

M.Sc. BOTANY LAB MANUAL

1st Semester



Prepared By
Biological Science Dept.
Botany

MIDNAPORE CITY COLLEGE



PREFACE TO THE FIRST EDITION

This is the first edition of Lab Manual for PG Botany first Semester. Hope this edition will help you during practical. This edition mainly tried to cover the whole syllabus. Some hard core instrument based topic are not present here that will be guided by responsive teachers at the time of practical.

ACKNOWLEDGEMENT

We are really thankful to our students, teachers , and non teaching staffs to make this effort little bit complete.

Mainly thanks to Director and Principal Sir to motivate for making this lab manual.

Laboratory Practice Safety Rules

1. Use safety glass when dealing with fire and chemical.
2. Should use front cover clothes during biochemistry practical.
3. Always use hand wash after dissection and any type of chemical use.
4. Carefully handle needles , forceps, microscope and any other dissecting instrument.

INDEX

Sl. No.	CONTENT	PAGE No.
BOT-105, Microbiology Phycology and Bryology Microbiology Gram Staining		
1.	Microbiology Phycology and Bryology Microbiology Gram Staining	01
2.	Negative Staining Technique	03
3.	Study of the curd organism	04
4.	Endospore Staining	06
5.	Phycology Description and workout of algal specimen: A	09
6.	Work Out of Specimen: B	10
6.	Work Out of Specimen: C	12
7.	Work Out of Specimen: D	14
8.	Bryology Work Out of Specimen: A	15
9.	Work Out of Specimen: B	17
10.	Work Out of Specimen: C	20
BOT-106, Mycology and Plant Pathology, Pteridophytes and Gymnosperms Mycology		
12.	Work Out of Specimen: A	23
13.	Work Out of Specimen: C	25
5.	Plant Pathology Study of Diseased Plant Specimen: A	26
14.	Work Out of Specimen: B	27
15.	Gymnosperms	36
15.	Gymnosperms	37

BOT – 105

Microbiology
Phycology and Bryology
Microbiology
Gram Staining

Principle : Different staining requires the use of at least three chemical reagent that are applied sequentially to a fixed smear. The first reagent is called primary stain. Its function is to impart its colour to all cells. In order to establish a colour contrast, the second reagent used is the decolouring agent. Based on the chemical composition of cellular components, the decolouring agent may or may not remove the primary stain from the entire cell or only from certain cell structure. The final reagent, the counter stain has a contrasting colour than that of the primary stain. The following decolourization, if the primary stain is not washed out the counter stain cannot be observed and the cells on their components will retain the colour of the primary stain. If the primary stain is removed, the colourized cellular components will accept and assume the contrasting colour of counter stain. In this way, cell types or their structure can be distinguished from each other on the basis of the stain that the cells retained.

Purpose : to become familiar with;

1. The chemical & theoretical bases for differential staining procedure.
2. The chemical basis of gram stain.
3. Performance of the procedure for differentiating between the two principle groups of bacteria:
 - Gram +ve bacteria
 - Gram –ve bacteria

Materials :

Cultures: 24 hours nutrient agar stain culture of sample 1.

Reagents:

Crystal violet (Primary stain)

Gram's iodine (mordant)

70% Ethyl alcohol (Decolourising agent)

Safranin (Counter stain)

Equipments:

Bunsen burner, inoculating loop, staining tray, glass slide, bibulous, Paper, lens paper and microscope.

Procedure:

1. obtain 4 clean glass slides.
2. using sterile technique prepared smears of each of the 2 sample organisms. the smear is prepared by placing a drop of water on the slide, then transferring each organism separately to the drop of water with a sterile cooled inoculating loop. mix and spread the organism by the means of circular motion by inoculating loop.
3. Allow smears to air dry then heat fix the smear by the Bunsen burner.
4. Gently flood the smear with crystal violet and let stand for 1 min.
5. Gently then wash it with tap water.
6. Gently flood smear with the gram's iodine mordant and let it stand for 1 min.
7. Gently wash it again with tap water.
8. decolourised it with 90% ethyl alcohol.
(caution: do not over decolourise, add reagent drop by drop until alcohol runs almost clear, showing only a blue colouration.)
9. Gently wash it with tap water when done, then the next step is to apply gently or counter stain gently with safranin for 45 sec.
10. Blot dry with bibulous paper and examine under oil immersion microscope.

Observation and result:

Draw a representative field.

Cell morphology;

- 1) Shape — Round shaped
- 2) Arrangement — single
- 3) Cell colour — crystal violet (purple)
- 4) Gram Reaction — gram positive

Comment:

According to the above result, the sample contains gram positive stain, because it retains the crystal violet stain colour, hence it is a gram positive bacteria.

NEGATIVE STAINING TECHNIQUE

Principle: In negative staining, the background but not the bacteria are stained by the use of an acidic stain which carries a negative charge on its surface and is used by the bacteria, that too carry a negative charge on their surface. In negative staining the bacterial growth is mixed with a loopful of the stain on a clean slide and the cover laid with a coverslip. The capsule appears as a clear one between the cell wall and the background under high-dry and oil-immersion objective.

Requirements:

- 1) 36-48 hours culture of *Lactobacillus fermentum*.
- 2) Indian ink.
- 3) Absorbent or blotting paper.
- 4) Clean glass slides.
- 5) Glass cover slip.
- 6) Inoculating loop.
- 7) Bunsen burner.

Procedure:

1. Take a clean glass slide.
2. Put a drop of Indian ink close to one end of the clean glass slide.
3. Add two loopfuls of a broth culture into the drop of stain and mix it with the loop.
4. Prepare a smear of the suspended organism using the edge of the second slide held at a 30 degree angle and push away to the other end of the slide.
5. Air dry the smear.

Observation:

Examine the slide under oil immersion objective.

Result:

Bacilli will appear as a clear zone with dark background.

Comment:

In the staining, Indian ink is repelled by the bacterial cell, therefore, the cell appears as a clear zone against the blue background.

Study of the curd organism

Introduction: Basically curd is a milk product produced due to fermentation i.e. microbiological activity in milk. The bacteria i.e. *Lactobacillus* produce lactic acid in the milk and causes souring and milk is ultimately converted into curd. In addition to *Lactobacillus* sp. Curd also contains the *Streptococcus* and Yeast. Curd organisms are studied by gram staining procedure which is a double staining procedure discovered by Christian Gram applicable for bacteria, not in any microbes.

Procedure of Gram staining

Requirements:

- a) Grease free slide
- b) Crystal violet solution (Gram stain)
- c) Lugol's iodine
- d) 70% ethanol
- e) Safranin solution (counter stain)
- f) Oil immersion
- g) Compound microscope
- h) Gentle tap water
- i) Spirit lamp
- j) Needle

Procedure:

- a) At first a grease free slide is taken
- b) A drop of curd suspension is taken on this slide
- c) Smear the suspension by using needle
- d) Then the smear is dried or heat fixed by placing it in the gentle flame of the spirit lamp
- e) A few drops of crystal violet solution are added over the smear and kept for 30 sec.
- f) Then a few drops of iodine are added for 90 sec. safranin.

But in case of gram (-ve) type, bacteria took crystal violet stain, & due to cell wall composition, they lose this stain by ethanol treatment. so, they are stained by safranin i.e. counter stain & finally they look red or pink.

- g) Now, the stain is poured and the slide is washed with 70% or 90% ethyl alcohol and dried.
- h) Then a few drops of safranin solution are added to the smear and kept for 2 mins.
- i) Now, the slide is washed under gentle stream of tap water and let it dry
- j) Then the dry slide is observed under 10X, 40X, 100X (oil immersion) microscopic view

Observation:

i.e, Lactobacillus sp. retain colour of crystal violet, so there gram positive.

Discussion of result:

Actually gram(+ve) type of bacteria, they took crystal violet stain as they form crystal violet +iodine complex(CVI)& when treat with ethanol then they do not loose their stain &there is no effect of second counter stain i.e

Precautions:

- i) The timing of staining & washing done in appropriate manner.
- ii) The slide must be grease free
- iii) Heat fixation should not provide high temp. as bacteria from the endospore.

ENDOSPORE STAINING

Principle:

Some bacteria are capable of changing in to dormant structure that are metabolically inactivate and do not grow or reproduce since these structure are formed inside the cells hence are known as endospore .

During sporulation a vegetative cell give rise to a new intracellular structure termed as endospore that is surrounds by a impermeable layer called as spore coat.

Example: Bacillus, Clostridium, Coniella, Desulfohalobium, thermophilum and spore.

These spore are differentially stained by using special procedure that help the dyes to penetrate the spore wall. An aqueous primary stain (malachite green) is applied and is steamed to enhance penetration of the impermeable spore coats ,once endospore are stained they do not readily decolorize and appear green with red cells .

Requirements:

- i) 48 hours nutrient agar cultures of Bacillus subtilis and Staphylococcus aureus.
- ii) Malachite green(5%aqueous)
- iii) Safranin(0.5%aqueous)
- iv) Staining tray
- v) Glass slides
- vi) Inoculum loop
- vii) Blotting paper
- viii) spirit lamp
- ix) microscope.

Procedure:

- i) Make smears of Bacillus subtilis and staphylococcus aureus on separate clean slide.
- ii) Air dry and heat fixed the smear
- iii) Flood the smear with malachite green
- iv) Heat the slide to steaming and steam for 5 minute adding more stain to the smear from time to time.
- v) Wash the slides under slowly running tap water.
- vi) Counter stain with safranin for 30 seconds.
- vii) Wash the smear with distilled water.
- viii) Bolt dry the slides that is wet with distilled water

Observation :

Examine the slides microscopically under oil immersion objects from a representative microscopic field of each preparation. Make drawings indicating the position and size of the endospore within individual cell as well as the size and also the exact shape of the free spore.

Result:

In *Bacillus subtilis* the endospore staining and the vegetative cells stain red. The vegetative cells are rod-shaped each containing an elliptical centrally located spore.

Comment:

From observation and result we can conclude that the supplied sample contains endospore staining. Some cells have taken malachite green stain and from greenish colour and some cells have taken safranin colour and from pink colour. So from above experiment we can conclude that greenish cells are spore and pink cells are vegetative cells.

Staining procedure for algal specimen

1. Put the species in clean slide and one drop cotton blue taken on it
2. After preparation the slide heated by sprit lamp and waiting for 3-5 minutes.
3. Added lacto phenol on the slide and spread well.
4. After preparation the specimen is covered by cover slip and given pressure on the cover slip for clean the bubbles.
5. At last seal the cover slip.

Phycology

Description and workout of algal specimen: A

Thallus Structure:

plant body is blue – green in colour, free floating colonial or attached to a substratum. Each colony contains a number of trichomes embedded within a matrix (common sheath) forming little blue Nostoc balls. The trichomes consist of a single series of uniform, often torulose, bead-like, ellipsoidal cells more or less depressed which are often contorted and some times form densely interwoven masses. Cells of each trichome are joined end to end to form moniliform (bead-like) chains. Sheaths of individual trichomes are heterocysts are present. Heterocysts are distinguished from the vegetative cell by thick walls, transparent contents, larger size and two polar nodules at two ends. Heterocysts separate the hormogonium.

Reproductive structure:

Akinetes – They are different in appearance from vegetative cells. Akinetes are spherical or oblong and much larger than vegetative cells with dense Protoplasm.

IDENTIFICATION:

Thallus blue green in colour, cells devoid of conspicuous nucleus, absence of any sex organ, presence of gelatinous sheath around the cells (in most cases) absence of organised cell organelles like plastids.

Thallus unbranched, filamentous, presence of hormogonium, heterocyst present some genera only.

The specimen belongs to the class: CYANOPHYCEAE.

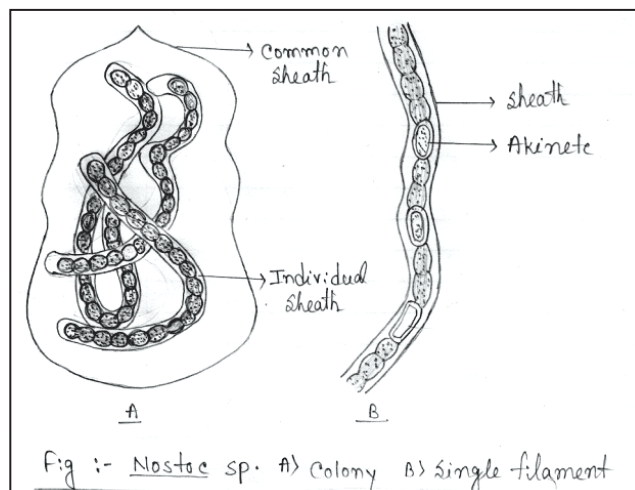
Trichomes unbranched, interwoven and surrounded by sheath presence of heterocyst and akinetes.

The specimen belongs to the order : NOSTOCALES.

Trichomes with single series of uniform, ellipsoidal (bead-like) cells presence of intercalary heterocysts and akinetes.

The species belonging to the family: NOSTOCACEAE.

Genus : *Nostoc*



WORK OUT OF SPECIMEN: B

Materials :- The specimen supplied from laboratory.

The plant body:-

- I) Plant body of this specimen is filamentous, much branched coenocytic and saponaceous thallus.
- II) The coenocytic body contains many nuclei, septa may form during injury on the development of sex organ.
- III) In terrestrial species the plant body remains attached to the soil surface with much branched thread like structure, the rhizoid are either absent or ill-developed.
- IV) The filamentous body has a thin outer wall, which is less elastic. It is made up of outer pectin and inner cellulosic layers.
- IV) In the centre of the filament a continuous vacuole is present except at the apical region, which is filled with cell sap.

Sexual Reproduction :-

- I) The sexual reproduction in specimen is of organous type.
- ii) It takes place by antheridium, the male sex organ and oogonium, the female sex organ.

Development of the sex organ:

Antheridium or male sex organ:

- I) The nuclei of antheridium aggregate in the centre and divide mitotically.
- II) Each nucleus along with some cytoplasm metamorphoses into sigmoid spindle shaped biflagellate antherozoid.
- III) The flagella are unequal in length, dissimilar, one whiplash and the other tinsel and laterally inserted.
- IV) The antherozoids are generally liberated through an opening developed at the apical region of antheridium.

Oogonium or Female sex organ :-

- I) Identically a small protuberance develops at or near the base of antheridial branch, due to accumulation of cytoplasm.
- (II) The cytoplasm of this region is colourless which has many nuclei and without any chromatophores.
- III) The mature Oogonium contains a large nucleus at the centre with many chromatophores and all droplets dispersed throughout the cytoplasm.
- IV) The protoplast along with nucleus round off and forms a single ovum or egg.
- V) It has a hyaline area towards the anterior known as receptive spot.

IDENTIFICATION :-

- I) Thallus yellowish green in colour filamentous coenocytic branched.
- II) Presence of oil as reserve food sexual reproduction complex Oogamous type.

Hence, the specimen is under the class – XANTHOPHYCEAE.

- I) Plant body filamentous a sexuality reproduce by multiflagellate zoospore

Hence the supplied specimen is under the order – HETROSIPHONALES

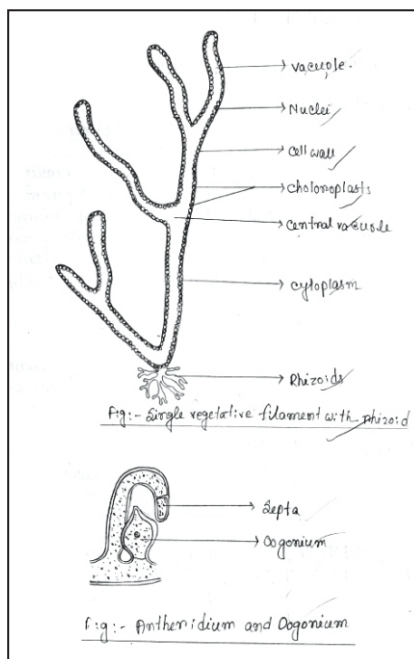
- I) Filaments irregularly branched.
II) Antheridia curved cylindrical and Oogonia.

Hence the specimen under the family –VAUCHERTIACEAE.

- I) Filament long tubular spirally branched.
II) Antheridia hook like curved and oogonium sessile on short stalked with a beak.

Hence the specimen is under the Genus – VAUCHERIA

So the supplied specimen is *Vaucheria* sp



WORK OUT OF SPECIMEN –C

Specimen: Supplied the specimen from the laboratory

Thallus structure:-

- I) The plant body is prominent, branched laterally or dichotomously and brownish red to purple red in colour.
- II) The main axis and branches possess a polysiphonia appearance as the central axis cell is surrounded by pericentral cells of a definite number.
- III) The cell is a prominent cell to cell organism connection and each cell has one nucleus and many discoid plastids embedded in dense cytoplasm. Ultimate branches are uniseriate structure and are known as trichoblast.

Reproductive structure :

Spermatangium :

The lateral branches of the male plant bear antheridium known as spermatangia in dense thallus. The spermatangia are short stalked, colourless and spherical oval structure. It contains a single nucleus.

Cystocarps :

It is an unshaped structure formed by gonimoblast filament surrounded by sterile filaments. The terminal cells of the gonimoblast filament produce a carposporangium with one carpospore.

Tetrasporangium :

It is borne on the central axis of the specialised filament called tetrasporangium filament. Each sporangium contains four tetraspores.

Identification :

Presence of gelatinous material in the thallus cells contains chloroplast with pyrenoid. Presence of characteristic post lytic structure called cystocarp.

Hence the specimen belongs to the class-RODOPHYCEAE.

Plant body heterotrichous uniaxial in the growing and multi axial in the mature region. Carposporangium developing on filamentous gonimoblasts derived directly from the fertilized carposporangia. Uni or multi axial construction of thallus presence of tetrasporangia with tetraspores.

Hence the specimen belongs to the order –SERPENTALES.

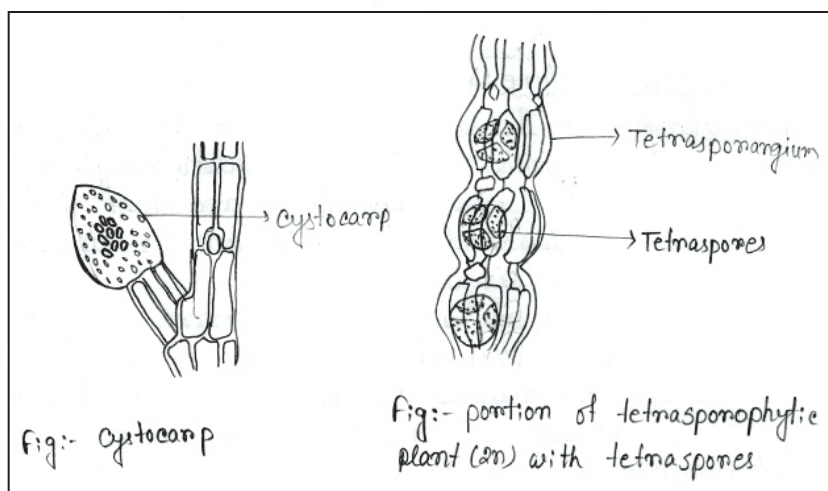
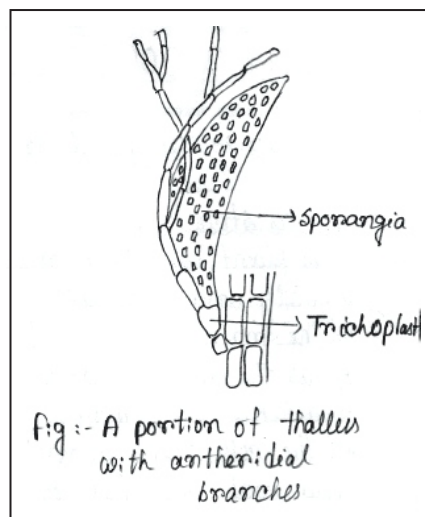
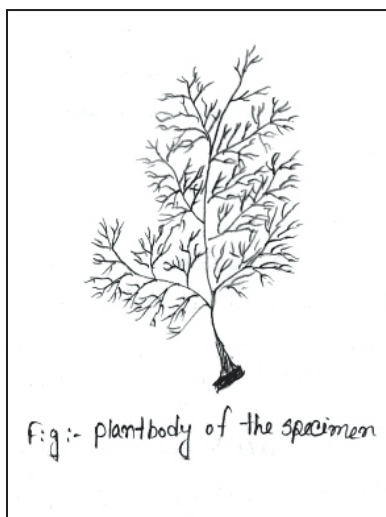
Polysiphonous branched thallus cystocarp urn shaped with a pore tetrasporangia with tetraspores

Hence the specimen belongs to the family-RHODOMELACEAE

Cells of the central axis polysiphonous surrounded by pericentral cells presence of spermatangia on separate filaments

Hence specimen belong to the genus **POLYSIPHONIA**

So the supplied specimen is *Polysiphonia* sp.



WORK OUT OF SPECIMEN –D

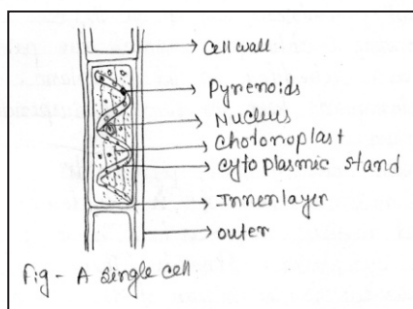
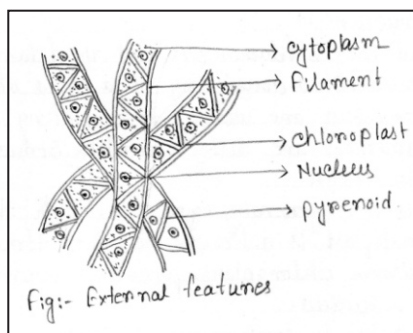
Material:-

- I) The plant body is filamentous and the filament are green, simple unbranched consisting of row of cylindrical cells
- II) The filamentous are usually free flating
- III) The filamentous are silky hair like structures which are smooth to touch.
- IV) The cells are generally more is length then in breath.
- V) The protoplast is differentiated into structures such as plasma membrane chlolooplasts pyrenoids central vascular other cytoplasm is nucleus.
- VI) The cytoplasm is surrounded by plasma membrane and it encloves a long vacuole fillec with tannin containing cell shape.
- VII) The most prominante feature of this cells the presence of spinal or rtibbow shaped chloroplast which are partial in position and remain cubedded in the cytoplasm.
- VIII) Chloroplast contain man pyrenoids.

Identifying character:-

- I) Unbranched filamentous type.
- II) The filamentous forms are free floating
- III) Filamentous forms are Silky hair like structure which are smooth touch.
- IV) Cells are generally more in breath than in breadth.
- V) Chloroplasts contain many pyrenoids

So, the supplied specimen is belongs the genus-*Spirogyra* sp.



Bryology

WORK OUT OF SPECIMEN: A

Material:

Specimen are supplied from the laboratory.

Vegetative structure:-

Plant body is like thallus dorsiventrally differentiated prostrate with dichotomous branching. There is a conspicuous longitudinal furrow on the dorsal side. The ventral surface bears a corresponding ridge and a transverse row of scales. One cell is thickest lower down the scale are in two marginal rows violet in colour. In addition there are two types of rhizoids smooth and tuberculate being on the ventral ridge of the thallus.

T.S of Thallus :-

In T.S through the thallus the following layers can be seen-

- a) Dorsal side bears a tissue in which there are a few vertical rows of chlorophyllose cells separated by narrow vertical air canal. This is chlorophyllose or assimilatory tissue.
- b) Ventral region of the thallus is formed by compact colourless parenchymatous tissue unicellular rhizoid are developed from the outer most layer with colourless cell. Air pores are bounded by four epidermis cells lower epidermis cells lower epidermis is a continuous layer

Reproductive Structure:-

Plants are mostly monoecious but rarely dioecious. Sex organs develop singly and acropetally in a linear row on the dorsal median furrow.

Archegonium :

The mature antheridium is stalked pear shaped and its single layered jacket encloses a number of sperm or androcyte mother cells.

Sporophyte:

Sporangium is some round sac like a single layered jacket enclosing many spores which are often in tetrads. Mature spore 3- layers exosporangium megasporangium and endosporium spores after remain in tetrads.

Identification :

Thallus dorsiventrally flattened and prostrate sporophytes simple and always of limited growth collumella absent inside capsule.

Hence the specimen belong the class HEPATICOPSIDA

Plant body ribbon shaped, dichotomously branched dorsal tissue layers green and with air canals or chamber thallus with scales and rhizoid on ventral surface sex organs on dorsal surface.

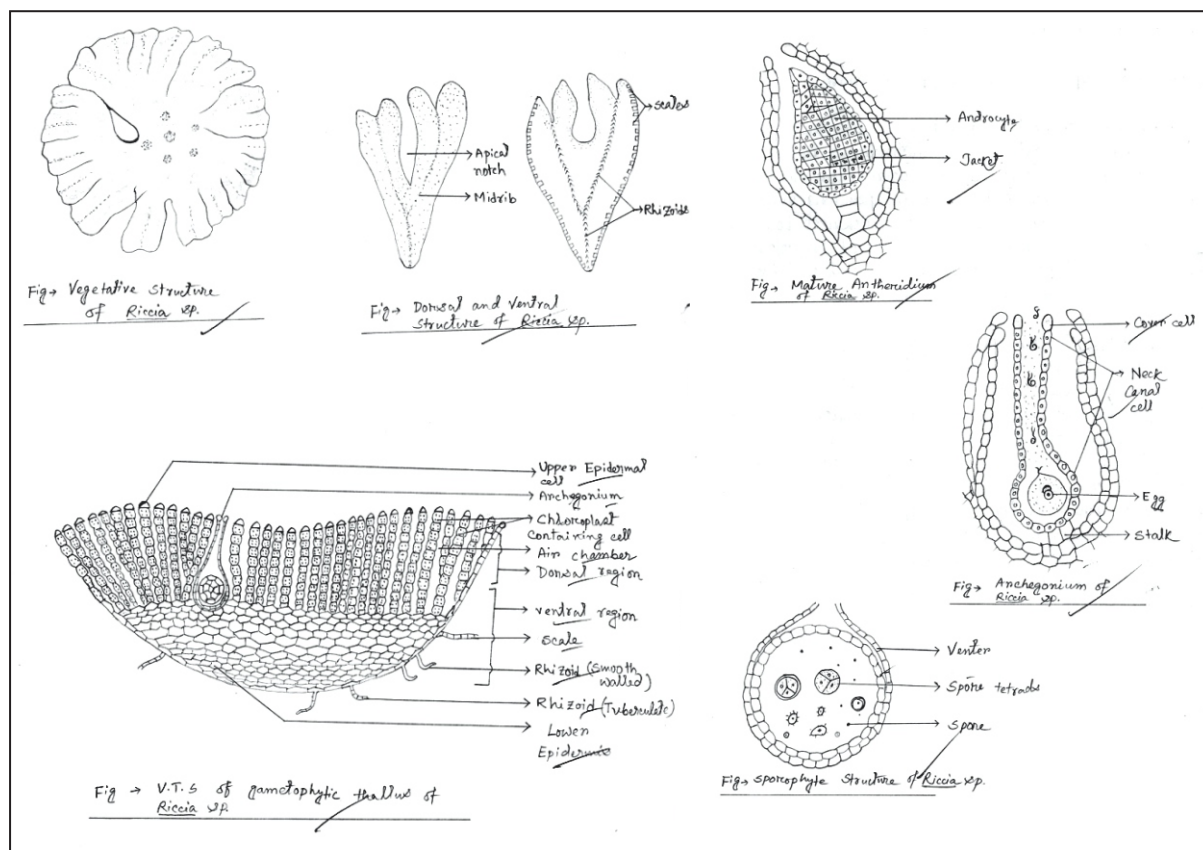
Hence the specimen belongs the order MARCHANTIALES.

Thallus dichotomously branched with sex organ along the entire length of the medium furrow.

Hence the specimen belongs the family **RICCIACEAE**

Thallus linear to wedge shaped air canal present in between two vertical rows of cells .sporophyte sac like and having one iayered jascked surrounding spores and nurse cells.

Hence the specimen belongs the genus is *Riccia* sp.



WORK OUT OF SPECIMEN – B

THE PLANT BODY:

a) External structure :

The gametophyte of *Marchantia* is dichotomously branched prostrate dorsiventral thallus. The dorsal surface of the thallus show many regular rhomboidal or polygonal areas. Each area has a pore at the centre. A distinct midrib is present on the thallus with a corresponding ridge on the ventral surface. The branches grow out by means of a growing point situated in the terminal groove.

The scales are membranous one layered thick usually violet in colour due to the presence of anthocyanin pigments. Beside the scales the ventral surface on the thallus bears rhizoids between the scales. The rhizoid performs the function of anchorage to the substratum as well as absorption of water and nutrient from soil.

The sexually mature thalli bear specialised erect branches called gametophores and gametophores which bear sex organ. These branches are umbrella shaped and arise from the apical notch. The thallus is dioecious or heterothallic either antheridiophore or archegoniophore.

b) Internal feature :

A section of the thallus shows three distinct regions: the epidermal region, the photosynthetic region and the storage region.

The epidermal region consists of a well defined upper and lower epidermis. The epidermis is formed of quadrate cells containing a few chloroplasts. An air chamber of schizogen origin is present just below the polygonal area.

The photosynthetic region; The upper dorsal epidermis contains a few chloroplasts. The air chamber is demarcated from the other by single layered partitions of cells containing chloroplasts. These horizontal rows of cells form the main photosynthetic tissue of the *Marchantia*.

The storage region: The ventral tissue lies immediately below the air chamber from the storage region it is a compact zone comprised of several layers of thin walled polygonal parenchymatous cells devoid of chloroplasts. The midrib of the thallus is made up of cells elongated transverse showing reticulate thickening.

Structure of female gametophores :

In *Marchantia* the production of sex organs is dependent on environmental conditions like day length, humidity, excess or nitrogenous substance etc. The sex organs are borne on special receptacles bearing male (Antheridium) and female (Archegonium) sex organs are called antheridiophore and archegoniophore or carpocephallum respectively. These are developed on separate plants, so that the *Marchantia* is dioecious or heterothallic although the gametophores are erect branching they are actually the direct extension of the prostrate vegetative thallus. This is clearly evident from the dorsiventral nature of the erect shoot with air chamber, air pores and rhizoid.

Archegonium: Like Riccia the archegonium of specimen –B also develop from a single superficial dorsal cell called the archegonial initial. The conical shaped initial cell become conspicuous, increase in size and project above the surface. The initial cell divides by a transverse wall to form an upper primary archegonial cell and a lower primary archegonial cell by a few irregular division forms a short but distinct multicellular stalk of the archegonium. The primary archegonial initial undergoes several phases or regular division to form the archegonium.

A mature archegonium is a pendulous flask shaped structure with a short stalk which attach to the lower (ventral) surface of the archegonial disc. A single archegonium has a basal swollen venter containing an egg cell a ventral canal cell and row neck canal cell inside the elongated neck.

Identification :

Thallus dorsiventrally flattened and prostrate sporophyte simple and with limited growth columella absent inside the capsule.

Hence the specimen belong the class HEPATICOPSIDA

Plant body prostrate ribbon shaped dichotomously branched presence of dorsal chamber sex organs on dorsal surface sporophyte devoid of columella or elaterophore.

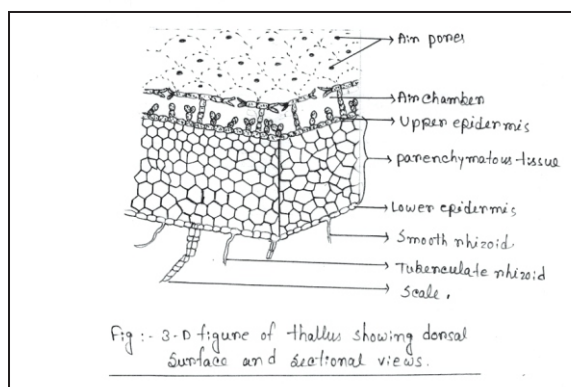
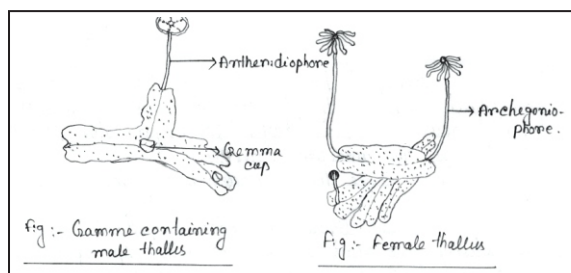
Hence the specimen belong the order MARCHANTIALES

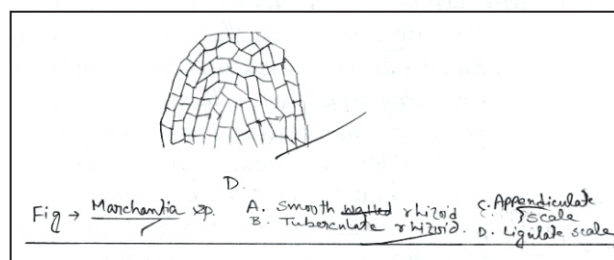
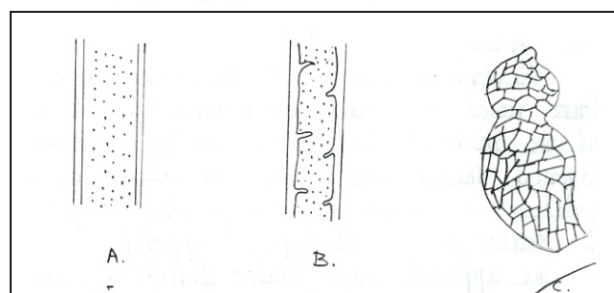
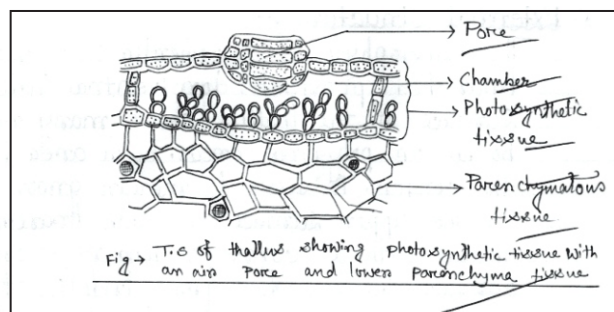
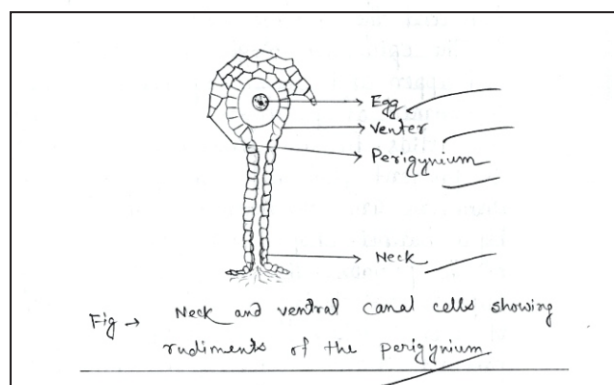
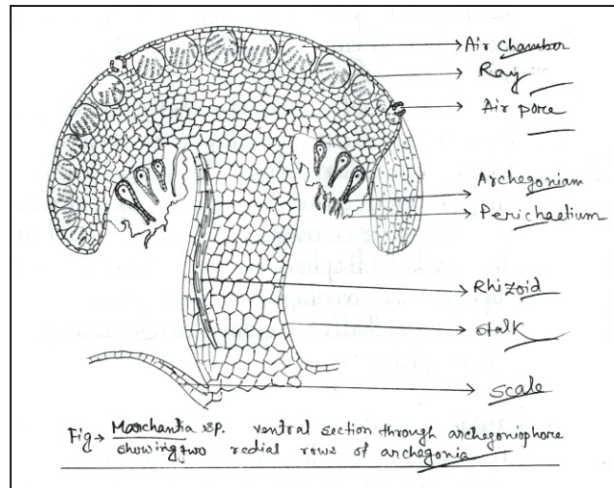
Antheridia and archegonia localized on special branches antheridiophore and archegoniophore sporophyte differentiated into foot seta and capsule elaters present.

Hence the specimen belong the family MARCHANTIACEAE.

Air chambers present in a now pores elevated photosynthetic filament present presence of antheridiophore and archegoniophore, sporophyte with foot seta and capsule. capsule bears spores and elaters

Hence the specimen belong the genus is *Marchantia* sp.





WORK OUT OF SPECIMEN – C**Material:**

Supplied from the laboratory.

The plant body**External feature:**

- a) The erect gametophytic plant is about an inch high with an erect leafy axis or stem attached to the substratum by rhizoid.
- b) The axis is radial often branched axially or extra axillary.
- c) The axis is covered with spirally arranged leaves which are more crowded near the apex forming a rosette
- d) The leaves are simple sessile ovate with pointed apex smoothed margin attached to the stem by a broad base
- e) The mature leaves have midribs but younger leaves are devoid of midrib.

Structure of the mature sporophyte:

The immature sporophyte is differentiated into foot along seta and a pear shaped tip of the tip.

Foot: It is a poorly developed conical structure embedded in the apex of archegonial branch.

Seta

- i) Seta is a long green in colour when young but becomes reddish brown at maturity.
- ii) A central conducting strand of thin walled cells surrounded by a cortex made up of comparatively thick walls.
- iii) Seta helps in conducting strand of thin walled to capsule.

CAPSULE:

- i) The mature capsule is pear shaped material.
- ii) It is divided into three distinct parts the sterile basal region the apophysis, the central fertile region the apical region.

CAPSULE WALL:

- i) The capsule wall is many layered.
- ii) The single layered outer most wall forms the epidermis which is followed by 2-3 layered parenchymatous hypodermis.
- iii) The inner 2-3 layered of parenchymatous cells are chlorophyllous which constitute the photosynthetic tissue of the capsule.

Spore sac

- i) The columella is surrounded by two elongated spore sacs
- ii) The spore sac has an inner wall of one layer of cells and an outer wall of 3-4 layers of such cells
- iii) The spore sacs are formed from the single layered archesporium.

AIR CHAMBER:

- i) The outer of the spore sac is followed by a big cylindrical air chamber.
- ii) It is transverse by string of filaments of elongated green cell known as trabiculle.
- iii) Trabecule is bridge the air space between the outer wall of the spore sac and the innermost layer of the capsule wall.

COLUMELLA

- i) It is the central axial part of the fertile zone comprising of thin walled colourless compact parebchymatous cells constricted of the base just above the apophysis.
- ii) The distal part of the columella is cone shaped which projects into the connectivity of the operculum.

THE APICAL REGION:

- i) The operculum is an obliquely placed dome shaped lid that closes the mouth of the capsule.

STRUCTURE OF PERISTOME TEETH:

- i) The peristome teeth lie that below the operculum and are attached beneath the edge of the diaphragm
- ii) It consists of two ring of long triangular teeth one within the other
- iii) The teeth are not cellular in nature and are made up of cuticle.
- iv) Each ring of peristome possesses 16 teeth.

Identification:

Absence of vascular tissue without any differentiation on root stem and leaf .sporophyte dependent on gametophyte presence of archegonia.

Hence the specimen is under the division –BRYOPHYTA

Gametophyte erect, rhizoids are multicellular presence of peristome teeth.

Hence the specimen is under the order-Funariales

Sporophyte differentiated from foot, seta and capsule

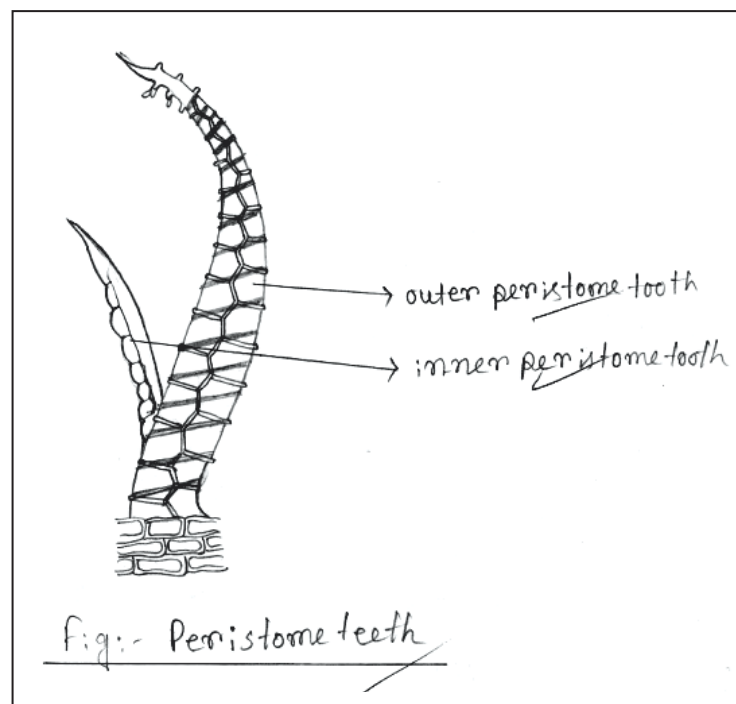
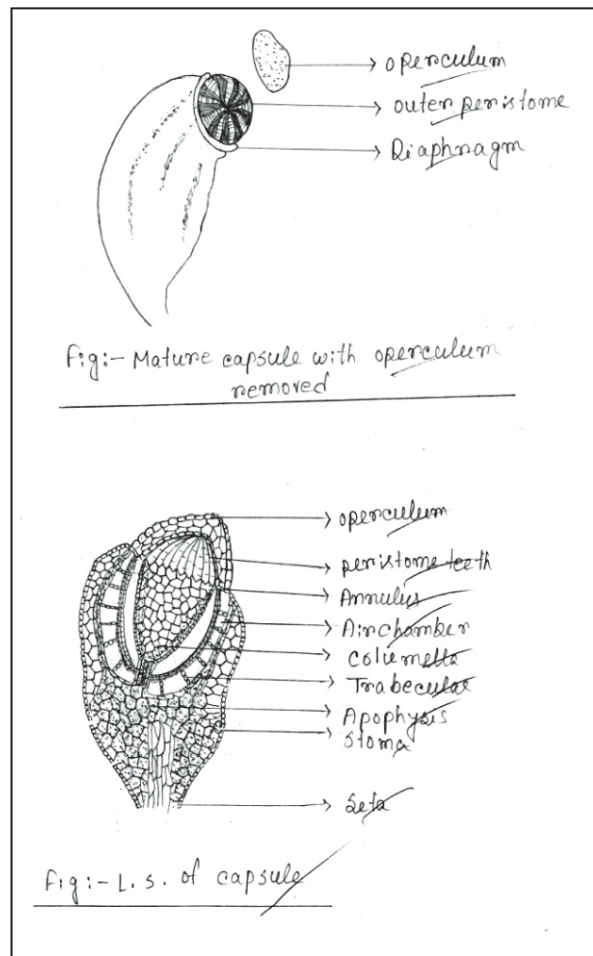
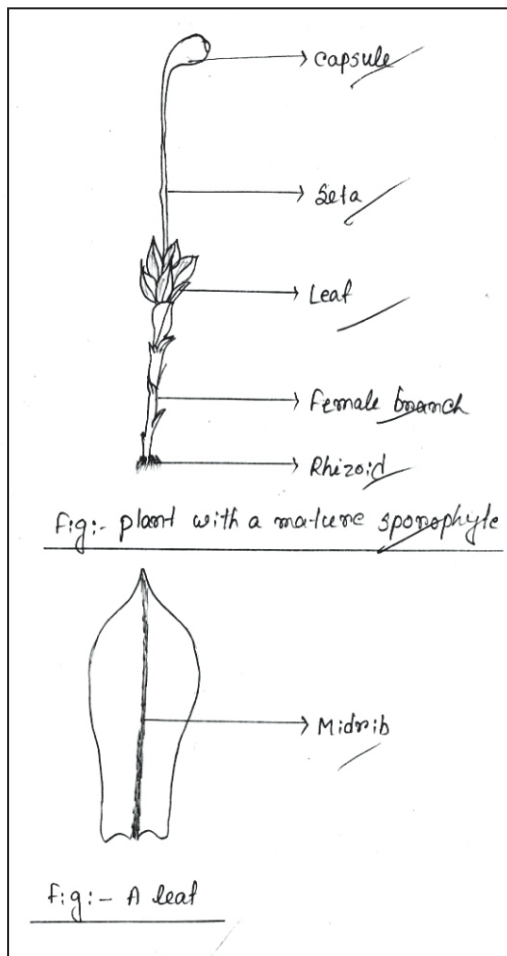
Hence the specimen under the family-Funariaceae.

Septa presence on the capsule has a lower sterile part called apophysis, trabiculae present.

Hence the specimen belong the genus-Funaria.

Plants prefer moist shady place and in close tufts on damp or alkaline soil.

Hence the supplied specimen is the specimen is *Funaria hygrometrica*



BOT – 106

Mycology and Plant Pathology, Pteridophytes and Gymnosperms Mycology

Work Out Of Specimen- A

Specimen: Supplied from the laboratory.

Somatic Structures:

1. Thallus is mycelial type.
2. Mycelium is profusely branched and septate.
3. Mycelium ramifies along the surface and sub-surface of the substratum producing sex organs at maturity.

Reproductive Structures:

1) Ascocarp-

It is apothecial type. The apothecia are sessile or subtipitate grow superficially or partially immersed in the substratum. They are soft, fleshy or waxy. A T.S through the ascocarp shows pseudo parenchymatous hypothecium and concave hymenium, dotted with ends of asci.

2) Ascus-

Asci are relatively broad, cylindrical or clavate, unitunicate and operculate. They are protruding at maturity from the hymenial layer. Each ascus bears 4-8 ascospores which are arranged in two rows.

3) Ascospore-

They are brownish to blackish at maturity and ellipsoidal to subglobose.

4) Paraphyses-

They are septate, hyaline, slender, adhering together or scarcely extended upwards.

Identification:

Thallus mycelia type, mycelium septate, presence of sac like ascus with endogenously formed ascospores.

—hence the supplied specimen belongs to the class- **Ascomycetes**.

Asci produced from ascogenous hyphae and enclosed in well developed Ascocarps.

—hence, the supplied specimen belongs to the sub-class- **Euascomycetes**

Ascocarp fleshy, coloured, asci unitunicate and operculate, ascospore elliptical.

—hence, the supplied specimen belongs to order- **Pezizaces**.

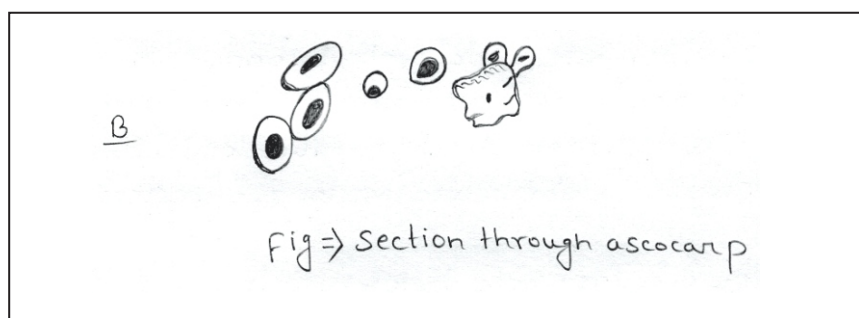
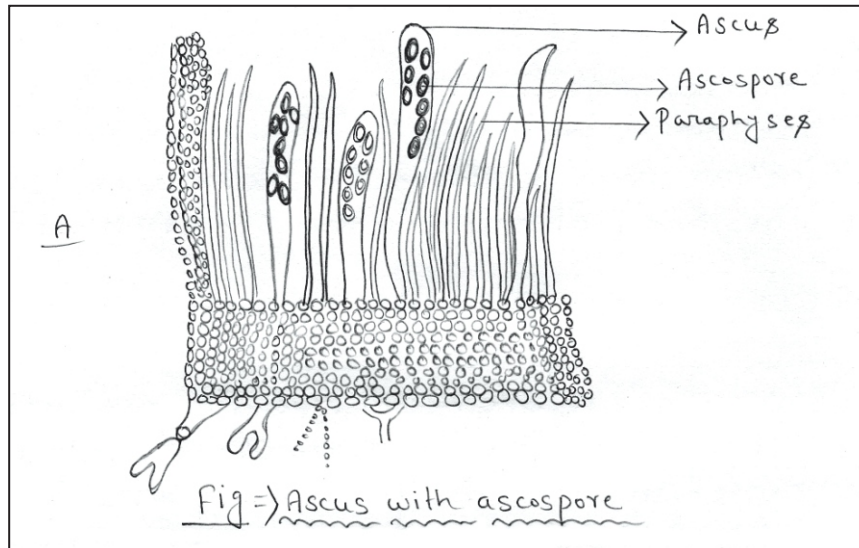
Asci relatively broad and protrude above the general level of the hymenium as they mature. ascospores usually lie in 2 rows in the ascus.

—hence, the supplied specimen belongs to family- **Ascobolaceae**.

Ascocarp cup-shaped, ascospores dark coloured, ellipsoidal to sub-globose.

—hence, the supplied specimen belongs to genus-*Ascobolus*.

~ So, the supplied specimen is *Ascobolus* sp.



Work Out Of Specimen –C

Material: supplied from the laboratory.

Somatic Structures: The thallus is mycelial type. It is composed of numerous, slender, branched and aseptate hyphae. There are two kinds of hyphae—**aerial hyphae** producing stolons and **sporangiophores** and **prostrate hyphae** producing rhizoids.

Reproductive Structures:

Sporangiophore: It is unbranched and arises in tufts from the aerial mycelium. It is terminated by a sporangium.

Sporangium: It is small, round and black. Each sporangium has a conspicuous dome-shaped columella, overarched by sporangiospores.

Zygospore: It is dark coloured, rounded and thick-walled. Its surface is warty. It is formed by the fusion of two similar gametangia.

Identification:

Thallus mycelial type, mycelium aseptate, spores formed within sporangia, oospore formed sexually.

—hence, the supplied specimen belongs to class—**Phycomycetes**.

Gametangia morphologically not distinguishable as male and female, zygospore formed by gametangial copulation, asexual reproduction by sporangiospores.

—hence, the supplied specimen belongs to subclass—**Zygomycetes**.

Asexual reproduction by sporangiospores formed within sporangium.

—hence, the supplied specimen belongs to order—**Mucorales**.

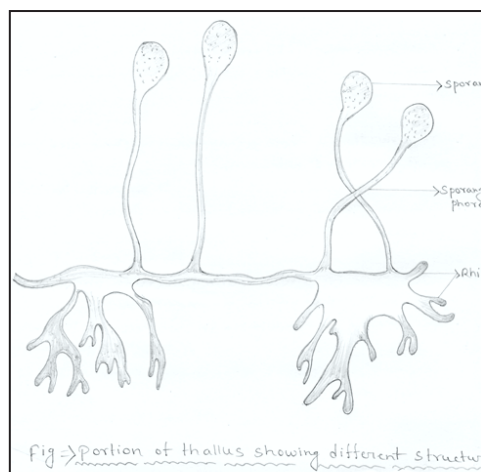
Sporangiospores liberated by breaking of sporangial wall.

—hence, the supplied specimen belongs to family—**Mucoraceae**.

Presence of conspicuous stolon forming aerial mycelium, sporangiophores formed in tufts from the aerial mycelium.

—hence, the supplied specimen belongs to genus—**Rhizopus**.

~ So, the supplied specimen is ***Rhizopus sp.***



Plant Pathology

Study Of Diseased Plant Specimen- A

Symptoms:

The symptoms appear on the stem, leaf sheaths. The symptoms are,

- 1) Leaf sheaths and also on leaves as elongated brown pustules, the uredosori containing huge number of uredospore.
- 2) The uredosori burst and expose the brown powder like uredospores.
- 3) Teleutospores develop towards the end of the season in new sorus, the teleutospores contain the teleutosorus or in the same sorus along with uredospores-the mixed sorus.
- 4) The teleutosori are black in colour and expose the mass of black spores-the teleutospores.

Causal Organism: *Puccinia graminis tritici* Erikss and Henn, is a macrocyclic, polymorphic and heterocious rust.

Under microscopic view the causal organism i.e *Puccinia graminis tritici* shows the following spores.

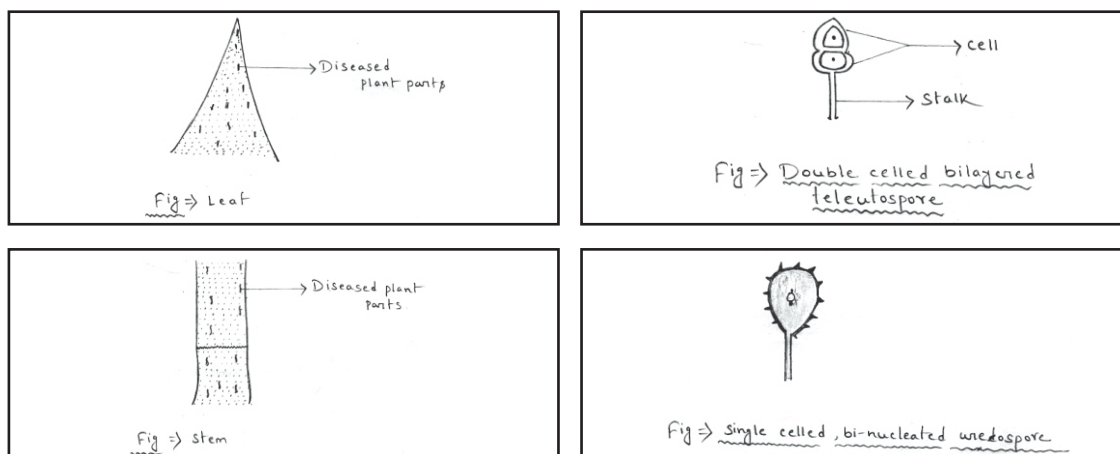
Teleutospores: The teleutosori contain teleutospores which are two celled, black, having thick and smooth wall with 2 germ pores. the germ pore of the apical cell is situated at the round apex, while in the lower cell is at one side just below the septum.

Uredospores: Uredosori contain uredospores which are one celled, dikaryotic, brown in colour, oval in shape. its having thick echinulate wall and four equatorially placed germ pores.

Identification of diseased plant:

- 1) Uredospores are one celled, dikaryotic, brown in colour and oval in shape, having thick echinulate.
- 2) Uredosori and teleutosori develop on plant and basidiospores on infected plant debris.
- 3) Teleutospores are two celled, black, having thick and smooth wall with two germ pores.
- 4) Elongated brown pustules are found on leaves and leaves sheath.

~Hence, the supplied specimen A is Black stem rust of wheat caused by *Puccinia graminis tritici*.



Study Of Diseased Plant specimen – B

Symptoms : The symptoms are visible on stem and also on leaf.

On Stem:

- 1) In early stage of disease development, the affected green stem shows purplish colouration on ring.
- 2) During the later part of rainy season or still later, the root primordial at the nodal region tends to convert into black dots, the acervuli.
- 3) The infected plant shows the death of 3rd or 4th leaves and the entire crown becomes dry in severe attack.

On Leaf:

- 1) The symptom on leaf appears as elongated bright red lesions on the midrib of leaf blade and reddish patches on leaf sheath.
- 2) The lesions gradually spread throughout the length of midrib.
- 3) The bright red lesion gradually changed with greyish centre, surrounded by dark reddish brown margin.
- 4) The margin become occupied by black dots, the acervuli of the fungus.

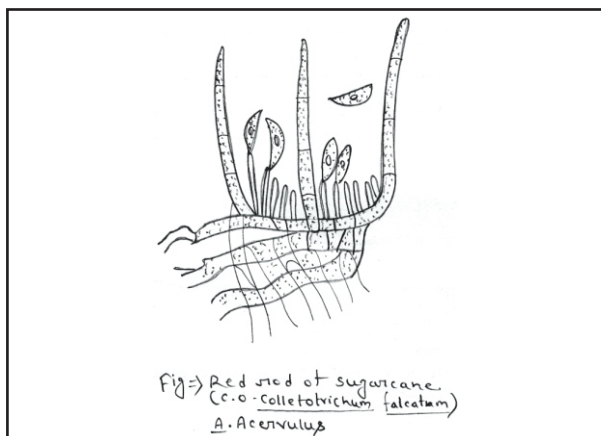
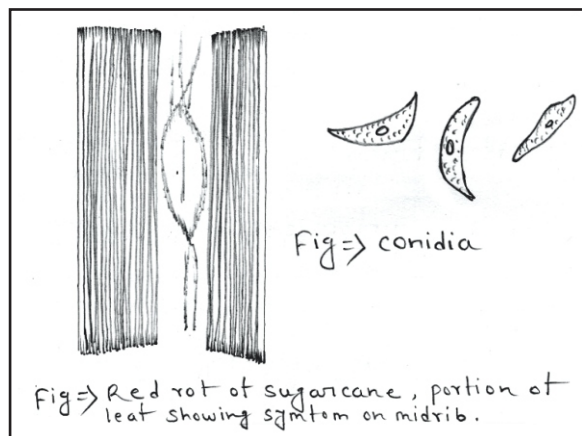
Causal Organism:

The causal Organism i.e, the pathogen is *Colletotricum falcatum*. Its perfect stage is *Glomerella tucumanensis*.

Identification:

1. Conidia develop on hyphal tip and also inside the acervuli.
2. In early stage of diseased developments, affected green stem shows purplish colouration.
3. Reddish purplish colour changed into black colour during maturity.
4. Root primordial at nodal region tends to convert into black dots, the acervuli.
5. Acervulus consists of numerous setae, setae also able to develop conidia like conidiophores.

~Hence, the diseased plant specimen B is Red rot of Sugarcane caused by the pathogen *Colletotricum falcatum* (perfect stage- *Glomerella tucumanensis*)



Pteridophytes

Material:- supplied from the laboratory.

Sporophytic Structure:-

A. Root:-

1. Morphological Features:-

- a) The primary root is ephemeral & is replaced by a large number of adventitious roots developed all over the surface of the rhizome.
- b) The roots are small & branched.
- c) Branched, black coloured adventitious roots are present.

B. Rhizome:-

1. Morphological Features:-

- a) The rhizome is usually subterranean & creeping but it is erect & semi erect.
- b) The rhizome is present, dichotomously branched & covered with scales.

2. Anatomical Features:-

- a) The outer layer is epidermis, it is composed of cutinized cells from which multicellular hairs arise.
- b) The hypodermis is made up of compactly packed sclerenchyma.
- c) The stele is amphiphloic siphonostele.

C. Leaf:-

1. Morphological Features:-

- a) The leaves are borne on upper surface of the rhizome.
- b) The pinnately compound leaves are produced in acropetalous succession on the creeping rhizome.
- c) The leaves simply uni-pinnate, while the leaves found same sp. the venation is open dichotomous type.

D. Lamina:-

2. Anatomical Features:-

- a) It is bound on both the sides by a layer of epidermis, the cells of which contain chloroplast.
- b) Paleae are present on the epidermis of some species.
- c) In between the upper & lower epidermis layers is present an undifferentiated mesophyll which is made up of spongy parenchyma.

E. Petiole:-**3. Morphological Features:-**

- a) The petiole is blackish & brownish in colour.
- b) It is soft, erect, branched shaped.

4. Anatomical Features:-

- a) It is differentiated internally into epidermis, hypodermis, cortex & stele.
- b) The epidermis is single layered & has a thick cuticle on its outer surface.
The stellar region consists of a single layered endodermis followed by single layered pericycle.

Sporophyte Structure:-(Reproductive)

- a) There is no differentiation of the sterile & fertile leaves.
- b) Many sporangia are grouped together towards the distal regions.
- c) The sori are born superficially towards the distal regions.
- d) The sporangia bearing leaves are called sporophylls.

A. Structure of the sporangium:-

- a) Each sporangium is oblong in shape & consists of a long multicellular stalk & head or capsule.
- b) The stalk is made up of three rows of cells, each row about four cells in length.
- c) The wall has a distinct vertical annulus of cells long.
- d) Few thin walled cells on one side of annulus of from stomium.

B. Spores:-

- a) The spores is the first stage of the gametophytic generation.
- b) The spores of sp. are tetrahedral.
- c) The spores of having a tri radiate ridge with concave sides.

Identification:-

- I. Plant body differentiated into rhizomatous stem.
- II. Leaf & root, leaf megaphyllous.
- III. Sporangia aggregate mostly on the leaf margin.

Hence, the supplied sp. belong to the class.....Filicopsida.

- I. Plants homosporous.
- II. Leaf 1-2 pinnate.
- III. Sporangia in sori on the ventral side of the leaf.

Hence, the supplied sp. belongs to the order.....Polypodiales.

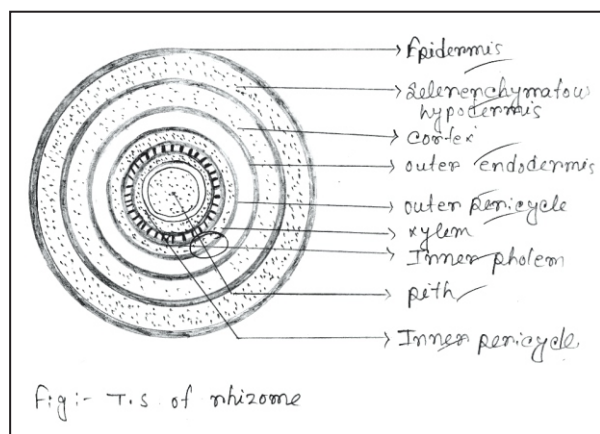
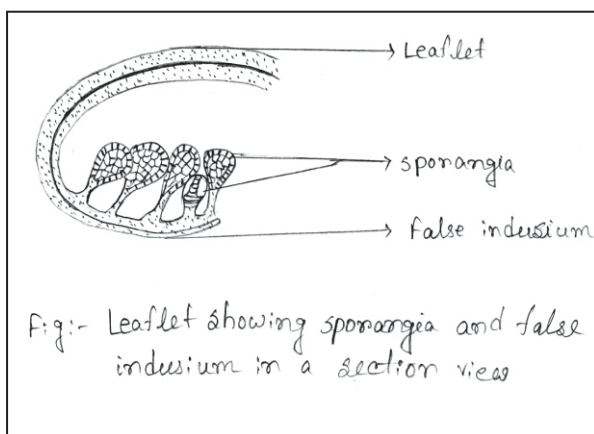
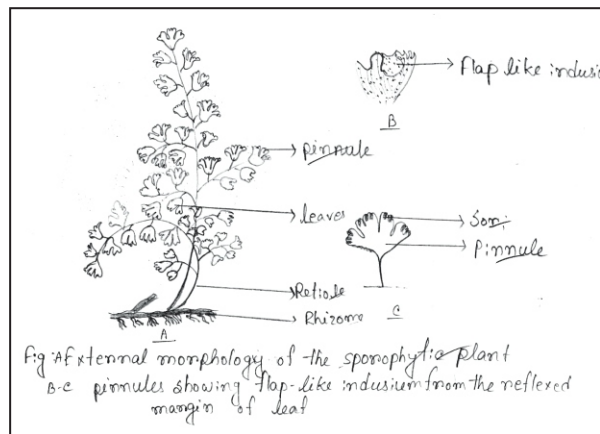
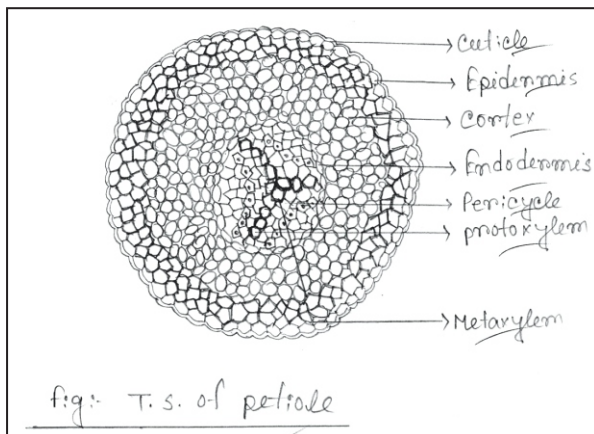
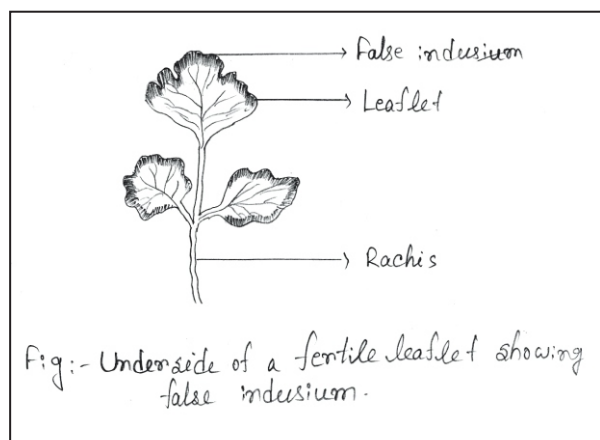
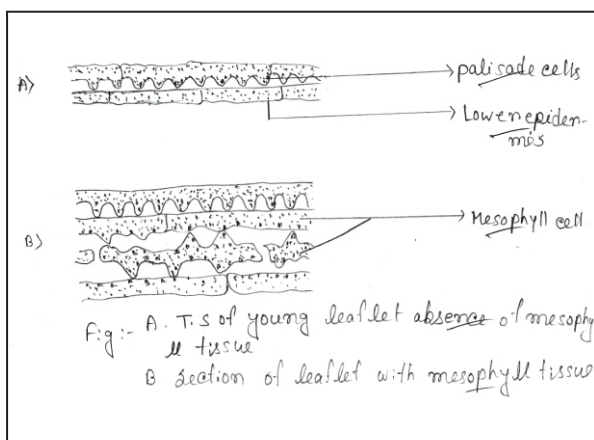
- I. Leaves unipinnate.
- II. Sori of true indusium.
- III. Sori protected by leaf margin.

Hence, the supplied sp. belongs to the family.....Pteridaceae.

- I. Leaves unipinnate.
- II. Pinnate long with marginal sori.
- III. Sori without true indusium.

Hence, the supplied sp. belongs to the genus.....*Adiantum*.

So, the supplied specimen 'C' is *Adiantum* sp.



Material:- Supplied from laboratory.

Sporophytic structure: (Vegetative)

Root:-

Morphological Features:-

- a) The primary roots are short lived(ephemeral) & are replaced by adventitious roots.
- b) Adventitious roots develop on the outer side at each nodes at the rhizome

Anatomical Features:-

- a) In T.S. the root shows tree distinct regions epidermis,cortex & stele.
- b) The epidermis is composed of single layered,parenchymatous cells,outer walls are thickly cuticularised.
- c) The cortex differentiated into 3 zones & stele is protostelic with diarch & exarch xylem.

Leaves:-

Morphological Features:-

- a) The leaves arise from the nodes & are arranged alternately in two rows on the upper side of the creeping rhizome.
- b) A mature leaf shows a long petiole on the tip of which there are four leaflets.
- c) A young leaf shows circinate vernation the leaflet margin is entire,serrate to crenulate.

Anatomical Features:-

- a) In T.s. the petiole differentiates into epidermis,cortex & stele.
- b) Epidermis composed of a single layered rectangular parenchymatous cells.Cortex is differentiated into outer & inner cortex.
- c) Stele is protostelic with diarch & exarch xylem.

Stem:-

Morphological Features:-

- a) Stem is rhizomates,dichotomously branched & creeps on just below the surface at the substratum.
- b) Stem is differentiated into nodes & internodes.

Anatomical Features:-

- a) In T.S. the stem shows epidermis,cortex & the stele from periphery to the center.
- b) Sporocarps are soft & green,but turns dark brown.
- c) Cortex differentiated into three layers & stele is amphiphloic solenostelic.
- d) Epidermis composed of compactly arranged thick walled cells.

Sporophytic Structure:-(Reproductive)**Sporocarp:-**

- It is a heterosporous fern. It produces two types of spores i.e. microspores & megaspores.
- Sporocarps are soft & green, but turn dark brown & hard at maturity.
- The sporocarp wall is hard, thick, thus resistant against mechanical injury.
- Anatomically, the wall is differentiated into three layers. The outer layer is epidermis made up of single layered cuboidal cells with sunken stomata.
- A longitudinal horizontal section of the sporocarp cuts each sorus transversely & it is seen that each sorus is elongate, covered by a delicate indusium.
- Gradate basipetal in arrangement with a row of larger sporangia (megasporangia) on top & two rows of smaller sporangia (microsporangia) on two sides. Mucilage rings are present at the two ends.

Identification:-

- Plant body differentiated into rhizomes, stem, leaf & root, leaf megaphyllous.
- Sporangia aggregate mostly on the leaf margin as sori.

Hence, supplied sp. Belongs to the class Filicopsida.

- Stem creeping, slender, often branched & rooted in the soil.
- Leaf petiolate sori borne within the sporocarp, indusiate, heterosporous.

Hence, supplied sp. Specimen belongs to the order Marsileales.

- Leaflet veins dichotomous, anastomosing at the tips.
- Sporocarp hard, stalked, sori gradate.

Hence, supplied sp. Belongs to the family Marsileaceae.

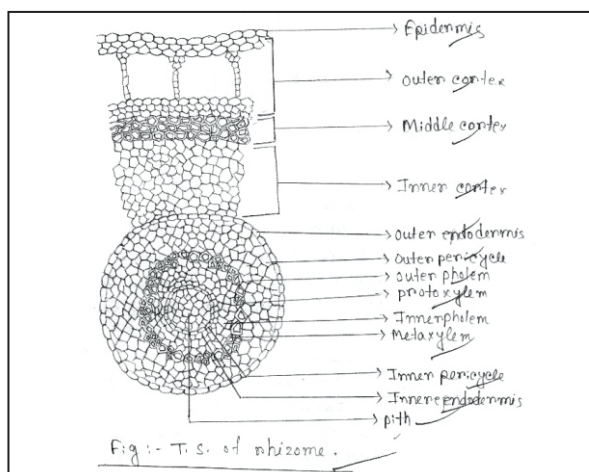
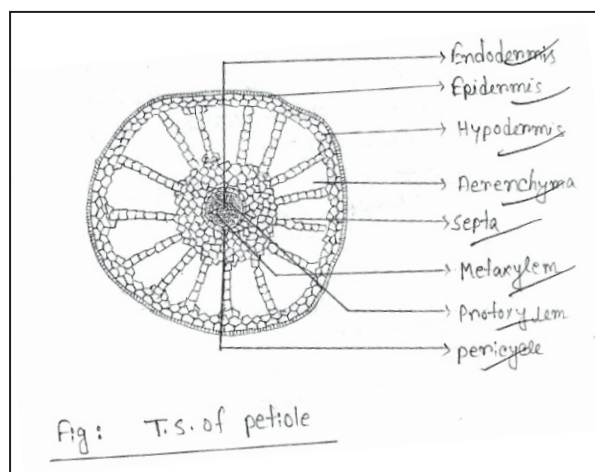
- Leaflets cut the apex of the petiole.
- Leaflets 4 in number.

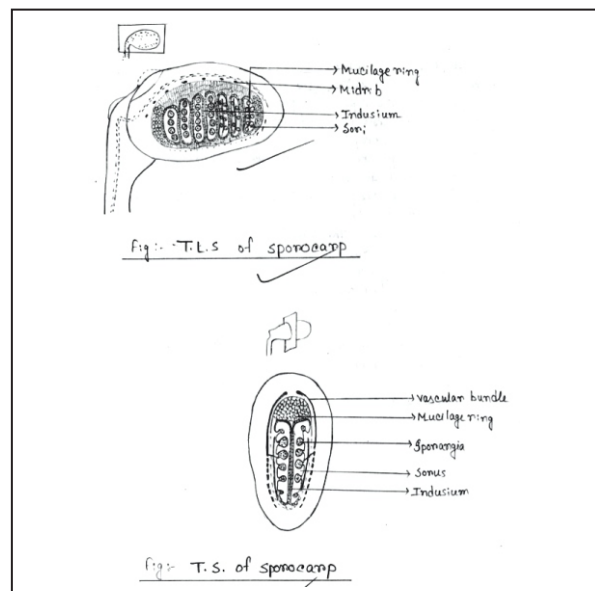
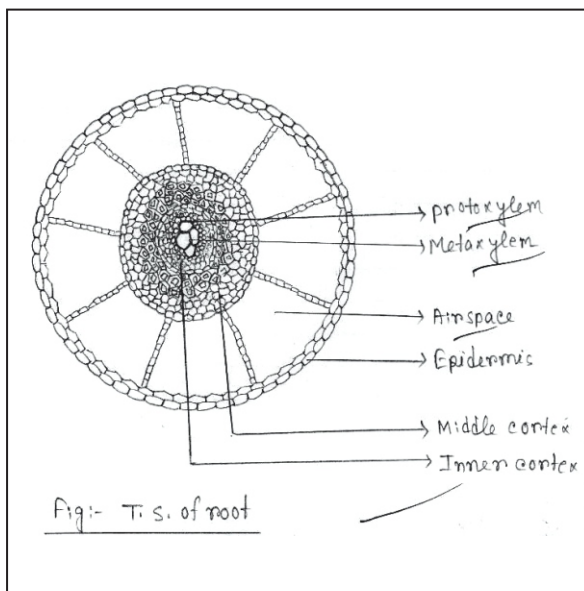
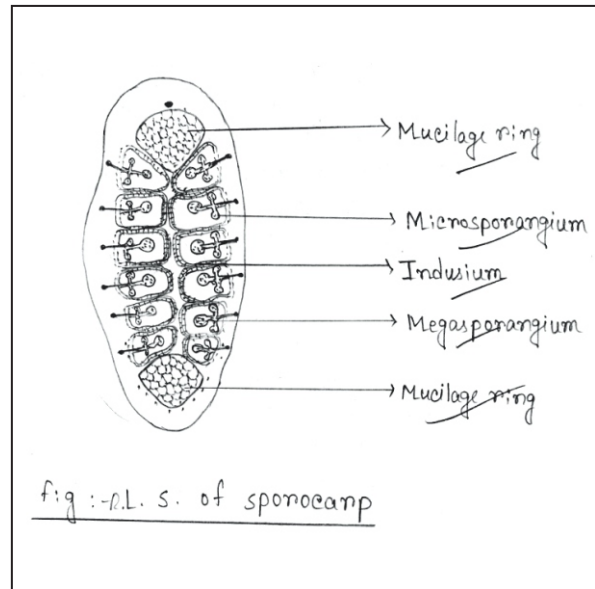
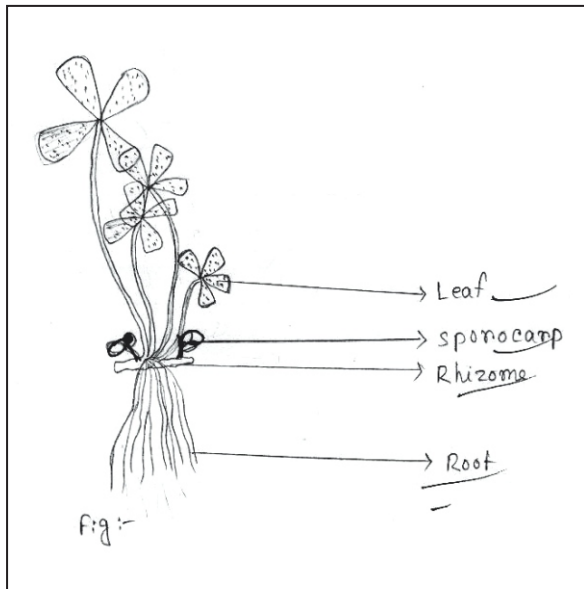
Hence, supplied sp. belongs to the genus Marsilea.

- The leaves are long, petiole & pinnate compound & each having 4 leaflets.
- The edge of the leaflets are alternatively with ridge & furrow.

Hence, supplied sp. belongs to the species *quadrifolia*.

So, the specimen 'A' is *Marsilea quadrifolia*.





Material:- supplied from the laboratory.

Sporophytic Structure:-

A. Root:-

1. ***Morphological Features:-***

- a) The primary root is ephemeral, replaced by a large number of adventitious roots.
- b) The roots are small & branched.

2. ***Anatomical Features:-***

- a) In T.S. the roots shows an outer piliferous layer, cortex & central stele.
- b) Cortex is differentiated in a parenchymatous outer cortex & sclerenchymatous inner cortex.
- c) The stele is protostelic with diarch & exarch xylem.

B. Rhizome:-

1. ***Morphological Features:-***

- a) The rhizome may be creeping or erect which may or may not show branching.
- b) The rhizome is differentiated into nodes & internodes.

2. ***Anatomical Features:-***

- a) The rhizome shows an outer single layered epidermis a few layered thick hypodermis, cortex.
- b) The stele is solenostelic or dictyostelic.
- c) Each stele is bound by its own endodermis.

C. Leaf:-

1. ***Morphological Features:-***

- a) The leaves are borne on upper surface of the rhizome.
- b) Young leaves are spirally coiled & show circinate vernation. Leaf pinnately compound, imparipinnate.
- c) All leaves are fertile, bearing sori along the ventral margin of pinnae, except the apices of the segments.

2. ***Anatomical Features:-***

- a) The rachis is transverse by a single C/U/V shaped leaf trace.
- b) The lamina is bifacial, hypostomatic.

Reproductive Structure:-

A. Spore producing organ:-

- a) It produces by means of spores.
- b) It is a homosporous fern.
- c) The sorus of pairs is called coenosorus. Coenosorus sori are marginal, borne continuously on a vascular commissure connected with vein ends.

B. Mature Sporangium:-

- A mature sporangium has a long stalk that terminates in a capsule.
- The jacket of the capsule is single layered, but with three different types of cells.
- The spores are dispersed through air to a moderate distance.

Identification:-

- Plant body differentiated into rhizomatous stem, leaf & root.
- Leaf megaphyllous.
- Sporangia aggregate mostly on the leaf margin as sori.

Hence, the supplied sp. belongs to the class.....Filicopsida.

- Plants homosporous, leaf 1-2 pinnate.
- Sporangia in sori on the ventral side of the leaf.
- Sporangia with indusium, sporangial development mixed.

Hence, the supplied sp. belongs to the order.....Polypodiales.

- Stem surface covered with scales, sori devoid of true indusium.
- Sori protected by leaf margin.
- Sporangia stalked, annulus vertical, homosporous spore trilete.

Hence, the supplied sp. belongs to the family...Pteridaceae.

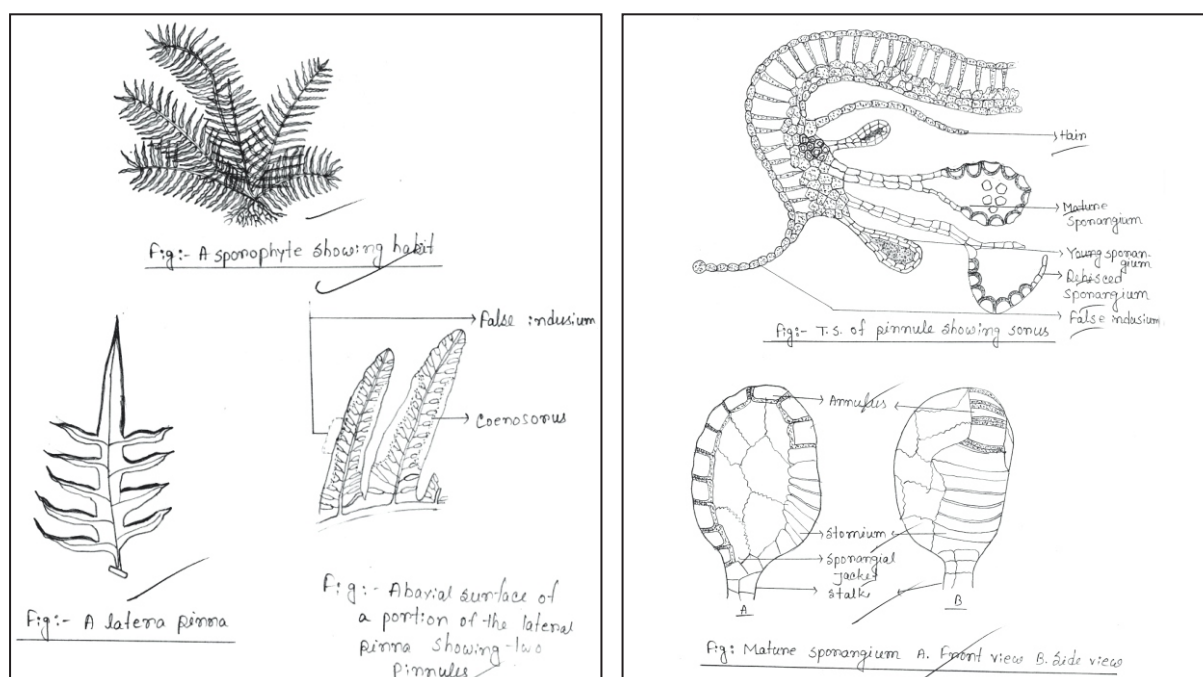
- Leaves unipinnate, pinnae long with marginal sori.
- Sori without true indusium, annulus oblique.
- Spores tetrahedral, trilete surface reticulate.

Hence, the supplied sp. belongs to the genus.....Pteris.

- Indusium is single layer.

Hence, the supplied sp. belongs to the species.....vittata.

So, the supplied specimen 'B' is *Pteris vittata*.



Gymnosperms

Material: supplied from the laboratory.

Vegetative structure: The plants are mostly evergreen, branched trees. Branchlets are slender, tough, divided near the apex into fine sprat. Smaller branchlets deciduous with the leaves reasons. Leaves are small, scale like, overlapping in 4 rows of 2 opposite sets, the upper & lower ranks flattened or groved the side ranks rounded or keeled. The foliage is usually glandular & aromatic. Thin bark is present, fissured only on old trees, outer bark normally shows scaling in patches of irregular shape but inner bark is fibrous.

Reproductive Structure: Strobili or cones are globose or sub globose. they are formed on different branchlets of the same tree.

Male Strobilis: It is some what reddish, cylindrical or globose. It is normally situated on branchlets near the base of the shoot & composed 3-6 pairs of microsporangia. Pollen grains are not winged.

Female Strobilis: It arises from short terminal branchlets. It is small green to purplish, composed of a few opposite pairs of leaf like scales. Mature cones are solitary & made up of 3-10 pairs of ovuliferous scales. Bract scales are fused with the ovuliferous scales. Cones are dry & ovoid to oblong. Seeds are small, thin & wingless, but attached to the edge of the ovuliferous scale. Cone scales posses thickened or ridged apex & a very fine spine.

Identification: Plant branched trees, leaves simple, microphyllous, well developed.

Hence, The sp. Belongs to the class ... Coniferapsida.

Plant evergreen, stem with secondary wood & resin canals, male & female strobili compact & cone like.

Hence, The sp. Belongs to the order ... Coniferales.

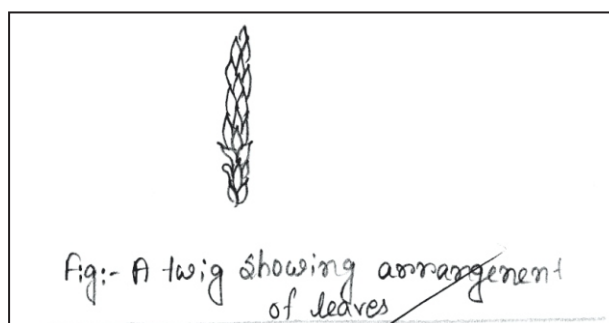
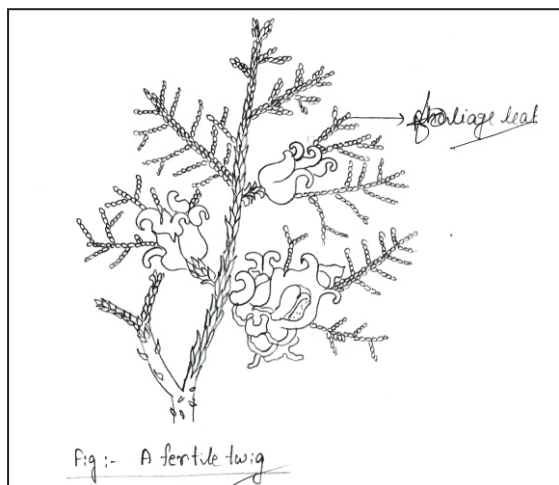
Leaves persistent, small like, usually in rows or whorled, bract scale fuses with the ovuliferous scale, pollen grains not winged.

Hence, the sp. Belongs to the family ... Cupressaceae.

Leaves scale like, usually arranged in 4 ranks of 2 opposite sets, foliage glandular & aromatic female cone ovoid too belong, cone scale with thickened or ridged apex a fine spine seed small thin attached to the edge of the scale.

Hence, The sp. Belongs to the genus ... *Thuja*.

So, The supplied specimen is *Thuja* sp.



Gymnosperms

Specimen:-Supplied from the laboratory.

Sporophytic Structure:-

- 1) Plant looks like a palm tree with a columnar aerial trunk & a crown of pinnately compound leaves.
- 2) Generally, the stem is unbranched, older trees exhibit branching.
- 3) The stem is covered by an amount of alternating bands of large & small rhomboidal leaf bases.
- 4) These leaf bases are persistent of which the large bases belong to foliage leaves, while the small ones belong either to scale leaves or to sporophylls.

Leaf (structure):-

External morphology:

- 1) Leaf dimorphism in possessing green foliage leaves & scale leaves. Scale leaves are small, dry, non green & triangular in shape, covered withramenta.
- 2) They have small persistent leaf bases, their only function is to provide protection to apical meristem & other aerial parts.
- 3) The foliage leaves are large, unipinnately, compound. A single leaf bears 75-100 pairs of leaflets which are arranged on either side of rachis in opposite or alternate manner.
- 4) Leaflets are sessile, elongated having revolute or flat margins.
- 5) Each leaflet is provided with a single unbranched mid vein without having any lateral veins.
- 6) The young rachis as well as leaflets are circinate coiled like those of ferns.

Internal structure or anatomy:-

- 1) The T.S. of rachis shows circular outline. The outer most layer is the epidermis which consists of cuticularised thick walled cells interrupted by sunken stomata.
- 2) The epidermis is followed by hypodermis, which is composed of a variable mixture of chlorenchyma & sclerenchyma followed by a parenchymatous spongy parenchyma.
- 3) The lower part of the mesophyll consists of spongy parenchymatous cells with intercellular spaces.
- 4) The vascular bundle is diploxylic in nature, made up of a broad triangular centripetal exarch metaxylem & two small patches of centrifugal endarch primary xylem. The centripetal xylem is separated from centrifugal xylem by a few parenchymatous cells.
- 5) Some tracheidal cells situated on either side of the centripetal cells situated on either side of the centripetal metaxylem are termed transfusion tissue.
- 6) The phloem is situated on abaxial side below xylem which is made up of sieve cells & parenchyma.

Identifying Character:-

- 1) Green foliage leaves & scale leaves are present.
- 2) Scale leaves are small, dry, non green, triangular in shape, covered by stomata.
- 3) Foliage leaves are large, unipinnately compound. A single leaf bears 75-100 pairs of leaflets.

- 4) Hypodermis is mixture of chlorenchyma & sclerenchyma followed by the parenchymatous ground tissue with many mucilage canals.
- 5) Two endarch bundles enters the leaf base & then they split up into several bundles. There are then dispersed in the ground tissue & arranged in an inverted green letter "omega" like are.
- 6) The vascular bundles are conjoint, collateral & open bearing both the centrifugal & centripetal xylem, so they are diploxylic or pseudomesarch in nature. Phloem lies on the side of the protoxylem.
- 7) T.S. of leaflet shows a thick walled, highly cuticularised epidermis. Upper epidermis is continuous, while lower epidermis is interrupted by stomata.
- 8) Stomata are haplocheilic, situated in pits with overarching rims. The two guard cells are bordered by 8-10 subsidiary cells arranged in a ring.
- 9) The hypodermal cells are thick walled, highly lignified. The mesophylls are differentiated into palisade & spongy parenchyma.
- 10) Transfusion tissue present.

Hence, the supplied specimen 'B' is the leaf of Cycas sp.

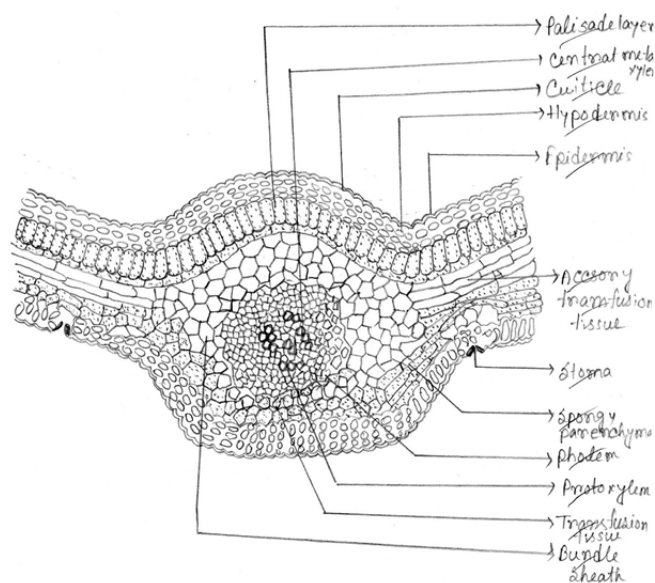
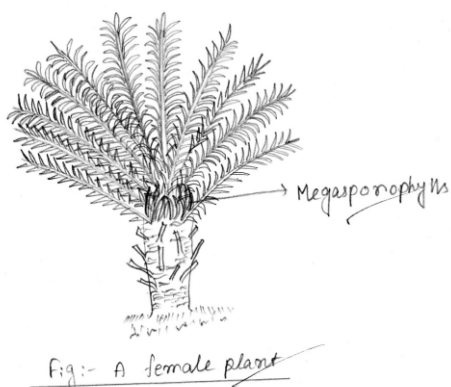


Fig:- T.S of leaflet of cycas