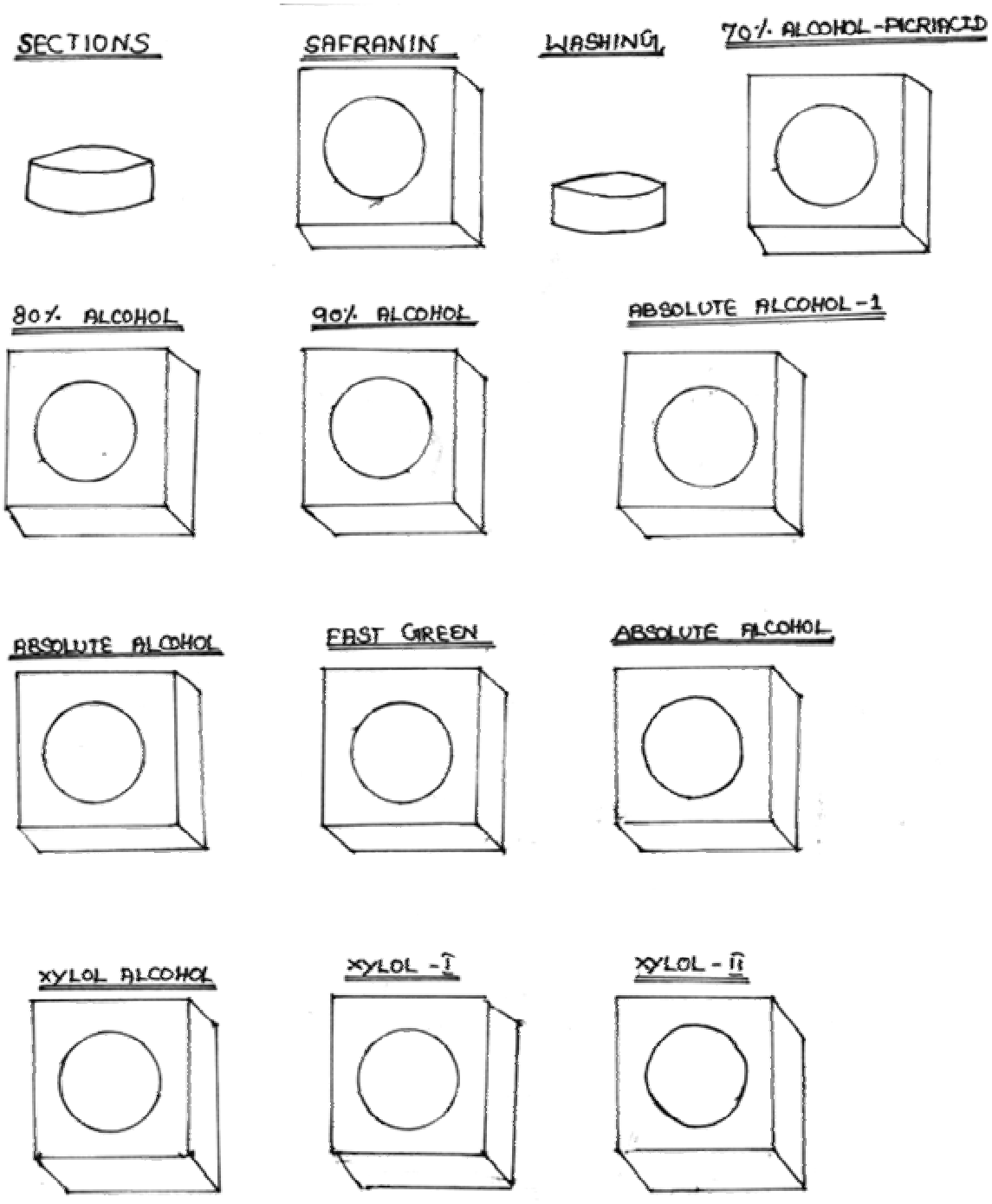
↓

Xylol II [5 minutes]

↓

Mount the section in DPX mountant.

STANING PROCEDURE FOR HAND SECCTION
DOUBLE STAINING



PAPER CHROMATOGRAPHY

AIM

To separate the leaf pigments by paper chromatographic technique.

REQUISITES

Fresh grass leaves, pestle and mortar, Whatmann filter paper no:1, glass jar, one holed rubber cork, micropipette, 10ml measuring jar, acetone and petroleum ether.

PROCEDURE

Fresh grass leaves are homogenised in the pestle and mortar with equal amount of petroleum ether and acetone and the extract is prepared. A narrow strip of Whatmann paper no.1 is taken. Near the pointed end, the extract is spotted and it is called as loading spot. The spot is loaded again and again and air dried. The pointed tip of the Whatmann paper is dipped in a glass jar containing solvent mixture of petroleum ether and acetone in the ratio of 9:1. The loading spot should not touch the solvent.

OBSERVATION

As the solvent ascends the pigments are carried with the solvent. Depending upon the adsorption capacity, the components get separated and form distinct zones. The orange colour indicates the carotene which lies near the solvent front followed by yellow colour xanthophyll, bluish green chlorophyll a and yellowish green chlorophyll b. The solvent front (S.F.) is marked and the distance travelled by the pigment is measured by using this, Rf value is calculated.

Rf value is calculated by using the formula.

$$\mathbf{Rf} = \frac{\text{Distance travelled by the pigment from the origin spot}}{\text{Distance travelled by the solvent from the origin spot}}$$

S.F. =

Rf value of carotene =

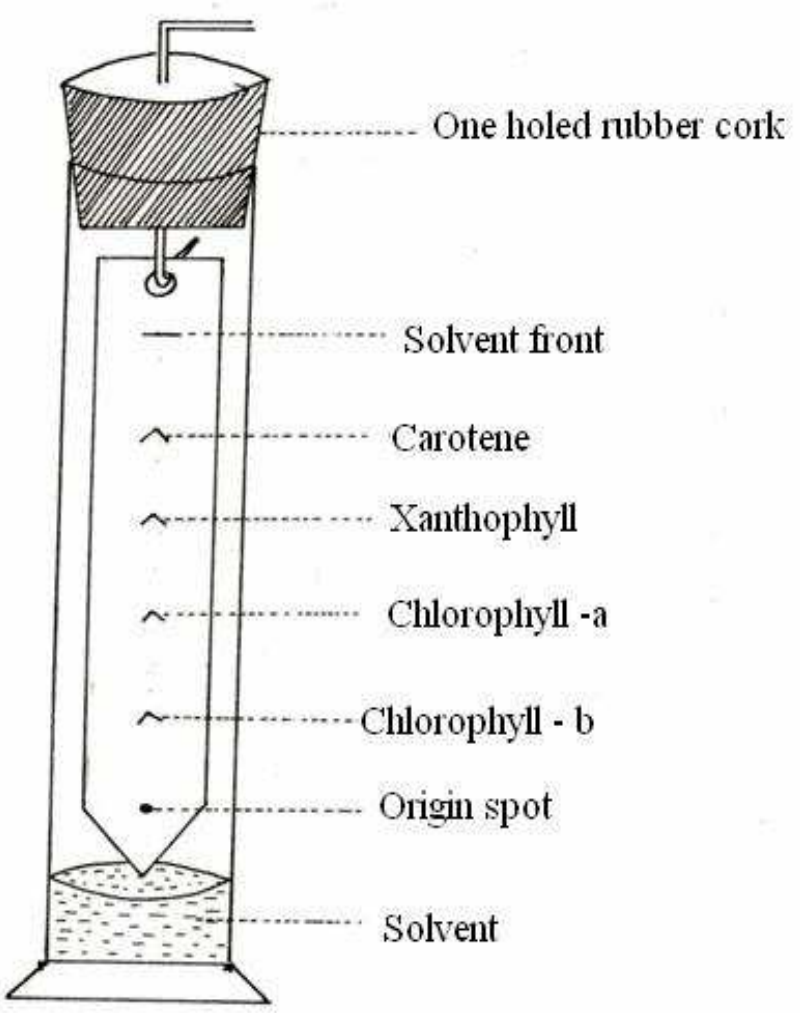
Rf value of xanthophyll =

Rf value of chlorophyll a =

Rf value of chlorophyll b =

INFERENCE

Rf value indicates the differential solubility and differential adsorption characteristic of the leaf pigments.



THIN LAYER CHROMATOGRAPHY

AIM

To separate the leaf pigments by thin layer chromatographic technique.

REQUISITES

Fresh grass leaves, pestle and mortar, chloroform, methanol, micropipette, 10ml measuring jar, acetone and petroleum ether, slides and coupling jar.

PROCEDURE

Fresh grass leaves are homogenised in the pestle and mortar with equal amount of petroleum ether and acetone and the extract is prepared. A uniform suspension of silica gel is prepared by dissolving it in a mixture of chloroform and methanol in the ratio of 2:1. The suspension is stirred well with glass rod. Two slides are dipped into the suspension and slowly drawn out and drained to the excess. The slides are completely dried. The extract is spotted towards the corner of the slide leaving space from the edge. Spot is loaded again and again and dried. The slides are kept immersed in a coupling jar containing a solvent mixture of petroleum ether and acetone in the ratio of 4:1. The setup is kept undisturbed.

OBSERVATION

As the solvent ascends the pigments are carried with the solvent. Depending upon the adsorption capacity, the components get separated and form distinct zones. The orange colour indicates the carotene which lies near the solvent front followed by yellow colour

xanthophyll, bluish green chlorophyll a and yellowish green chlorophyll b. The solvent front (S.F.) is marked and the distance travelled by the pigment is measured by using this, Rf value is calculated.

Rf value is calculated by using the formula:

$$\mathbf{Rf} = \frac{\text{Distance travelled by the pigment from the origin spot}}{\text{Distance travelled by the solvent from the origin spot}}$$

S.F. =

Rf value of carotene =

Rf value of xanthophyll =

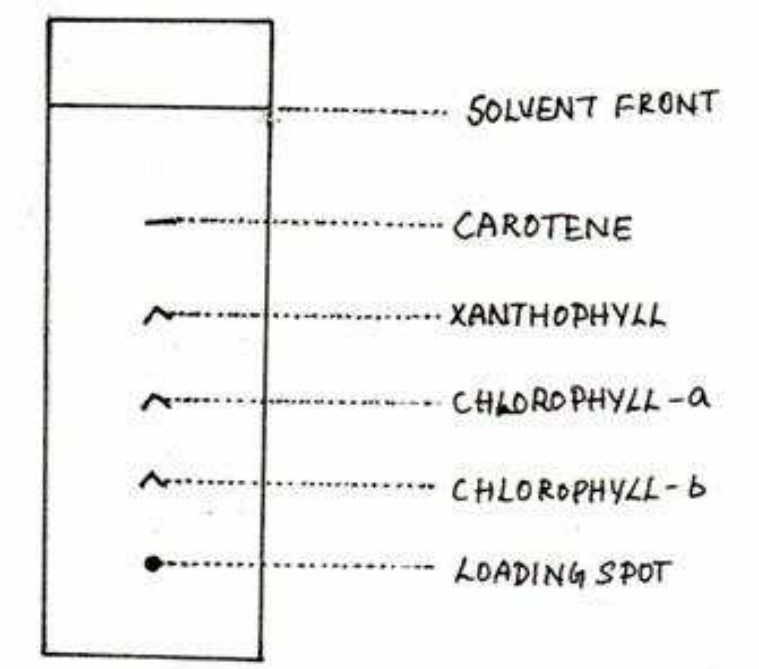
Rf value of chlorophyll a =

Rf value of chlorophyll b =

INFERENCE

Rf value indicates the differential solubility and differential adsoption characteristic of the leaf pigments.

.



COLORIMETER

AIM

To find out the absorption of different coloured solutions.

REQUIREMENTS

Colorimeter, distilled water, different coloured solutions - red (safranin), green (leaf extract) and blue (ink).

PROCEDURE

The colorimeter is set to zero with water (for safranin and ink) and acetone (for leaf extract) as blank.

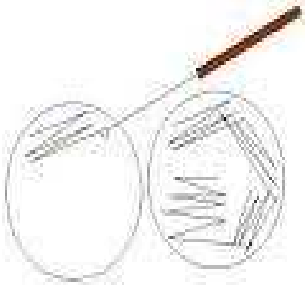
The wave length is adjusted 670 nm in that wave length the colorimeter is adjusted to zero with the blank.

Cuvette with different coloured solutions is inserted in the light path of colorimeter.

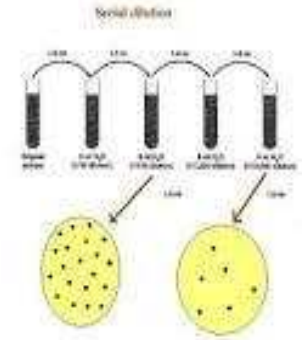
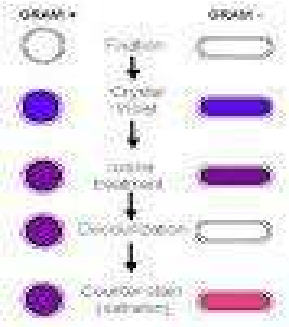
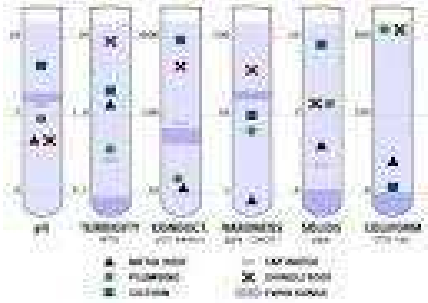
The optical density is noted down. Care is taken to see that the cuvettes are cleaned before inserting into the colorimeter.

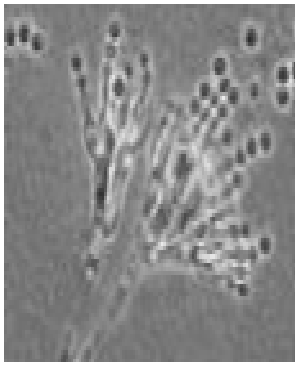
The observed OD is tabulated.



GENERAL MICROBIOLOGY




BASIC RULES OF A MICROBIOLOGY LABORATORY

- Always wear an apron (a white coat or gown) before entering the microbiology laboratory to protect from microbial contamination and laboratory hazards.
- Cut nails regularly.
- Tie long hairs back to avoid contamination and fire hazards.
- Keep your working laboratory bench clean.
- Never keep books, purses, bags etc on the working bench.
- Always wash your hands with soap in running tap water before and after the work.
- Clean your working bench with ethanol.
- Don’t put your fingers in your eyes, ears and mouth. It may facilitate the chance of infection by pathogenic microorganisms.
- Don’t eat, drink or talk while working with microorganisms.
- Always maintain aseptic condition while working with microorganisms.
- Always use flame-sterilized inoculation loop/needle.
- Don’t open the culture tubes/plates directly and never inhale them nor observe with naked eyes.
- While working with broth culture, don’t suck the suspension with mouth. Always use pipette sucker.
- After completion of work always label the cultures with names, code and date of work. It will help recording the data. Record your results at time.
- Clean the working table/ bench when the work is completed.
- Clean the lenses of objective with tissue paper.
- For any difficulty, ask you laboratory assistant or concerned teachers.

CLEANING AND CARE OF LABORATORY GLASS WARES AND EQUIPMENTS

AIM

To clean the glass wares and equipments with chromic acid which is the cleaning solution.

PRINCIPLE

Chromic acid is used to get rid off contaminants from the inner walls of glass wares like test tubes, petriplates, conical flasks, pipettes, measuring jars.

REQUIREMENTS

Potassium dichromate crystals -75gm

Concentrated Sulphuric Acid -400ml

Distilled water -600ml

Plastic tray with cold water

Beaker/conical flask

Bunsen burner/spirit lamp

Measuring jar

Glass wares

Blotting paper etc.

PROCEDURE

75 gm of potassium dichromate crystals are dissolved in 500 ml of warm distilled water in a beaker, stir using a glass rod. Add 400 ml of concentrated sulphuric acid slowly with continuous stirring. During this procedure the beaker is kept in a basin of water to absorb a large amount of fumes evolved. After sufficient cooling the chromic acid

thus formed is kept in acid bottle for cleaning the glass wares. Pour small amount of chromic acid inside the glass wares, petri plates, conical flask etc. Rotate the glass wares in such a manner so that the chromic acid is uniformly get distributed throughout the surface of the glass wares. The excess solution is poured off from the glass wares and is kept for 24 hours, as such. After 24 hours wash glass wares with distilled water and are kept ready for sterilization by inverting them to air dry.

PRECAUTIONS

- Chromic acid being corrosive to almost every material but porcelain and plastic should be handled with care.
- The outer wall of glass wares should not be rinsed with acid solution but cleaned only with distilled water.

STERILIZATION

AIM

To sterilize all the glass wares equipments and other materials before culturing.

PRINCIPLE

Sterilization is done to get rid off all living organisms and so as not to be a contaminated while growing the culture.

REQUIREMENTS

Autoclave/pressure cooker

Dry oven

Bunsen burner/spirit lamp

Ultra violet radiation in a inoculation chamber

Glass wares like test tubes, flask, petriplates, pipettes etc.

Non absorbent cotton

Spirit, formalin, distilled water

Cover glass, slides

Needle, foreceps

Scalpel, pair of scissors

Platinum loop

PROCEDURE

BY AUTOCLAVING

Autoclave consists of a horizontal or vertical elongated cylinder with a diameter of above 11.5 to 21 inches. It has two concentric metal cylinder in which 15 lbs pressure is built up. Water is boiled in a separate cylinder below, the steam raises up in the outer jacket. This

builds up the pressure with in the inner jacket and hence the boiling point increases. This apparatus is used normally for the sterilization of glass wares containing cultural media and petridishes.

A pressure of 15 lbs is applied for every square inch for all liquid media. Hence the temperature rises about 121°C after which the apparatus is kept for sterilization for about 15 minutes. The steam is then let to cool in the autoclave before opening and taking out the sterilized wares. While sterilizing the soil media the apparatus should be kept for one hour at 121°C for 15 lbs pressure.

PRECAUTIONS

- The autoclave should be fitted air tight.
- All the air inside the autoclave should be expelled completely.
- Sterilizing materials should be wrapped before.

PRESSURE COOKER

If an autoclave is not available or only a few materials are to be sterilized, a pressure cooker of the type used at home for cooking can be used for sterilization. Add just enough water to cover the bottom sieve plate. Place the wrapped materials and the plugged flasks with the contents given. Close the lid properly and apply heat. When the steam starts coming out of the nozzle on the top, wait for two minutes to allow the steam completely replace the air inside the cooker. Then place the weight on the nozzle down to the right position. The weight will rotate freely on the nozzle. Wait for few minutes to hear the hissing noise of the steam. When the pressure reaches 15 lbs, note that time. Allow for 20 minutes and then put off the flame. Allow it cool for a few minutes and then open the cooker.

HOT AIR OVEN: (DRY HEAT)

The temperature is raised to above 160°C and maintained for two hours. This method is used for sterilizing materials like pipettes, glass wares etc.

UV RADIATION

Ultra violet radiations are usually used for sterilizing the inoculation chamber where in media etc are kept. Before inoculation, the UV light is switched on for about 45 minutes, before using the chamber for inoculation and other purposes.

PRECAUTION

- The chamber should not be used while changing radiation.
- Avoid seeing the UV lamp on radiation.

- It is dangerous to the eye sight.
- Before and after inoculation the platinum loop should be heated to red hot in the flame of Bunsen burner/spirit lamp. It is also advisable to dip the platinum loop in the spirit before heating to red hot for sterilizing the needle foreceps, scalpel, slides, cover slip etc. Pass them over the flame for few seconds.

FORM ALCOHOL

This is used to sterilize the floor and sides of the inoculation before using the chamber.

PREPARATION

Ethyl alcohol

Distilled water

Formalin

Form alcohol is used for cleaning by moistening the cotton with form alcohol and simply rubbing the surface of the portion to be cleaned.

PREPARATION OF STAINS

AQUEOUS METHYLENE BLUE

REQUIREMENTS

Methylene blue (90% dye = 0.3 gm)

Ethyl alcohol (95% (or) rectified spirit = 30 ml)

PROCEDURE

Dissolve the dye in alcohol and add the solution to 100ml of distilled water.

LACTO PHENOL COTTON BLUE

Phenol crystals - 20 gms

Lactic acid - 20 gms

Glycerol - 40 gms

Water - 20 ml

Cotton blue - 0.05 gm

PROCEDURE

Warm phenol with water until the phenol crystal gets dissolved. Then add the lactic acid and glycerol. The resulting solution is called lacto phenol. This is commonly used as mounting medium for normal mycological studies. For the preparation of cotton blue stain add 0.05 gm of cotton blue in 100 ml of the lacto phenol. This is then kept in brown bottle.

GRAM'S STAIN

Crystal Violet Solution

0.5 gms of crystal violet in 100ml of distilled water.

Gram's Iodine Solution

One gram of iodine and two grams of potassium iodide in 300ml of distilled water.

Safranin Solution

0.25 gms of safranin in 100ml of distilled water.

Ethyl Alcohol (95%)

95 ml of ethanol is mixed with 15 ml of distilled water.

PREPARATION OF MEDIA

NUTRIENT AGAR

AIM

To prepare a nutrient agar media which is a good medium for the bacterial culture.

REQUIREMENTS

Nutrient agar mixture

Bacto-beef extract -3 grams

Bacto-peptone - 5 grams

Bacto agar - 15 grams

Distilled water - 100 ml

Conical flasks, Measuring jar,

NaOH, cotton, pH paper.

PROCEDURE

Since the nutrient agar is available as readymade mixture, it is easy to prepare medium. 23 g of nutrient agar is dissolved in 100 ml of distilled water in a clean sterilized conical flask. Adjust the pH of the solution to 6.8. If it is less than 6.8 add small amount of NaOH. Pour the medium in three 500 ml conical flasks. Close the mouth of the flask with cotton plug and wrap by brown paper.

POTATO DEXTROSE AGAR (PDA)

AIM

To prepare the potato dextrose agar medium which is a simple but common medium for many microbial cultures.

PRINCIPLE

PDA medium is a semi-synthetic medium. The principle involved in this is by supplying the necessary nutrients in the medium. The micro organisms can be grown easily *invitro* condition.

REQUIREMENTS

Conical flask (100 ml)	-	2
Conical flask (500 ml)	-	3
Measuring jar (500 ml)	-	1
Peeled potatoes	-	200 gm
Agar-Agar	-	15 gms
Dextrose	-	20 gms
Distilled water	-	1000 ml
Cotton		

PROCEDURE

200 gms of peeled and sliced potatoes are steamed for half an hour in 500 ml of distilled water in a conical flask. At the same time, 15 gm of chopped agar strips are steamed in another 1000 ml flask for 30 minutes. Potato extract is obtained by filtering through a muslin cloth. Dextrose is added to this extract. This solution is then mixed with agar solution. Mix them well by thorough shaking. Then pour the medium is three 500 ml flasks in equal amount. Close the mouth of the flask with cotton plug and wrap them with thick brown paper. The flasks are kept in an autoclave (or) pressure cooker for sterilization. After sterilization the medium is kept in the refrigerator.

TECHNIQUES IN MICROBIOLOGY

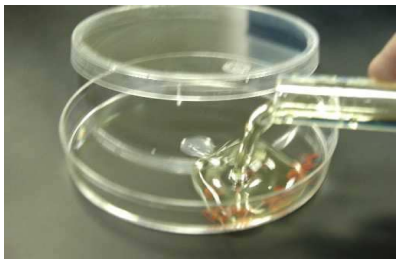
POUR PLATE TECHNIQUE

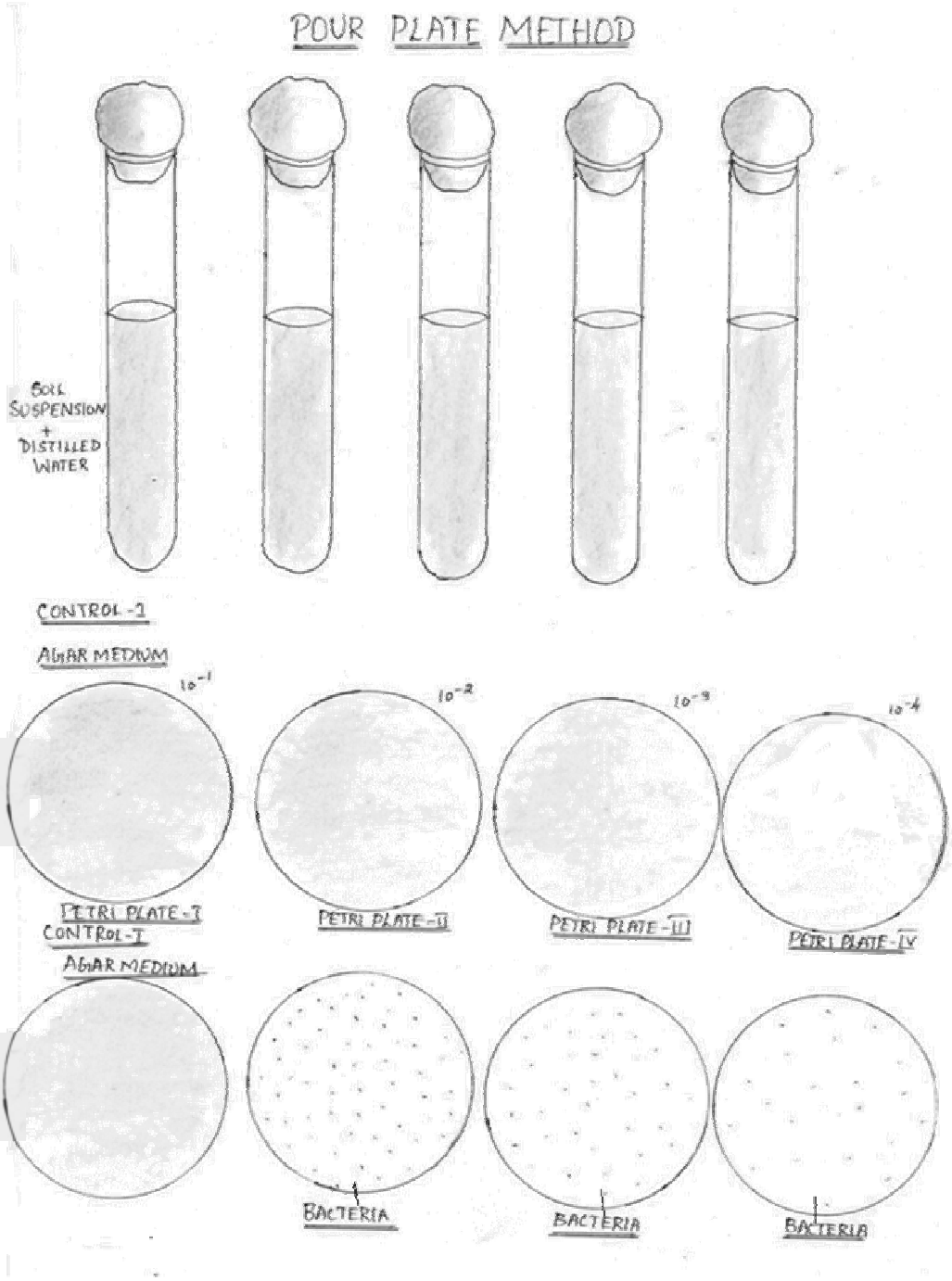
Melt the agar medium by heating it thoroughly in boiling water and cool it to 45°C to 47°C. [The cooling lessens the amount of condensation of moisture when the liquid agar is solidified in the petridishes]. Then pour 12 to 15 ml of this cooled (but still liquid) agar into a sterile covered petridish.

PRECAUTIONS

Several precautions are necessary to prevent contamination.

- When we take the melted agar from the water bath, wipe the outside of the tube with a cloth or paper towel, otherwise the water will run into the plate and introduce contaminants.
- When we remove the cotton plug or cap to pour the agar, flame the mouth of the test tube to kill the micro organisms on the outside lip.
- In pouring the agar from the tube to the plate raise the cover of the plate only one side just sufficiently to admit easily the mouth of the tube.
- After pouring the agar into the plate lower the cover and immediately, pick up the plate and gently tilt it from side to side to distribute the agar uniformly over the bottom. Then allow the agar to solidify placing it on the table undisturbed.

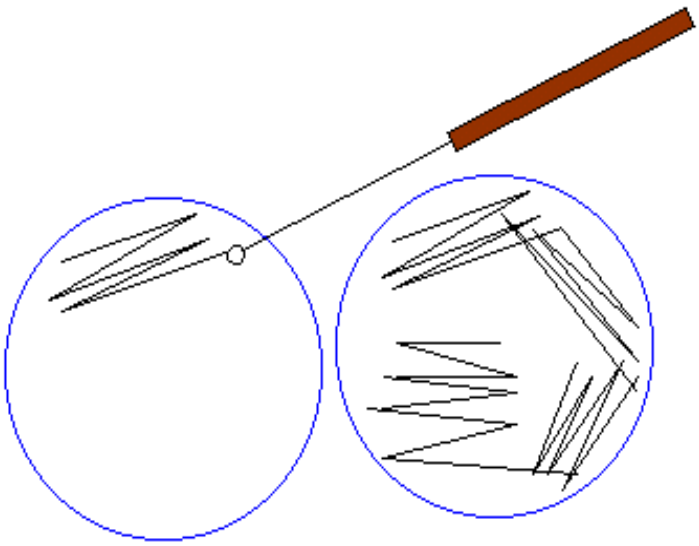



POUR PLATE METHOD
SOIL SUSPENSION + DISTILLED WATER
CONTROL-1
AGAR MEDIUM
10^{-1}
10^{-2}
10^{-3}
10^{-4}
PETRI PLATE-I
PETRI PLATE-II
PETRI PLATE-III
PETRI PLATE-IV
CONTROL-I
AGAR MEDIUM
BACTERIA
BACTERIA
BACTERIA

STREAK PLATE TECHNIQUE

The immediate objective of the streak plate is to produce well separated colonies especially of bacteria from concentrated suspension of cells. During inoculation the closely packed cells at the start of the streak continue. Fewer and fewer cells remain in the droplets being carried on the table. As this fall off and grow on the surface, well separated colonies develop. Flame a streaking needle [Bent like a hockey stick] or a loop and cool by jabbing it into the edge of the agar with a drop of culture. Streak the culture back and forth from edge to edge in parallel lines moving towards as.

When we reach the centre of the plate spin it around 180°C and continue streaking row moving away from us. [This reverse avoids interference with needle by the tip of the plate]. Hold the petriplate cover in our left hand, partially covering the agar. Streaking may also be done both vertically as well as transversely.



ISOLATION OF MICRO ORGANISMS BY THE SOIL DILUTION METHOD

PRINCIPLE

Soil contains plenty of micro organisms such as bacteria, fungi, protozoa, algae and nematodes. This technique will help us to isolate, bacteria, fungi and actinomycetes from the soil.

MATERIALS REQUIRED

Nutrient agar medium

Sterilized test tube

Sterilized petridishes

Spirit lamp

Sterilized cotton plug

Garden soil

PROCEDURE

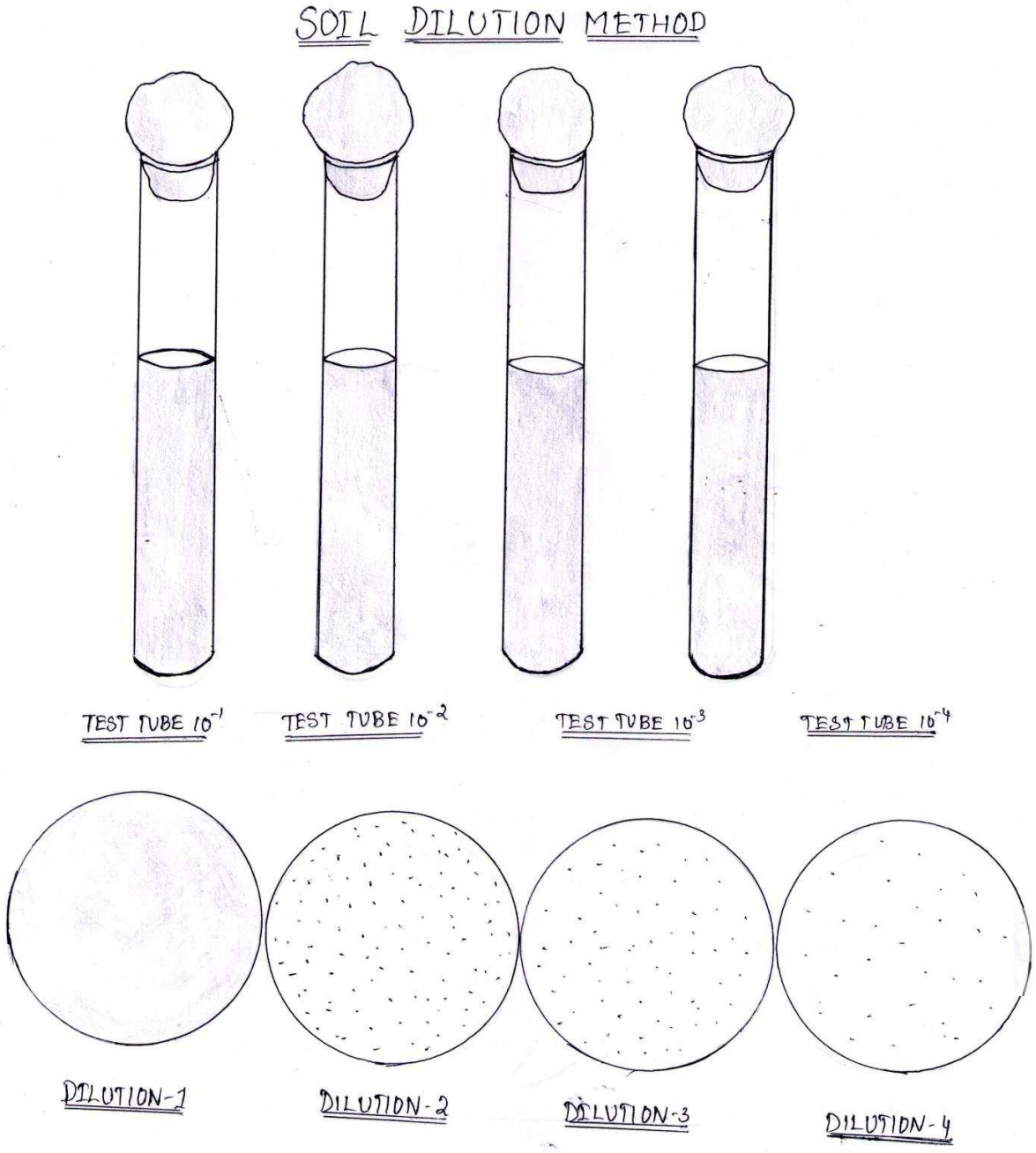
Take one gram of garden soil, 9 ml of sterilized water is added and shaken well. 3 sterilized test tubes are taken with 9 ml of distilled water. Soil solution is shaken well and 1 ml of soil solution is transferred aseptically to the first test tube. 1 ml is taken and is transferred to the second test tube aseptically and this will make 10^{-2} dilution. Now from the second test tube 1 ml of 10^{-2} dilution is taken and transferred aseptically to third test tube and its dilution is 10^{-3}. 15 ml of cooled nutrient agar medium is added to each petridish and inoculum is mixed by gentle rotation of petridish. The contents of the test tube 1, 2 and 3 are transferred aseptically to 3 sterilized petriplates. The petriplates are numbered and are allowed to remain at room temperature for 24 hours.

OBSERVATION

In the first petriplates colonies are very much crowded and small where as second and third petriplates the colonies are more and more sparsely distributed and are larger with distinct and characteristic form, shape, colour, texture. This is because of progressive dilution of original sample.

INFERENCE

Micro organisms in soil belong to various groups like bacteria and fungi. Some of them are unicellular and others are multicellular having mycelial growth.


SOIL DILUTION METHOD
TEST TUBE 10^{-1}
TEST TUBE 10^{-2}
TEST TUBE 10^{-3}
TEST TUBE 10^{-4}
DILUTION-1
DILUTION-2
DILUTION-3
DILUTION-4

MICROBIOLOGICAL EXAMINATION OF DRINKING WATER

PRINCIPLE

The drinking water must be free from faecal contamination, because the pathogens responsible for various diseases are present in contaminated water. So the aim of water analysis [presumptive test] is to find out the presence of coliform bacteria in water.

MATERIALS REQUIRED

Water samples

Test tube

Cotton plug

Lactose broth

Pipette

Spirit lamp

PROCEDURE

- Water sample is taken into sterile screw cap tubes.
- Lactose broth is prepared and it is taken in a test tube. 10 ml of water sample taken in a test tube. It is added to lactose. The test tube is incubated at 37^0C for two days.

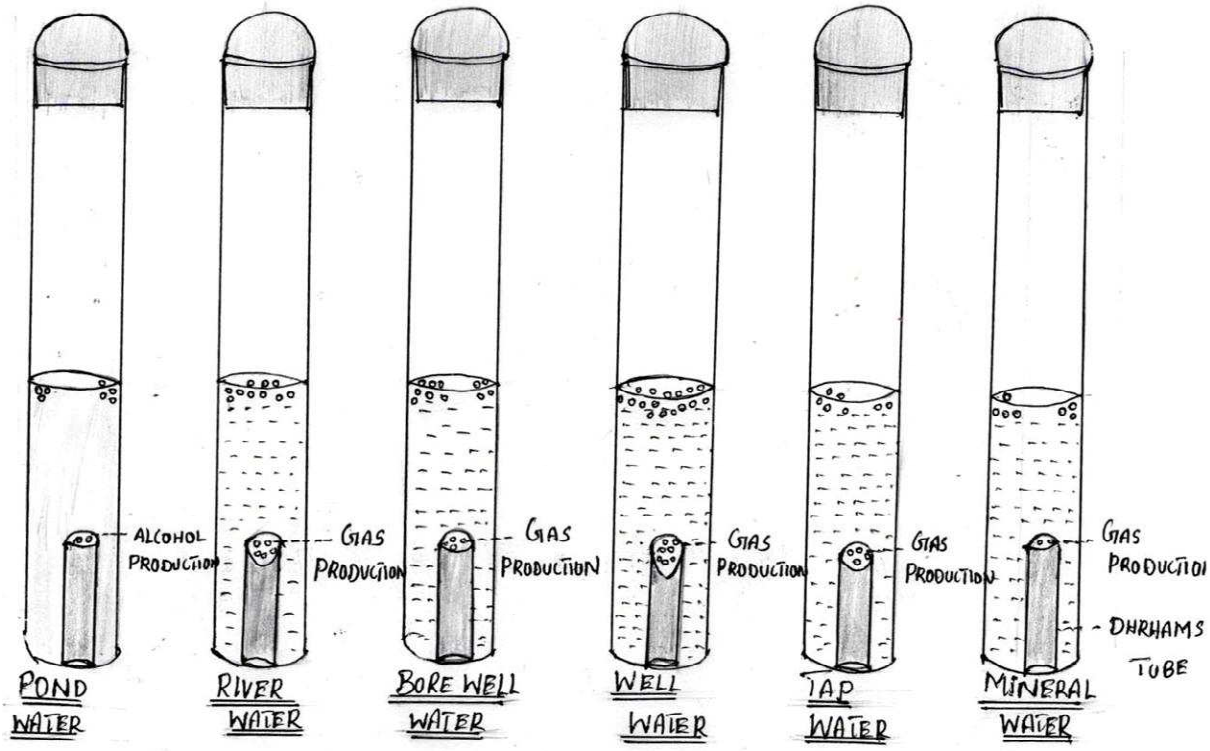
OBSERVATION

After 24 hours there is a formation of gas.

INFERENCE

The formation of gas at the end of 24 hours is a positive presumptive test for the presence of coliform bacteria.

MICROBIOLOGICAL EXAMINATION OF DRINKING WATER



GRAM'S STAINING

AIM

To study the bacteria using Gram staining a method which is a differential staining procedure.

REQUIREMENTS

Crystal violet solution

Gram's iodine solution

Safranin solution

95% alcohol

Butter milk

Glass slides

Spirit lamp

Blotting paper

Microscope

PROCEDURE

A drop of butter milk is smeared over the clean glass slides.

The smear is carefully air dried.

Fix it by passing through a spirit lamp.

Stain the fixed smear for one minute with crystal violet solution.

Drain the excess stain.

Wash gently in distilled water.

Flood with Gram's Iodine solution.

Flood with 95% ethanol. Treat repeatedly with fresh alcohol until the colour to come out.

Wash with water.

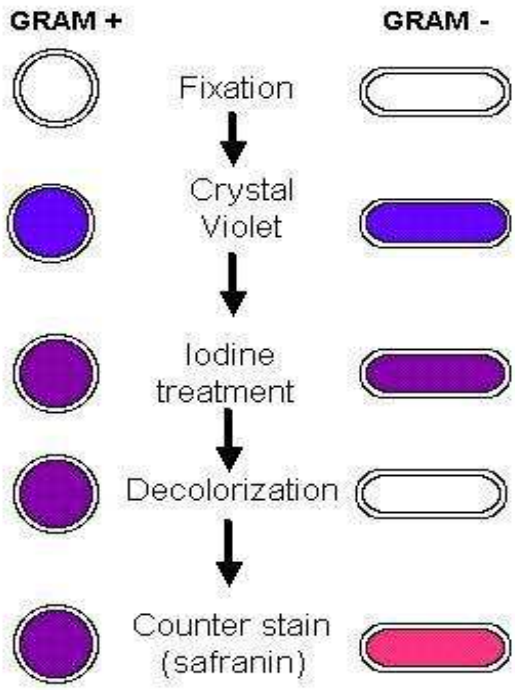
Counter stain with safranin for two minutes.

Wash with water and dry between sheets of blotting paper.

Examine under oil immersion objective.

OBSERVATION

Bacteria which retained the primary stain are Gram positive organisms and which took the counter stain are Gram negative. In the former bluish violet colour is seen while in the later reddish colour is seen.



MICROBIAL ANALYSIS OF MILK

METHYLENE BLUE REDUCTASE TEST

Milk sample contain a large population of micro organisms. This reduces the dissolved oxygen in the milk. Due to their vigorous growth the dimethylene blue loses its colour in reduced condition. The methylene blue reductase test is used to screen the quality of milk containing large population of organisms and streptococcus which reduces the dye.

REQUIREMENTS

Sample of milk from various sources.

Methylene blue solution.

Sterile screw cap test tube.

Sterile 1 ml pipette.

Water bath at 37°C.

Sterile distilled water.

Bunsen burner.

PROCEDURE

Methylene blue solution is prepared by dissolving methylene blue aseptically in 25 ml of sterile distilled water.

Each sample of milk is mixed thoroughly.

10 ml of each type of milk is transferred into the labelled test-tubes using separate sterile 10 ml pipette each time.

1 ml of methylene blue is added to each test tube using separate pipettes.

The test tubes are closed with the stoppers.

The contents of each test tube are mixed by gently inverting each tube 2-3 times.

The tubes are incubated in water bath at 37°C for six hours in a incubator at 37°C to 0.5°C in complete darkness.

Control tubes each containing 10 ml of boiled milk and 1 ml of methylene tube is also incubated.

OBSERVATION

The incubated milk tubes were observed for every 30 minutes for 3-6 hours for the reduction of methylene blue that is change in colour of the sample from blue into white. The time required was recorded for the decolourization in the samples.

RESULTS

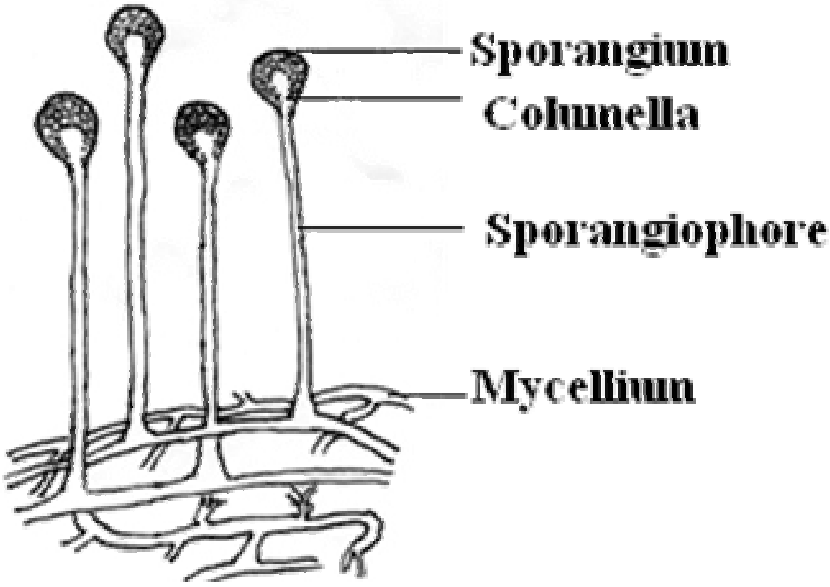
The quality of the milk samples is determined as very poor; fair and good.

S. No.	Milk Sample	MBRT (in Hrs)	Result
1			
2			
3			
4			

MICROBES IN SPOILT FOOD

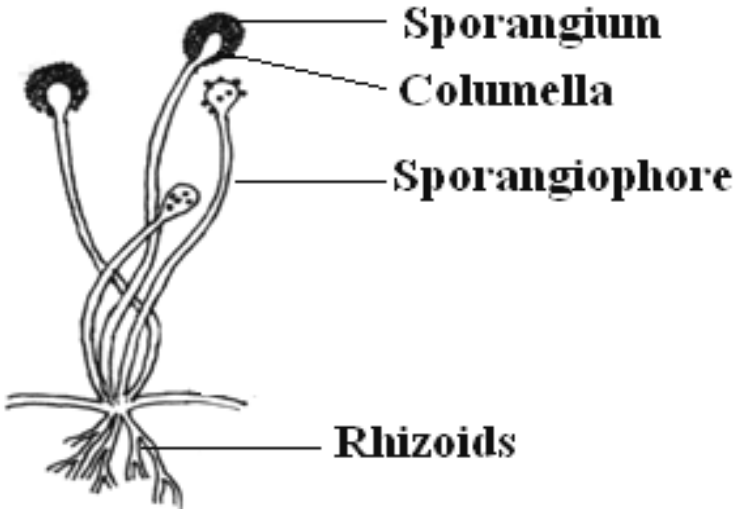
MUCOR

- Mucor is a saprophytic fungus.
- The aerial mycelium has highly branched hyphae over the surface of the substratum.
- The stolons are absent.
- The sporangiophore is branched.
- The erect sporangiophore enlarges to form a dome shaped structure over the vesicle.
- The columella remains intact after the rupture of sporangium.



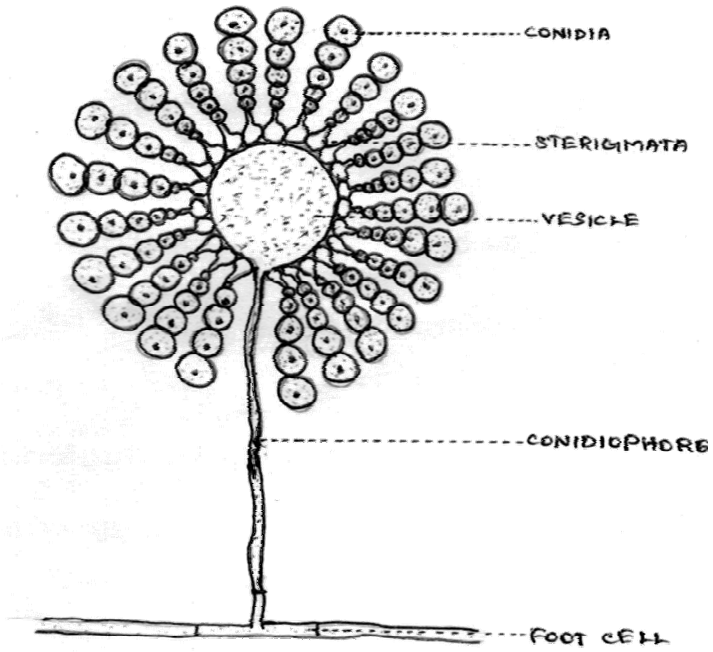
RHIZOPUS

- It is a saprophyte.
- The mycelium during vegetative phase is white and fluffy mass of loosely enlarged hyphae.
- The hyphae are concentric and aseptate.
- The aerial hyphae grow horizontally into stolons.
- From the surface of stolon, the rhizoidal hyphae arise which help in anchoring the fungus to substratum and for absorption.
- The unbranched sporangiophore arises in tufts in air from stolon.
- Asexual reproduction occurs by formation of non motile multinucleate spore produced endogenously inside sporangia.
- The sporangiophore enlarges to form a vesicular structure called columella.
- The columella assumes the shape of hat after the dispersal of spores.



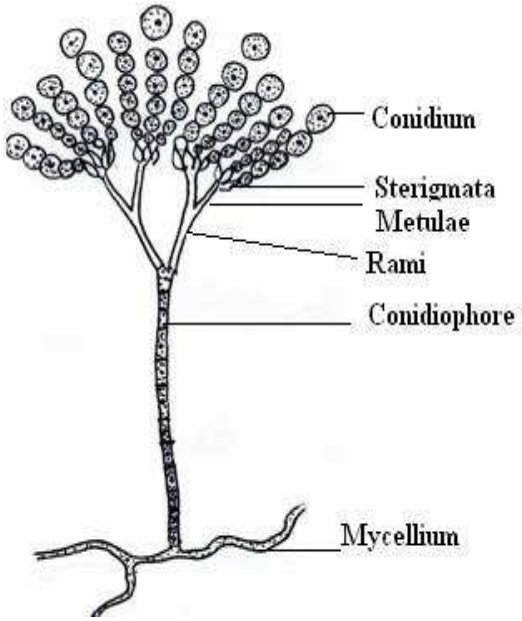
ASPERGILLUS

- Aspergillus is a saprophytic fungus.
- It appears in the form of greenish smoky patches on bread and decaying vegetable.
- The fungus reproduces asexually by the formation of conidia.
- The foot cell produces conidiophore which swells at its tip to form a vesicle.
- From the surface of multinucleate vesicle arise numerous radially arranged sterigmata.
- The sterigmata are produced in 2 or 3 layers.
- The sterigmata cut off a chain of basigenously arranged conidia.



PENICILLIUM

- Penicillium is a saprophytic fungus.
- It grows on decaying fruit, vegetables and food stuffs.
- Commonly known as green or blue mould.
- Mycelium is well developed copiously branched and septate.
- Vegetative mycelium bears upright and branched conidiophores [primary, secondary, tertiary].
- The branch which bears sterigmata or phialides is called the rami.
- The branch which bears rami is called metulae.
- Conidia are arranged on sterigmata in basipetalous succession.
- Each conidium is a globose and multinucleate structure.

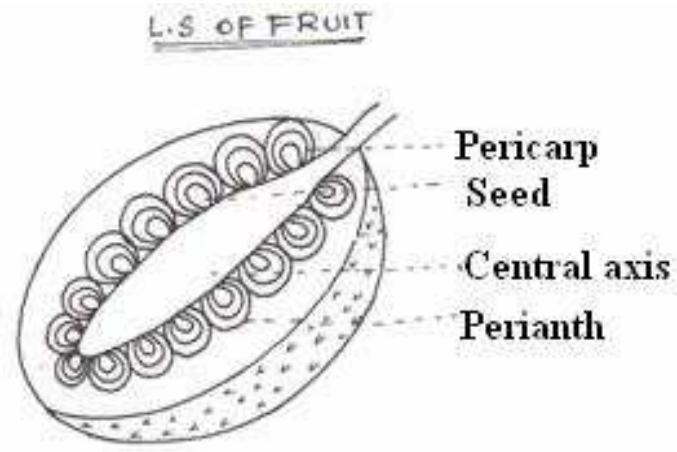


Write the binomial, family and useful parts of the following

NAME	BINOMIAL	FAMILY	USEFUL PARTS
Cotton	*Gossypium herbaceum*	Malvaceae	Seed-fibre
Mustard	*Brassica juncea*	Cruciferae	Seeds
Fenugreek	*Trigonella foenum-graecum*	Leguminosae Sub family-Papilionaceae	Seeds
Asafoetida	*Ferula assa-foetida*	Umbelliferae	Root-gum resin
Coriander	*Coriandrum sativum*	Umbelliferae	Seeds/fruits
Cumin	*Cuminum cyminum*	Umbelliferae	Fruits/seeds
Coffee	*Coffea arabica*	Rubiaceae	Seeds
Sugar	*Saccharum officinarum*	Gramineae	Stem
Sago	*Manihot utilissima*	Euphrobiaceae	Tuberous root
Tobacco	*Nicotianum tobacum*	Solanaceae	Leaf
Rubber	*Havea brasiliensis*	Euphorbiaceae	Stem latex
Sesamum	*Sesamum indicum*	Pedaliaceae	Seeds
Castor	*Ricinus communis*	Euphorbiaceae	Seeds
Mint/Pudina	*Mentha viridis*	Labiatae	Leaf
Chilli	*Capsicum frutescens*	Solanaceae	Fruits

SPOTTERS
MULTIPLE FRUIT / SOROSIS
E.g. *JACK FRUIT*

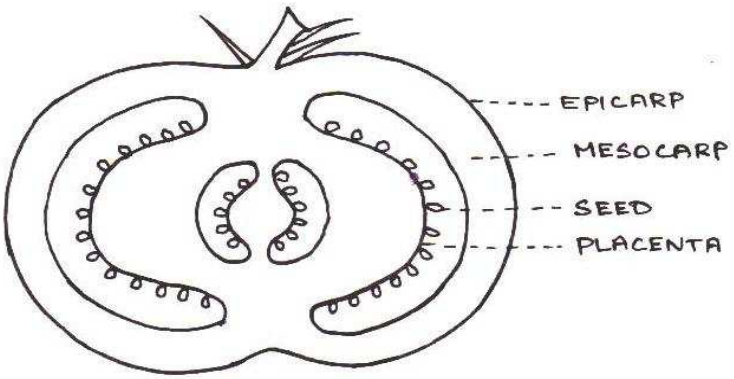
- Multiple fruit is derived from a whole inflorescence.
- The female spike develops into the fruit called as sorosis.
- In L.S, there is a long central axis, which is the inflorescence axis or peduncle
- The sweet edible flakes represent the perianth of the fertilized flowers
- Inside the edible portion, there is a membranous bag containing one big seed.
- The bag represents the pericarp of the fruit.
- Between the edible flakes there are numerous flat elongated whitish structures which are not edible represents the perianth of sterile flowers.
- The fruit is covered by a sheath and it has many spines represents the stylar portions.



BERRY
Eg. *TOMATO*

- Fruit is a fertilized and ripened ovary.
- The whole fruit is fleshy derived from syncarpous ovary.
- The pericarp is thick and succulent.
- Pericarp is differentiated in to outer epicarp and inner mesocarp.
- The epicarp is thin and form the skin of the fruit.
- The mesocarp form the inner pulp which encloses the seeds on swollen placenta.

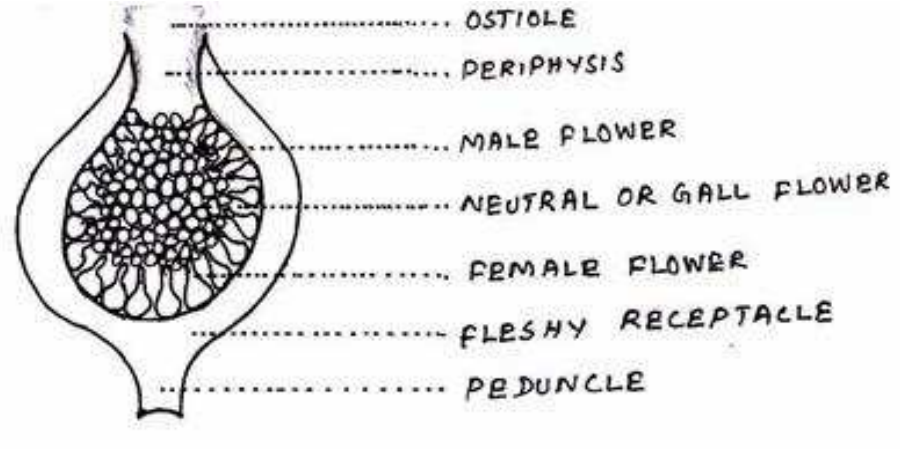
L.S OF FRUIT



HYPANTHODIUM

- It is a special type of inflorescence.
- It consists of a fleshy receptacle.
- The receptacle becomes hollowed out to form a cavity which encloses numerous unisexual sessile flowers.
- It is opened out by a hole called ostiole.
- Near the ostiole, numerous hairs called periphyses are present. eg. Ficus.

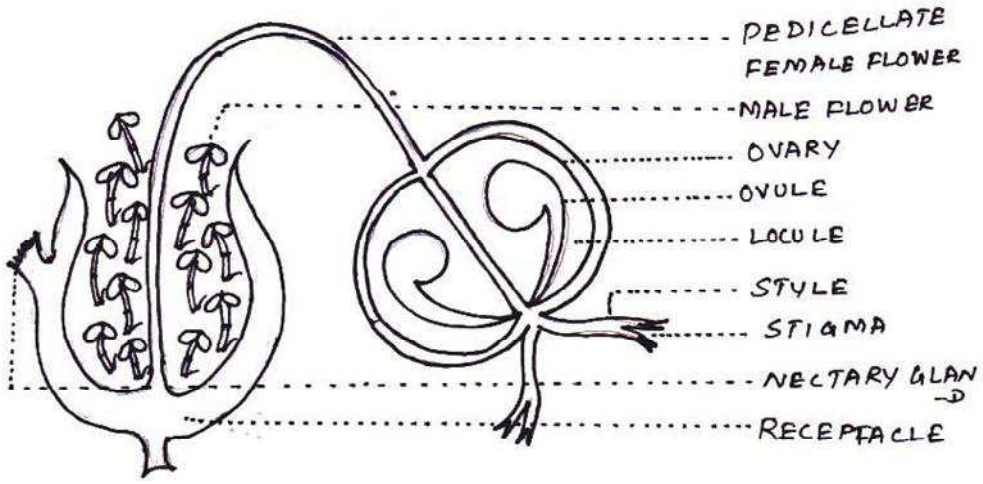
L.S OF INFLORESCENCE



CYATHIUM

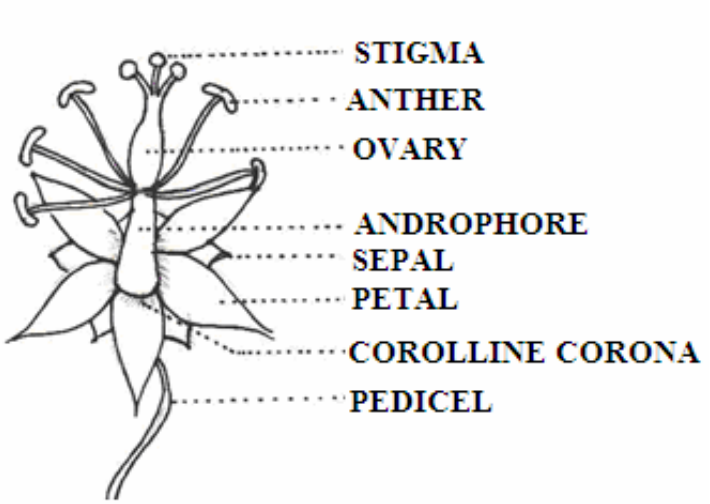
- It is a special type of inflorescence.
- It consists of five involucral bracts forming involucral cup.
- This cup encloses a solitary, central pedicellate, achlamydeous female flower surrounded by five scorpiod cymes of male flowers.
- An extra floral nectar gland is present on one side by the involucral cup. eg. Euphorbia heterophylla.

L.S OF INFLORESCENCE



TORUS MODIFICATION-ANDROPHORE
Eg. *PASSIFLORA*

- In Passiflora, the torus is elongated between the petals and stamens
- The elongated torus is called androphore.
- Prescence of corolline corona.
- Axilliary bud modified into tendrils.
- Bracteoles are large.
- Leaves – palmately lobed.
- Tendril climbers.



EYE PIECE/OCULAR

- It is a part of microscope.
- This is situated near the eye of the observer.
- It lies at the upper end of body tube.
- It magnifies (5x, 10x, 15x and 20x) the image produced by the objectives.

OBJECTIVE LENS

- It is a part of microscope.
- It is a biconvex lens.
- It is situated near the object.
- Body tube with the objective at lower end.
- It magnifies the object 10 times / 45 times / 100 times.

COLORIMETER

- Colorimeter is a device to determine the concentration of biochemical compound.
- It works under **Beer-Lambert's** law.
- It consists of steady source of light, a prism, a filter, a cuvette holder, a photocell, an amplifier and a galvanometer.
- OD adjustor between filter and a cuvette holder helps to adjust the OD.

CENTRIFUGE

- Centrifuge is an instrument.
- It can rotate the sample at the speed up to 30,000 rpm.
- This consists of a motor and a rotor.

- The centrifugal force passes the substances to move away from the axis more rapidly.
- The movement of particles in a centrifugal field is called sedimentation.
- Sedimented particles are called pellets.
- The solution above the pellet is called a supernatant.

USES

- Isolation and purification of protein and determination of their molecular weight.
- Isolation of organelles and macromolecules.

CHROMATOGRAPHY

- Chromatography is a biochemical technique.

PRINCIPLES

- Different rates of migration of components (differential solubility) of a mixture on the solvent system.
- Different rates of adsorption of the components (solutes) in the stationary phase.

PAPER CHROMATOGRAPHY: (Partition chromatography)

- Stationary phase: **Water (Liquid)** is held between the cellulose fibres of filter paper (supporting medium).
- Mobile phase: **Solvent** (**Liquid).**

THIN LAYER CHROMATOGRAPHY: (Adsorption chromatography)

- Stationary phase: **Solid (Silica gel),** glass plate act as a supporting medium.
- Mobile phase: **Solvent** (**Liquid).**

USES

Separation and identification of bio-molecules such as amino acids, fatty acids and carbohydrates etc.

AGAR SHREDS

- Agar is widely used **dried, mucilage** which occurs in the cell walls of red algae ***Gelidium, Gracillaria.***
- It is a polymer of galactose with sulphate groups.
- It is used for the preparation of **culture medium** for the growth of algae, fungi and bacteria.
- It has laxative properties.
- Used in the preparation of medicines, cosmetics and also in leather and textile industries.
- Acts as a **solidifying agent** in microbiological media.

STREAK PLATE TECHNIQUE

- A method of obtaining **discrete colonies and pure cultures.**
- A sterilized loop or transfer needle is dipped into a suitable dilute suspension of organisms.
- It is then streaked on the surface of a solidified agar plate, to make a series of parallel, non-overlapping streak (Z-streak).
- The aim of this method is to obtain colonies of Micro organisms that are pure. i.e. growth derived from a single cell or spore.

AUTOCLAVE

- Autoclave is an instrument used in the microbiological laboratory.
- It is an apparatus in which **saturated steam under pressure** effects sterilization (moist heat).
- Used for sterilizing solid and **liquid media, glass wares, heat resistant instruments and rubber products.**

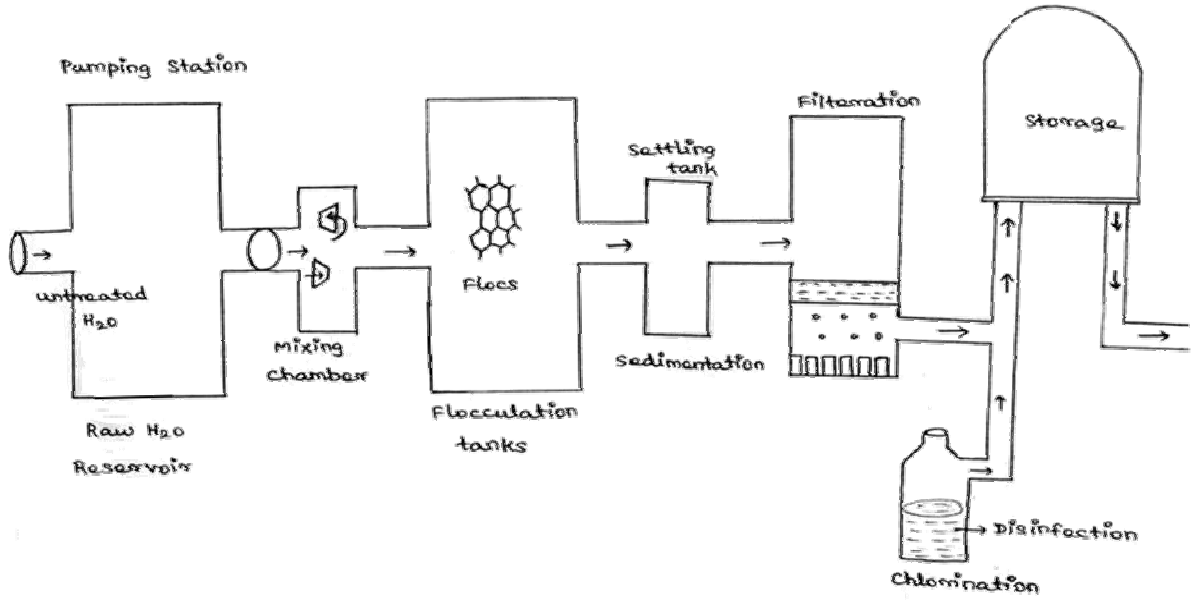
- It is not recommended for oils, powders, heat-sensitive fluids, plastics (that melts).
- It is a double walled cylindrical metallic vessel, made of thick stainless steel or copper.
- Autoclave lid is provided with **pressure gauge and exhaust valve.**
- The articles to be sterilized are kept loosely in a basket, provided with holes, for free circulation of the steam.
- Most of the organisms are killed at a temperature of **121°C (15 lbs/in^2) in 15 minutes.**

LAMINAR FLOW HOOD

- A type of **filtration** method.
- It is used for reducing the danger of infection with infectious Micro organisms and preventing contamination of sterile materials.
- It works on the principle of application of **High Efficiency Particulate Air Filter (HEPA).**
- Essential equipment in microbiological, clinical laboratories, medical (hospitals) and pharmaceuticals.

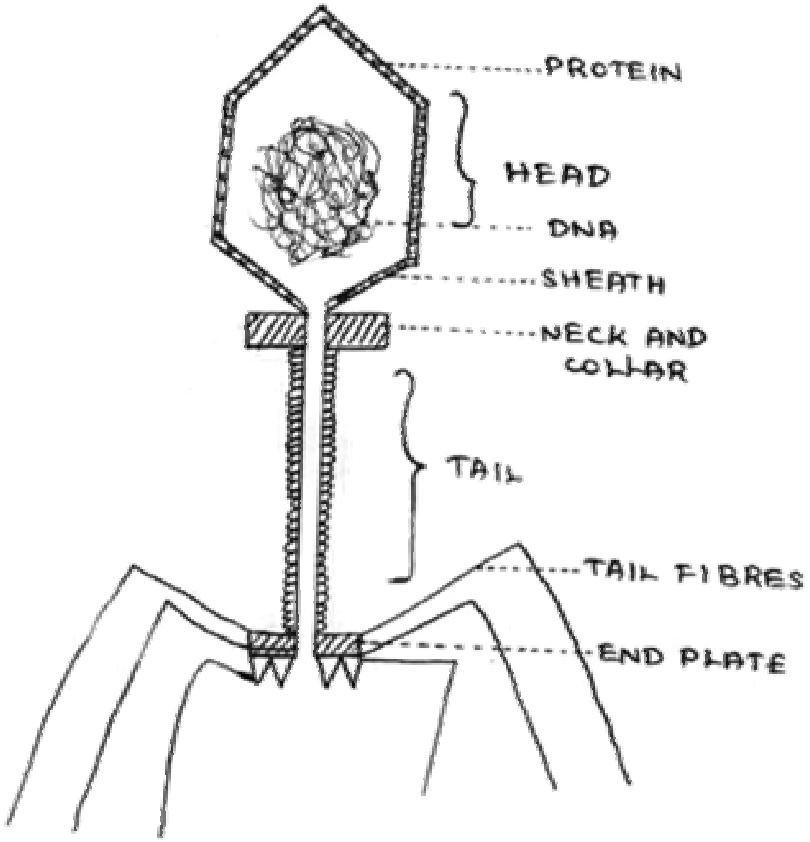
WATER PURIFICATION

- Steps involved in the purification of drinking water are:
    1. Sedimentation
    2. Filtration
    3. Disinfection
- **Sedimentation** results in partial reduction of microbes in water by removal of bulky objects such as leaves, sand, gravel particles.
- **Filtration** is effective means of removing microbes and other suspended material from water.
- **Disinfection** is the final step of water purification. It is carried out by:
    - **chlorination**
    - **ozonation**
    - **Irradiation – UV light**
    - **By boiling.**



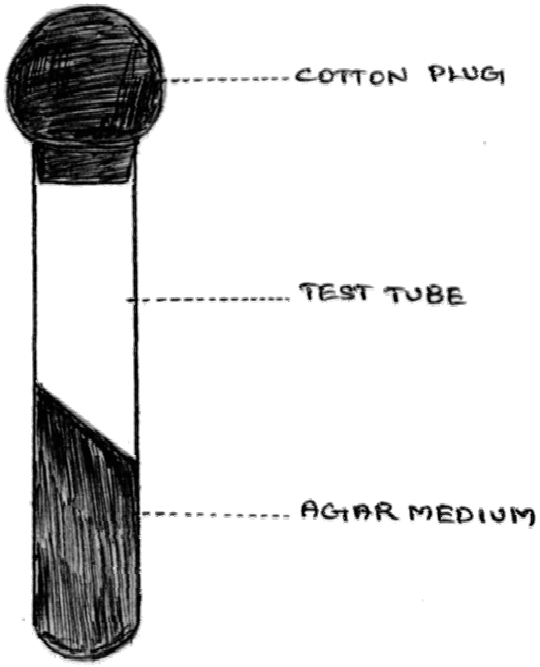
BACTERIOPHAGE

- Virulent bacteriophage is **T_4 phage.**
- It is tadpole shaped, consists of 3 parts: **head, tail and neck.**
- Head is polyhedral, covered by a protein coat called **capsid.**
- Double stranded **DNA** is present inside the head.
- Neck is short and contains a collar, connects the head with tail.
- Tail consists of central hollow core, surrounded by contractile sheath, attached to a hexagonal end plate.
- End plate has six long fibres and six short spikes, tail fibres serve as an organ of attachment.



AGAR SLANT

- Agar slants are used for maintaining pure cultures of Micro organisms for subculturing purpose.
- These are prepared by pouring solid media in test tube in liquefied state.
- Allowed to harden in the slant position.
- It provides more surface area for the growth to the Micro organisms.
- Easier to store and transport.
- Sloped surface is easier to streak than horizontal surface.
- Less contaminated.



ASPERGILLUS

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- The sterigmata are produced in 2 or 3 layers.

  The sterigmata cut off a chain of basigenously arranged conidia.

MODEL QUESTION PAPER

Time: 3 hrs **Max. Marks: 100**

1. Refer **A & B** to their respective **families giving reasons**.

   (Identification-1; Reasons-2) **(2 × 3 = 6)**

2. Describe specimen **'C'** in technical terms. Draw **L.S of flower**, construct the **floral diagram** and write down the **floral formula**.

   (Technical description-2; L.S-1; floral diagram -1; floral formula -1) **(1 × 5 = 5)**

3. Dissect and mount the **floral parts** of **D.** submit the slide.

   (1 × 2 = 2)

4. Prepare acetocarmine **squash of root tip 'E'.** Submit the slide.

   (Procedure-2; Slide-2; Diagram -1) **(1 × 5 = 5)**

5. Cut transverse section of specimen **'F'**. Prepare and submit **double staining slide**.

   (Slide-5; Procedure-2) (**1 × 7 = 7)**

6. Study the bacteria in the sample **'G'** using **Grams stain** and submit the slide.

   (Procedure-2; Slide-4; Diagram-1) **(1 × 7 = 7)**

7. Identify, draw diagrams and write notes on **H, I, J, K &L.**

   (Identification-1; Notes-3; Diagrams-2) **(5 × 6 = 30)**

8. Give the genus, species, family and morphology of **useful parts** of **M, N & O.**

   (Genus-1; Species-1; Family-1; Morphology-1) **(3 × 4 = 12)**

9. Spot at sight of **genus and family** of **P, Q & R**

(Genus-1; Family-1) **(3 × 2 = 6)**

80 marks

Herbarium	10
Record	10
Total	**100**

KEY

1. **A & B** – Annona / Murraya / Vinca / Hyptis / Morinda / Leucas.
2. **C** – Cassia / Daemia / Crotolaria / Euphorbia.
3. **D** – Prosopis / Achyranthes / Daemia.
4. **E** – Onion Root Tip
5. **F** – Double Staining
6. **G** – Grams Staining
7. **H** – Hypanthodium / Cyathium / Sorosis / Berry

   I & J – Ocular / Objective / Spherical aberration / Chromatic aberration / Colorimeter / Centrifuge / Chromatography

   K & L – Agar Shred / Streak Plate / Autoclave / Laminar Flow Hood / Water Purification / Bacteriophage
8. **M, N & O** – Fenugreek / Centella / Coriander / Sago / Sugar / Sesamum / Cotton / Nicotiana / Ferula / Sago
9. **P, Q & R** – Fresh / Herbarium Specimens