LABORATORY MANUAL

for

5-Year Integrated (*B.Sc. - M. Sc.)* Botany

VIRUSES, BACTERIA, ALGAE, FUNGI and BRYOPHYTES

DEPARTMENT OF BOTANY

UNIVERSITY OF KASHMIR, NORTH CAMPUS, DELINA, BARAMULLA

Contents:

VIRUSES, BACTERIA, ALGAE, FUNGI AND BRYOPHYTES

Purpose: To help students understand the diversity, structure, reproduction, and ecological roles of viruses, bacteria algae, fungi and bryophytes.

To acquaint them with the basic laboratory tools including microscopes etc.

To help them understand the procedure of staining and its purpose

To acquaint them with the basic format of recording lab exercises

Objectives: To identify species belonging to the above-mentioned groups, study their morphology, cellular structure, anatomy and observe their vegetative and reproductive structures.

List of Equipment and Material

Dissection Microscope

Compound microscope

Petri dishes

Dissection box

Staining solutions (safranin, cotton blue),

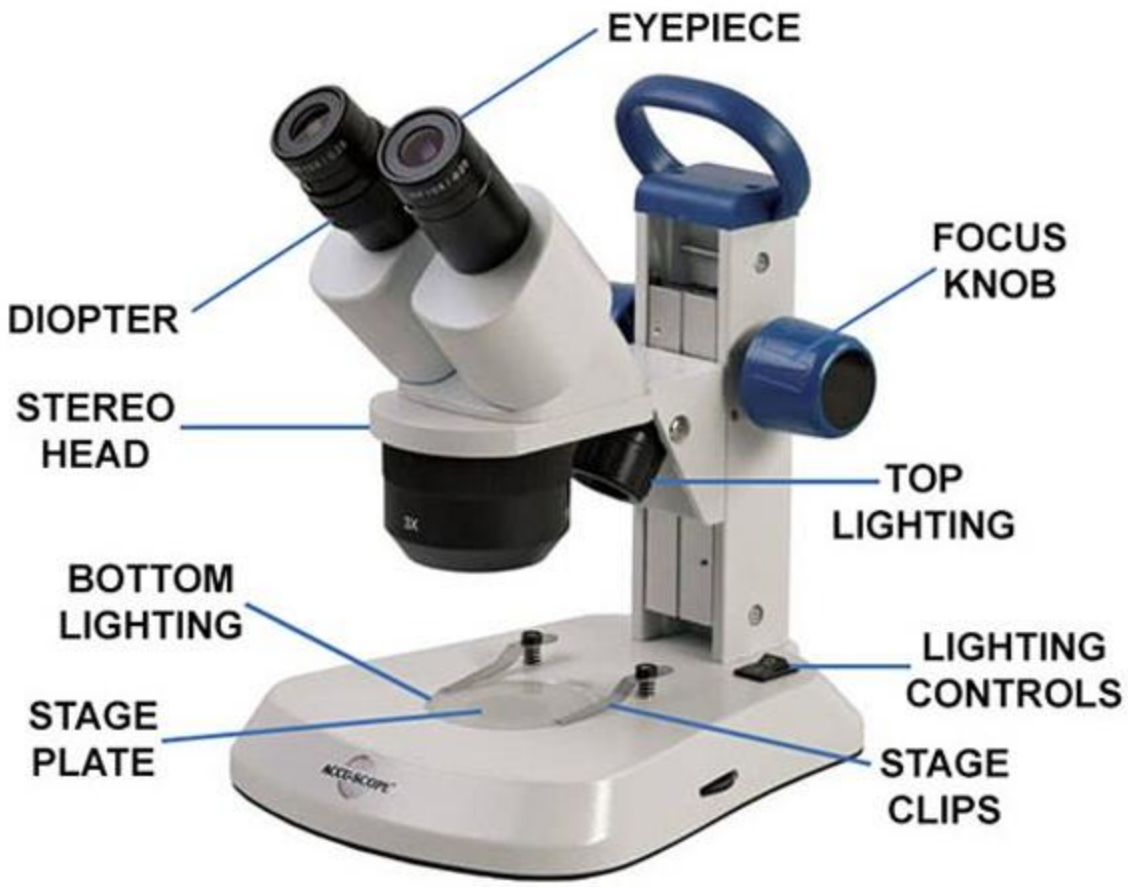
Slides Cover slips, etc.

Anatomy of microscope

A. Dissection Microscope

A dissecting microscope is a useful tool for viewing small features or fine details. Usually, the range of magnification is around 10x to 20x. This type of microscope uses incident light to see the specimen.

To view a specimen under the dissecting microscope, position the specimen, then look through the ocular lenses. You will have two knobs on the side of the microscope. One of these is a coarse focus/adjustment another one is fine focus. Coarse focus is used to determine how closely you'd like to view features of your specimen. You'll notice that it gets quite blurry very quickly. Once you have the magnification you want, use the fine focus to resolve the image (de-blur it).



Picture of dissection microscope

B. Compound Microscope

A compound microscope is an optical instrument used for magnifying small objects that are not visible to the naked eye. It uses multiple lenses to achieve high magnification and is commonly used in, biology research laboratories. A compound microscope has following parts:

1. **Eyepiece (Ocular Lens)** – The lens at the top that you look through, is known as eyepiece. It usually has a magnification of 10x (or more).

2. **Objective Lenses** – these are a set of lenses attached to the nosepiece. They may have 4x, 10x, 40x, and 100x magnification to further magnify the specimen.
3. **Revolving Nosepiece (Turret)** – Holds multiple objective lenses and allows to switch between them.
4. **Stage** – The flat platform where the slide is placed is known as stage.
5. **Stage Clips** – these hold the slide in place.
6. **Coarse and Fine Focus/adjustment Knobs** – these are used to adjust focus by moving the stage up or down.
7. **Light Source** –natural light, LED or halogen bulb are used as a source of illumination.
8. **Condenser Lens** – Focuses light onto the specimen.
9. **Diaphragm (Iris Diaphragm)** – Controls the amount of light passing through the specimen.
10. **Base and Arm** – These are the structural parts of the microscope.

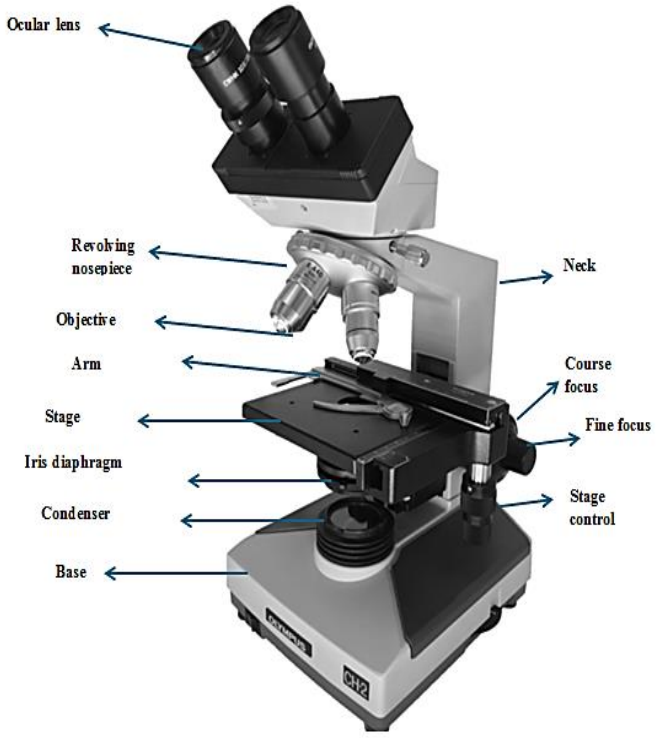
Working Principle:

A compound microscope uses two sets of lenses:

- The **objective lens** forms an enlarged real image of the specimen.
- The **eyepiece lens** magnifies this image further, making it visible to the observer.

Magnification Calculation:

Total Magnification = Eyepiece Magnification × Objective Lens Magnification
For example, if the eyepiece is 10x and the objective lens is 40x, the total magnification would be **10 × 40 = 400x**.



How to use a compound microscope

Preparation of wet mount illustrates how a compound microscope can be used

Place a drop of water onto a glass slide. Obtain a small plant material, for example a few stands of a filamentous algae, place it on the slide and tease it apart. Hold a glass coverslip at an angle, touching the base of the coverslip to the water droplet on your slide. Slowly lower the coverslip over the drop of water until it is flat against the slide.

II. Observe your Specimen

Place the slide you have prepared onto the microscope stage, holding it in place with the arm, and use the stage controls to place your specimen directly above the light source. Rotate the revolving nosepiece to the scanning objective (5x), adjust the light intensity (it should not hurt your eyes), and look through the ocular lenses (10x). For better visualization the specimens are stained using various staining solutions.

Purpose of Staining:

Staining is essential because visibility dramatically. The plant materials, in which there is no differentiation of tissues such as members of algae, fungi and bryophytes are stained by a single staining process. But pteridophytes, gymnosperms and angiosperms are stained by the double staining method due to the presence of differentiation of tissues. Staining improves tissue differentiation and also structures can be easily differentiated. Members of different groups can be stained in general as follows:

Algae:

Algal members are generally stained with a few drops of safranin for a few minutes (time is variable for different members) and mounting is done in 10% glycerine. Aniline blue, fast-green, acetocarmine and haematoxylin are some of the other stains used for algal preparations.

Fungi:

Cotton blue is considered to be very situatable for fungal. After staining with cotton blue, the material is mounted in lactophenol. Cotton blue serves as a staining as well as mounting medium if prepared in lactophenol. Aniline blue or haematoxylin also give satisfactory staining results in certain cases.

Bryophytes:

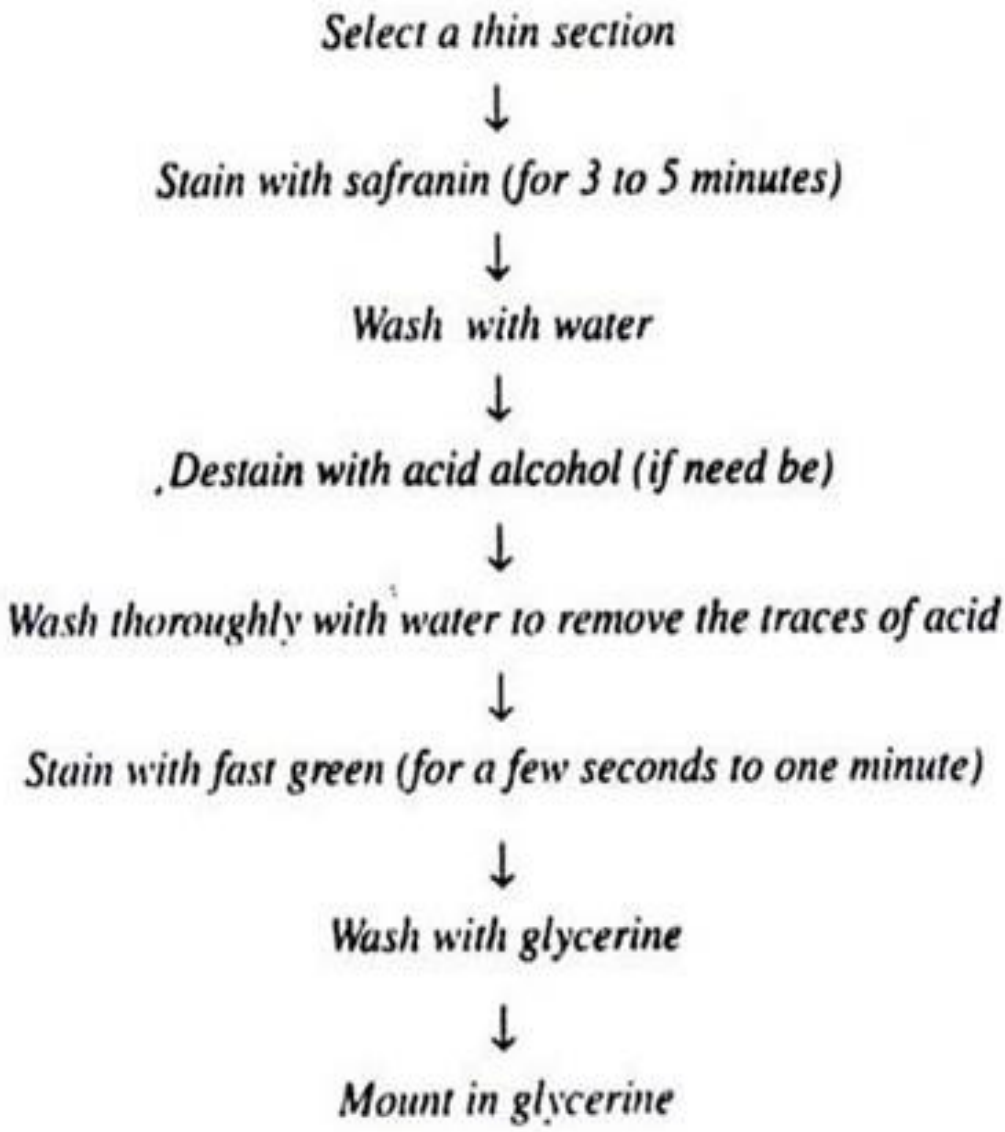
Thin sections of bryophytes are usually stained with safranin and mounted in 10% glycerine. Other stains that can be used for members of bryophyta, are Delafield's haematoxylin and fast-green.

Pteridophytes and Gymnosperms:

Representatives of these groups are stained by a double staining method.Some of the commonly used methods of double staining are as under

1. Safranin-Fast Green Method:

Keep the material to be stained in safranin for three to five minutes and then wash it with water. See under the microscope that only thick-walled cells are stained. Excess of stain is destained by acid alcohol. Again, wash the material very thoroughly with water so that even the traces of acid are removed. Now stain the material with few drops of fast green for few seconds. Time for keeping the material in fast green varies from few seconds to one minute for different materials. Wash the material with glycerine and mount in a drop of glycerine. With this method, all thick-walled cells get red stain and all thin-walled cells the green stain.The follow-chart makes a ready reference.



2. Safranin-Aniline Blue Method:

Follow exactly the same procedure as mentioned above except that in place of fast green use aniline blue.

3. Haematoxylin-Safranin Method:

Keep the sections in Delafield haematoxylin for four to five minutes and remove the excess of stain with water. Wash with ammonia. Wash the material very thoroughly with water. Now stain with safranin for few minutes. Wash the sections with glycerine for removing excess of stain and mount in glycerine. Here is the easy-to-follow flow chart.

Select a thin section

↓

Stain with haematoxylin (4-5 minutes)

↓

Wash with water

↓

Wash with ammonia water till stain turns blue

↓

Wash with tap water

↓

Stain with safranin (2-3 minutes)

↓

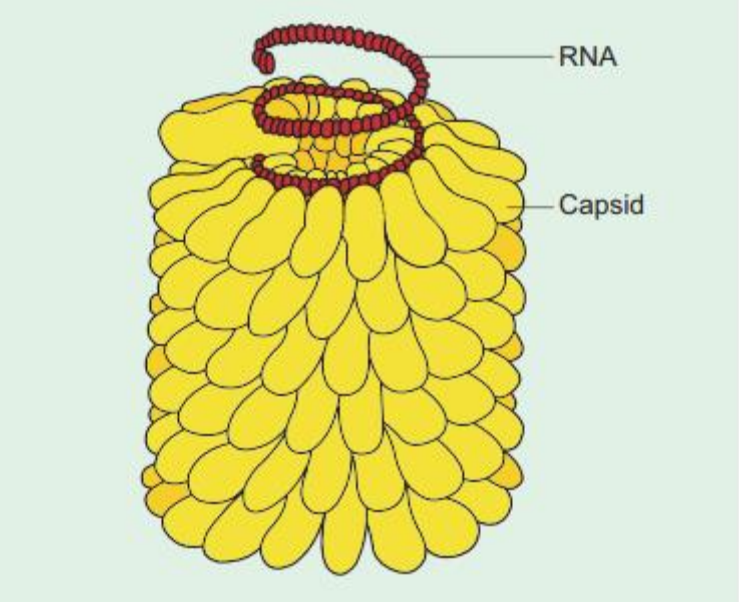
Wash in glycerine

↓

Mount in glycerine.

Tobacco Mosaic Virus (TMV)

1. Tobacco Mosaic Virus (TMV) is a positive-sense single-stranded RNA virus species in the genus Tobamo virus that infects a wide range of plants.
2. The infection causes characteristic patterns, such as mosaic-like mottling and discoloration on the leaves. 3. This virus has a rod-like appearance.
3. Its capsid is made from 2130 molecules of coat protein and one molecule of genomic single strand RNA, 6400 bases long.
4. The coat protein self-assembles into the rod-like helical structure (16.3 proteins per helix turn) around the RNA, which forms a hairpin loop structure.
5. The TMV genome consists of a 6.3–6.5 kbp single-stranded (ss) RNA.
6. The protein monomer consists of 158 amino acids which are assembled into four main alpha helices, which are joined by a prominent loop proximal to the axis of the virion.
7. It is the first pathogen identified as a virus. It was crystallized by W.M.Stanley.
8. TMV is spread mechanically by abrasion with infected sap.
9. Symptoms of virus infection include colour changes, dwarfing, and tissue distortion.
10. The appearance of streaks of colour in certain tulips is caused by virus.



GRAM STAINING PROCEDURE

PREPARATION:

Smear Preparation:

A drop of curd is smeared onto a clean glass slide and allowed to air dry.

Primary Stain (Crystal Violet): crystal violet, a basic dye, is applied to the slide for about 1 minute, staining all bacteria purple.

Rinsing: The slide is rinsed with water to remove excess crystal violet.

Mordant (Gram's Iodine): Gram's iodine is applied for about 1 minute, forming a complex with the crystal violet, which is then trapped within the cell wall.

Rinsing: The slide is again rinsed with water to remove excess iodine.

Decolorization: A decolorizing agent, typically alcohol or a mixture of alcohol and acetone, is applied for a brief period (3-5 seconds). Gram-positive bacteria retain the crystal violet-iodine complex due to their thick peptidoglycan layer. While as, Gram-negative bacteria lose the crystal violet-iodine complex due to their thin peptidoglycan layer and outer membrane, becoming colorless.

Rinse: The slide is again rinsed with water to remove the decolorizing agent.

Counterstain (Safranin): Safranin, a red dye, is applied for about 30 seconds, staining the colorless Gram-negative bacteria pink or red.

Rinse: The slide is rinsed with water to remove excess safranin.

3. Observation:

Drying: The slide is allowed to air dry or gently blotted with bibulous paper.

The stained slide is examined under a microscope using oil immersion (1000x magnification).

Results: Gram-positive bacteria appear purple while as Gram-negative bacteria appear pink or red.

Some laboratory exercises

Format of the exercise

1. Aim of the exercise
2. Materials required
3. Procedure
4. Observations
5. Conclusion

Section I: Algae

General characters of Algae

An introduction to algae

Algae are a diverse group of photosynthetic organisms found in various aquatic environments. They can be classified as simple plants or plant-like organisms. Algae are primarily aquatic organisms, found in both freshwater and marine environments. However, some can also grow in moist terrestrial conditions or on surfaces like rocks and trees.

Algae are autotrophic. They thrive in environments with sufficient light and water. Algae contain different types of pigments to absorb light. The most common is chlorophyll (mainly chlorophyll-a), but they can also contain other pigments such as:

- **Carotenoids** (yellow, orange)
- **Phycobilins** (in red algae)
- **Fucoxanthin** (in brown algae)

Algae can be microscopic (e.g., phytoplankton like *Diatoms* and *Green Algae*) or macroscopic (e.g., large seaweeds like kelp).

Their forms vary from single-celled to multicellular colonies, and they can also take forms like filaments or sheets.

Algae do not have complex vascular tissues (like those in higher plants). They lack structures like stems, roots, and leaves.

Their bodies are generally simple and can range from microscopic (e.g., phytoplankton) to large macroscopic forms (e.g., seaweeds).

They may be unicellular (e.g., *Chlorella*) or multicellular (e.g., *Laminaria*).

Algae reproduce both sexually and asexually.

Asexual reproduction usually occurs through binary fission, fragmentation, or spore formation.

Sexual reproduction involves the fusion of gametes and can vary greatly among species (e.g., isogamy, anisogamy, or oogamy).

Algae play a vital role in ecosystems, particularly aquatic food chains, as primary producers.

They are also responsible for producing a significant amount of the Earth's oxygen through photosynthesis.

Algae are primary producers, meaning they form the base of the food chain in aquatic ecosystems.

Some algae are also symbiotic, living in association with other organisms (e.g., lichens or corals).

Some laboratory exercises

Exercise: 1

Aim: To study prokaryotic algae *Anabaena* sp.

Materials: *Anabaena* culture or water sample containing *Anabaena*, Compound microscope, Glass slides and cover slips, Droppers, Staining reagents (e.g., methylene blue or safranin) Distilled water and Filter paper etc.

Procedure:

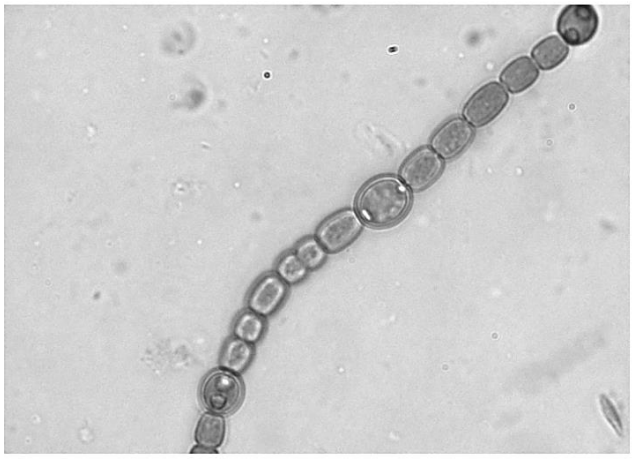
Take a clean glass slide and place a drop of *Anabaena* culture or water sample on it using a dropper. Add a drop of staining reagent (e.g., methylene blue) to enhance visibility and place a cover slip carefully to avoid air bubbles.

Adjust your slide on a microscope. Start with a low-power objective (10x) to locate *Anabaena* filaments.

Switch to high-power objective (40x) and then to oil immersion (100x) for detailed observation.

Observations:

It consists of a long, unbranched chain of cells arranged in a bead-like pattern known as trichome. The vegetative cells of trichome are Regular, greenish in color and perform responsible for photosynthesis. The trichome also consist of specialized cells with thick walls called heterocysts that help in nitrogen fixation by converting atmospheric nitrogen (N_2) into ammonia (NH_3). There are some thick -walled, dormant cells known as akinetes that help the organism survive in harsh conditions like drought or nutrient deficiency. A gelatinous outer layer protects the filament and helps in buoyancy.



Exercise 2: *Chlamydomonas* sp.

Aim: to study morphology of Chlamydomonas sp. through a temporary mount.

Requirement: microscope, dropper, petridish, glass slide, cover glass, culture/ material of the species

Procedure:

- Place a small drop of *Chlamydomonas* culture or water samples containing *Chlamydomonas* sp. on a clean microscope slide.
- Gently place a cover slip over the drop to avoid trapping air bubbles.
- Stain and add a drop of glycerine

Observe the Morphology first under lowest magnification and then increase magnification to view individual cells clearly.

Observations

Shape: *Chlamydomonas* is typically spherical or oval with a characteristic shape.

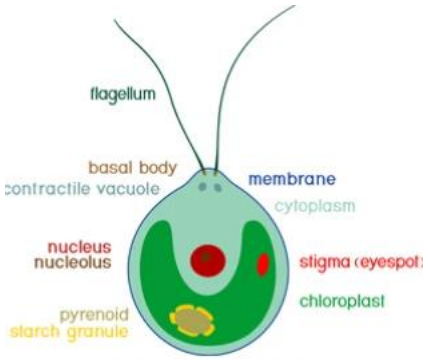
Flagella: Two long, whip-like flagella that help the cell move.

Chloroplast: A single, cup-shaped chloroplast within the cell that contains chlorophyll for photosynthesis.

Eyespot: A red-orange spot near the base of the flagella that is involved in phototaxis (movement towards or away from light).

Nucleus: Visible as a small, dark structure within the cell.

Record your Observations: Draw a detailed diagram of the cell, labeling the flagella, chloroplast, eyespot, and nucleus.



***Chlamydomonas* sp.**

Section II: Fungi

General characters of Fungi

Fungi are a diverse group of eukaryotic organisms that include **molds, yeasts, and mushrooms**. They belong to the kingdom **Fungi** and are distinct from plants, animals, and bacteria.

- They have a true nucleus with a nuclear membrane hence are eukaryotic.
- **Cell Wall** is composed of **chitin** (not cellulose like plants).

Mode of Nutrition: fungi are **Heterotrophic** and obtain nutrients by absorption (saprophytic, parasitic, or mutualistic).

- **Saprophytic** fungi decompose dead organic matter (e.g., *Rhizopus, Penicillium*).
- **Parasitic** fungi live on or inside a host and cause diseases (e.g., *Puccinia, Candida*).
- **Mutualistic** fungi form symbiotic relationships, such as **lichen** (fungus with algae) and **mycorrhizae** (fungus with plant roots).

Thallus structure: fungal thalli usually contains thread like filaments known as hyphae. A network of hyphae is called mycelium. Fungi may be unicellular or multicellular.

- **Unicellular** – Yeasts (e.g., Saccharomyces) are single-celled fungi.
- **Multicellular** – Molds and mushrooms have a filamentous structure.

Reproduction in Fungi: fungi reproduce by asexual as well as sexual methods

- **Asexual Reproduction** – By spores (conidia, sporangiospores), budding (yeasts), or fragmentation.
- **Sexual Reproduction** – By the fusion of gametes or specialized structures (e.g., zygospores, ascospores, basidiospores).

Importance of Fungi:

Beneficial Roles:

- Decomposers in ecosystems.
- Used in fermentation (bread, beer, wine).
- Produce antibiotics (e.g., Penicillin).

Harmful Effects:

- Cause diseases (e.g., Athlete's foot, Ringworm).
- Spoil food and crops (e.g., Rust and smut fungi).

Some laboratory exercises

Exercise: 1

Aim: To study morphology of *Rhizopus* spp.

Requirement: microscope, dropper, Petri dish, glass slide, cover glass, culture/ material of the species - *Rhizopus* culture or infected bread

Procedure: Using forceps or a needle, carefully take a small portion of the fungal growth.

Place it on a clean glass slide.

Add a drop of lactophenol cotton blue stain to enhance visibility.

Gently place a cover slip over the sample, avoiding air bubbles.

Observe the slide under a low-power objective (10x) to locate structures.

Switch to high-power objective (40x) to study detailed morphology.

Observations:

Rhizopus appears as a filamentous fungus with white or grayish-black mycelium.

It consists of three main structures:

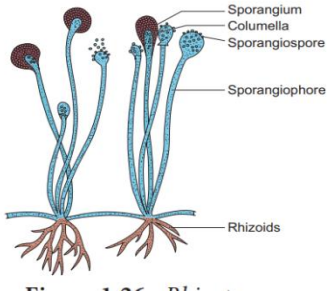
Sporangiophores – Erect, unbranched hyphae bearing sporangia.

Sporangia – Round, black spore-containing structures at the tips of sporangiophores. Spores inside sporangia may be seen as small, round structures. If the sporangium is broken, spores may spread out.

Rhizoids – Root-like structures anchoring the fungus to the substrate.

Conclusion:

The microscopic study of *Rhizopus* confirms its filamentous nature, presence of sporangia for asexual reproduction.



Exercise: 2

Aim: To study the morphology of *Alternaria* spp.

Materials: microscope, dropper, petridish, glass slide, cover glass, culture/ material of the species - *Rhizopus* culture or infected bread.

Procedure: Using a needle or brush, carefully transfer a small portion of fungal growth onto a clean glass slide after cutting section of the material infected with *Alternaria*.

Add a drop of lactophenol cotton blue stain for better visualization.

Gently place a cover slip over the sample to avoid air bubbles.

Observe the slide under a low-power objective (10x) to locate structures.

Switch to high-power objective (40x) to study detailed morphology.

Observations:

A*lternaria* spp. appears as a filamentous fungus with dark brown to black mycelium.

It produces distinctive **conidia** that are large, dark-colored, and multicellular.

- **Conidia** are formed in chains, with transverse and longitudinal septa.
- They may be oval or club-shaped, with a beak-like projection.
- The conidia may be scattered or clustered at the tips of conidiophores.

Conclusion

The microscopic study of *Alternaria* spp. confirms its characteristic conidia morphology, chain-like spore arrangement.

***Alternaria* sp.**

Section III: Bryophytes

General characters of bryophytes

Bryophytes are non-vascular, primitive land plants. They are also known as "amphibians of the plant kingdom" because they require water for reproduction.

Habitat: they are found in moist, shady environments such as forests, rocks, and damp soil. Some species grow in aquatic or extreme habitats like deserts and Polar Regions.

Structure and Organization: they lack true roots, stems, and leaves; instead, they have root-like **rhizoids**, leaf-like **phylloids**, and stem-like **cauloids**. They exhibit **thalloid** (liverworts) or **leafy** (mosses) forms.

Vascular Tissue: they lack vascular tissues (xylem and phloem), so they depend on diffusion for water and nutrient transport.

Alternation of Generations: they show **haplodiplontic** life cycle with two distinct phases:

Gametophyte phase: Haploid, photosynthetic, and independent.

Sporophyte phase: Diploid, dependent on the gametophyte, and produces spores.

5. Reproduction: they reproduce by asexual and sexual reproduction

Asexual Reproduction: it occurs through fragmentation, gemmae (e.g., *Marchantia*), or tubers.

Sexual Reproduction: sexual reproduction is oogamous (male gamete is motile, female is non-motile).

Antheridia (male) produce biflagellate sperm, and archegonia (female) produce egg cells.

Water is essential for sperm motility and fertilization.

Sporophyte Structure: it consists of three parts:

1. **Foot** – Anchors the sporophyte to the gametophyte.
2. **Seta** – A stalk-like structure.
3. **Capsule** – Produces spores through meiosis.

Spore Dispersal: spores are dispersed by wind and germinate to form new gametophytes.

Ecological Importance:

- They act as pioneers in ecological succession on barren lands.
- They help in soil formation and water retention.
- Some bryophytes e.g., *Sphagnum* act as natural water reservoirs.

9. Economic Importance:

Bryophytes are used as bioindicators of environmental pollution.

Peat moss (*Sphagnum*) is used as fuel, soil conditioner, and in wound dressin

Exercise: 1

To study the external morphology of *Marchantia* sp.

Specimen of *Marchantia*, Petridish or watch glass, Forceps, Dropper, Needle or dissecting needle, Compound microscope or hand lens, Glass slide and cover slip, Water, blotting paper

Obtain a fresh specimen of *Marchantia* (from natural habitat -a moist and shady place/ a botanical garden/lab sample).

Clean the thallus and place the it in a petridish or watch glass.

Add a few drops of water to keep it moist.

External Morphology:

Using a hand lens or microscope, observe the **dorsal surface** of the thallus.

Look for **midrib**, **polygonal markings (air pores)**, and **gemma cups**.

Turn the thallus and observe the **ventral surface**.

Note the **rhizoids** (smooth and tuberculate) and **scales**.

Identify and observe the **gemma cups** (for asexual reproduction) if present.

Look for **male (antheridiophores)** and **female (archegoniophores)** reproductive structures if available.

Draw diagrams of the dorsal and ventral sides and reproductive structures.

Observations:

The *Marchantia* thallus is **dorsiventrally flattened**, **green**, and **lobed**.

Dorsal surface shows polygonal areas with a central pore called air pores.

Gemma cups are cup-shaped and contain **gemmae** which help in vegetative reproduction.

Ventral surface bears numerous **rhizoids** for attachment and water absorption.

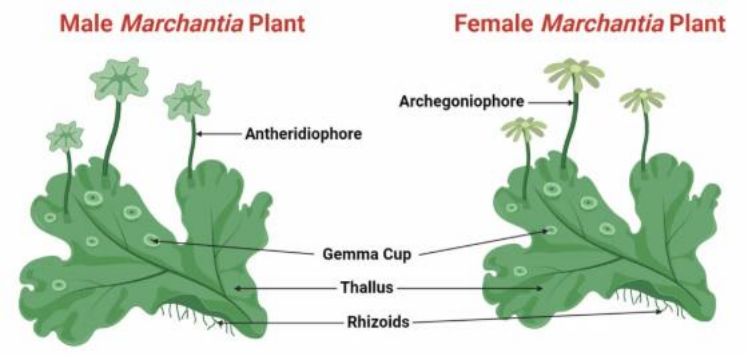
It also bears Scales arranged along the midrib region for protection and moisture retention.

Reproductive structures:

Antheridiophore: Male, stalked structure with a flat disc bearing antheridia.

Archegoniophore: Female, stalked with umbrella-like structure bearing archegonia on the underside.

The morphology of *Marchantia* shows adaptations for life in moist terrestrial habitats, including specialized structures for vegetative and sexual reproduction.



Marchantia sp. (male and female thalli)

Exercise: 2

Aim: to study the anatomy of *Marchantia* species through transverse sections (T.S.) of the thallus.

Materials required: fresh *Marchantia* thallus, Razor blade or microtome, Watch glass with water, Needle or forceps, Staining solution (e.g., safranin), Glycerin, Glass slide and cover slip, Compound microscope, Blotting/filter paper, Brush (for lifting thin sections).

Procedure: Take a fresh *Marchantia* thallus and make thin **transverse sections (T.S.)** using a razor blade or microtome. Transfer the thin sections into a watch glass containing water. Stain the sections with **safranin** for a few minutes. Rinse off excess stain with water. Place the stained section on a clean glass slide. Add a drop of **glycerin** and carefully place a cover slip over it. Remove excess glycerin using blotting paper. Observe the slide under a compound microscope. Note the different layers and tissues present.

Observations:

A T.S. of the *Marchantia* thallus shows the following structures:

The upper epidermis contains a single layer of compactly arranged cells.

The upper surface contains barrel-shaped **air pores** which lead to underlying **air chambers**.

Air chambers lie just below the dorsal epidermis.

They appear as large polygonal chambers arranged in rows.

They contain **photosynthetic filaments** hanging into the chamber.

This region is the **Assimilatory region** which is the primary site of photosynthesis.

Located below the assimilatory zone is the **Storage region consisting of parenchymatous cells.**

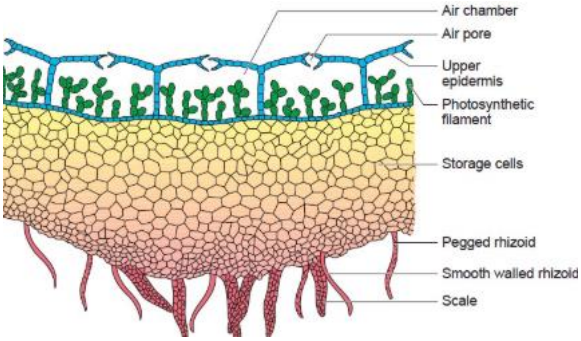
It is composed of several layers of **parenchymatous cells** with stored food (starch).

The lower epidermis bounds the storage regions and **gives rise to rhizoids**. The rhizoids are smooth walled and tuberculate.

Rhizoids help in anchorage and water absorption.

Conclusion

The anatomy of *Marchantia* shows clear tissue differentiation with adaptations for terrestrial life, such as air chambers for gas exchange and parenchymatous storage tissue for food storage.



Marchantia sp. Anatomy

Exercise: 3

To study morphology of *Funaria* sp.

Materials:

Materials Required: Fresh or preserved specimen of *Funaria,* Watch glass or petri dish, Forceps, Needle, Hand lens or compound microscope, Dropper, Water, Blotting paper, Slide and cover slip

Procedure:

Place the *Funaria* specimen in a watch glass with a few drops of water to moisten it.

Gently spread out the plant using a needle or forceps.

Begin by observing the entire gametophyte plant.

Note the rhizoids, stem, and leaves constituting the gametophytic phase.

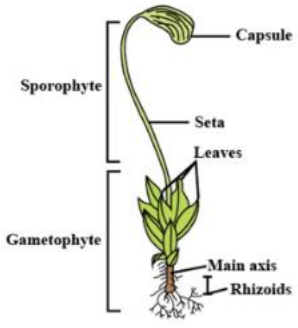
Observe the sporophyte attached to the gametophyte if present.

The Gametophyte small, green, leafy structure. It has a central axis bearing green leaves. The leaves are simple, without veins, and spirally arranged. **Rhizoids** are fine, branched and branched with oblique septa. **Rhizoids** help in anchorage and water absorption.

Sporophyte grows from the apex of the gametophyte.

It consists of **footseta** (long stalk), and **capsule** which is the spore-producing structure.

Capsule covered with hood-like **calyptra**



Funaria sp.