BGS Science Academy & Research Centre

Agalagurki, Chikkaballapura

Affiliated to Bangalore North University

DEPARTMENT OF BOTANY

LABORATORY MANUAL FOR I SEMESTER
I BACHELOR OF SCIENCE
CBCS SCHEME

DIVERSITY OF NON VASCULAR PLANTS
INTRODUCTION TO MICROBIOLOGY, VIRUSES, BACTERIA, CYANOBACTERIA AND PHYCOLOGY

Laboratory Instructions

- Do not forget to carry laboratory apron, observation book and other required accessories.
- Handle equipments, microslides, glasswares, chemicals and specimen bottles with proper care.
- Report breakages / damages to the batch incharge or laboratory assistants.
- Keep the laboratory clean and tidy.

SEMESTER I – PRACTICAL PAPER-I

DIVERSITY OF NON VASCULAR PLANTS
INTRODUCTION TO MICROBIOLOGY, VIRUSES, BACTERIA, CYANOBACTERIA AND PHYCOLOGY

Total Units - 13

1. Study of instruments: - Autoclave, Inoculation chamber, Hot air oven, Incubator and inoculation loop. **1 unit**
2. Sterilization of glassware and media preparation (Nutrient Agar, Martin Rose Bengal Agar). **1 unit**
3. Isolation of Bacteria from soil by pour plate method. **1 unit**
4. Colony characteristics of bacteria to identify colonies obtained. **1 unit**
5. Plant diseases- **1 unit**
   a. Bacterial disease – Citrus canker
   b. Mycoplasmal disease – Sandal spike disease
   c. Viral diseases – Tomato leaf curl, Papaya leaf curl and yellow mosaic of beans
   d. Other – Little leaf of tomato and *Vinca rosea*
6. Gram's staining: - a) *Rhizobium* from root nodules **1 unit**
   b) *Lactobacillus* from curds
7. Measurement of cell concentration by using Haemocytometer. **1 unit**
8. Type study of Cyanobacteria: - *Anabaena*, *Spirulina* and *Scytonema*. **1 unit**
9. Type study of Algae: - *Chlamydomonas*, *Hydrodictyon*, *Oedogonium*, *Chara*, *Sargassum* and *Polysiphonia*. **5 units**

PRACTICAL QUESTION PAPER-I

DIVERSITY OF NON VASCULAR PLANTS

INTRODUCTION TO MICROBIOLOGY, VIRUSES, BACTERIA, CYANOBACTERIA AND PHYCOLOGY

Max marks – 35

1. Identify the given specimens **A, B, C & D** with labeled diagram and reasons. **4×3=12**
2. Describe colony characteristics of given colony **E** and tabulate your observations. **-2-**
3. Prepare a temporary slide of **F**, identify, sketch, label with reasons. Leave preparation for evaluation. **-5-**
4. Stain given material **G** by Gram's Staining. Write the procedure and identify with reasons. Leave preparation for evaluation. **-3-**

OR

Calculate the population of fungal spores / yeast cells **G** using Haemocytometer. **-3-**

☐☐=☐

6. Record and submission. **5+3=8**

SCHEME OF VALUATION

1. Four specimens **A, B, C, D** – two from Algae, one from Cyanobacteria and one from specimen of diseases / Herbarium (Identification – **1** mark, labeled diagram with reasons **2** marks).
2. Colony characteristics of the given colony **E** – **2** marks.
3. Specimen **F** from Algae (mounting – **2** marks. Identification – **1** mark, sketch with reasons **2** marks).
4. Specimen **G** – Gram's staining (Staining, procedure and result – each **1** mark).

OR

Calculation of fungal spores / yeast cells **G** using Haemocytometer (Procedure **1** mark, calculation – **2** marks).

5. Two permanent slides **H & I** – from Algae / Cyanobacteria (Identification – **1**mark, sketch ☐☐ marks).
6. a) Record – **5** marks

   b) Submission of 3 algae / cyanobacteria materials – **3** marks

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DIVERSITY OF NON VASCULAR PLANTS PART I
Introduction To Microbiology, Viruses, Bacteria, Cyanobacteria And Phycology

STUDY OF INSTRUMENTS

Microscope, Autoclave, Inoculation chamber, Hot air oven, Incubator and inoculation loop.

Microscope

It is the most indispensable instrument in a biology laboratory, so much so that it comes to be called 'The primary instrument of the biologists'. It helps to increase the resolving power (property to distinguish objects lying very close as separate bodies) of human eye which fails to recognize objects lying closer between 0.01 to 0.25 mm.

Some common types of microscopes are listed below-

1. Dissecting microscope,
2. Compound microscope,
3. Binocular microscope,
4. Phase contrast microscope and
5. Electron microscope.

Of these, dissecting microscope and compound microscopes are very commonly used by the students.

[I] Dissecting microscope

It is used for dissection, specially during taxonomic studies, embryo separation, etc.

Construction. It consists of basal foot, a vertical limb, stage and a lens. The basal foot is a stand. The limb has an attached stage made of glass plate. A folded arm which can be moved vertically holds the lens. A mirror is attached at the base of the limb.

Mechanical operation.

1. Move the lens and adjust it over the object.
2. Illuminate the object suitably by adjusting the mirror.
3. Focus the object by using adjustment screw.

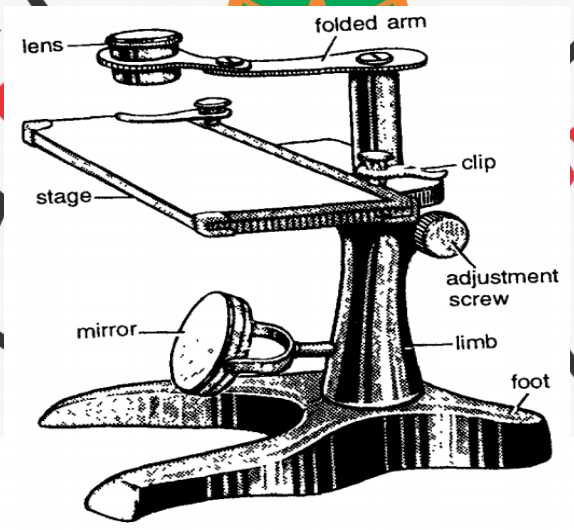


Fig. 1. A dissecting microscope.

[II] Compound microscope

It is one of the most commonly used and by far the most suitable microscope in the Botany Laboratory. At one time, it employs one ocular (eye piece) and one objective, in working position. As such, it is also known as monocular-mono-objective microscope.

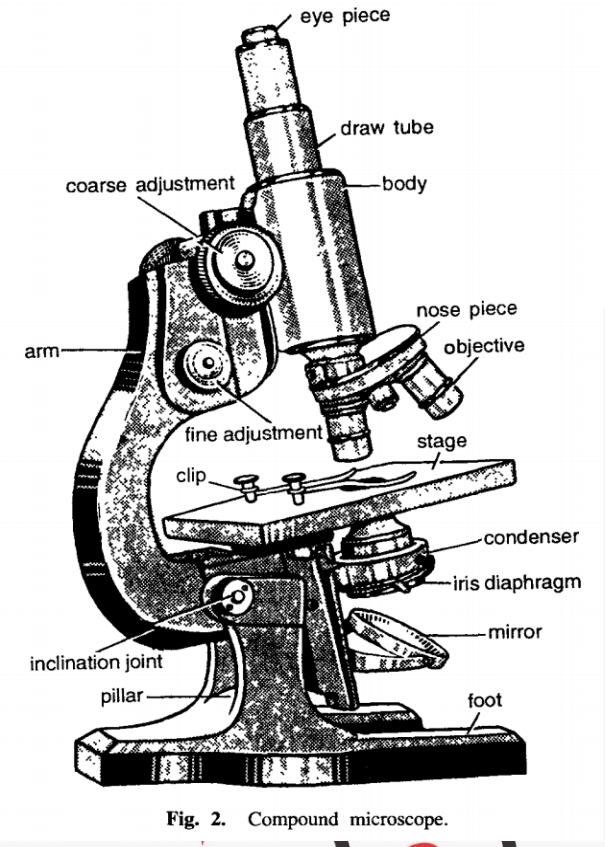


Fig. 2. Compound microscope.

Construction. The microscope is built around a strong basal foot and a vertical limb. The foot supports the vertical limb. A round, rectangular or square stage is fixed to the limb. It is provided with spring clips to hold the slide in position.

A movable or fixed sub-stage is situated directly below the stage. It is provided with an iris diaphragm and condenser lens. Iris diaphragm is a wheel-shaped metal disc to regulate the aperture, through which light rays reach the condenser and are passed to an object. Condenser is a system of two or more lenses under the stage which receives parallel light rays from mirror and converge them at the level of stage.

Mechanical operation.

1. Microscope is placed in maximum diffuse light. Direct sunlight is harmful for the eyes. The northern light is most suitable. If light source is artificial, filter (preferably blue coloured) is used.
2. Light is adjusted by turning the mirror towards the source of light and also by moving the sub-stage up and down, as well as with the help of iris diaphragm.
3. A prepared slide is placed on the stage. Object is adjusted just over the stage aperture.
4. The object is located and focused with a low power objective using coarse adjustment.
5. If higher magnification is desired, nose-piece is turned to next higher power. Fine adjustment can be used freely at this stage, while the use of coarse adjustment be avoided.
6. High power objective and subsequent higher powers are used only when object is properly mounted under coverslip.
7. The object should always be observed with both eyes open.

Care.

1. Before and after the use, all the lenses and metal parts including stage should be cleaned. The lenses are cleaned with tissue paper, muslin cloth or clean and soft handkerchief.
2. Microscope is kept covered when not in use. Proper wooden box, plastic bags, bell jars or even a clean cloth can be used.
3. Objectives should not be ordinarily removed from the nose-piece.
4. Operating screws, condenser, iris diaphragm, mirror and stage or stage clips should always be handled carefully.

AUTOCLAVE

Autoclave is an instrument used for sterilization in biology experiments. It was first developed by Chamberland in 1884. Autoclave is usually of pressure cooker type made up of double walled cylindrical metal vessel made up of stainless steel or copper. The lid is provided with the pressure gauze for monitoring the pressure, exhaust valve to remove the air and safety valve to avoid explosion during operation. The articles are kept loosely in the autoclave chamber in a basket. Water is boiled with the help of heater and steam is released into the autoclave's chamber. The exhaust valve is kept open till the air in the chamber is driven out. The exhaust valve is closed and steam pressure in the chamber is allowed to reach the desired value. The temperature of the steam inside the chamber depends on pressure in the autoclave. Generally, a pressure of 15 pound with temperature at 121°C is employed for 15-20 minutes which is enough to kill all the cells and spores of organisms. Steam condenses on the cooler surface of the object and transfers heat energy to the object and sterilizes it. Autoclave is used for sterilizing culture media, glass wares, other heat resistant instruments, etc., but not for oils, powders and plastics.

INOCULATION CHAMBER

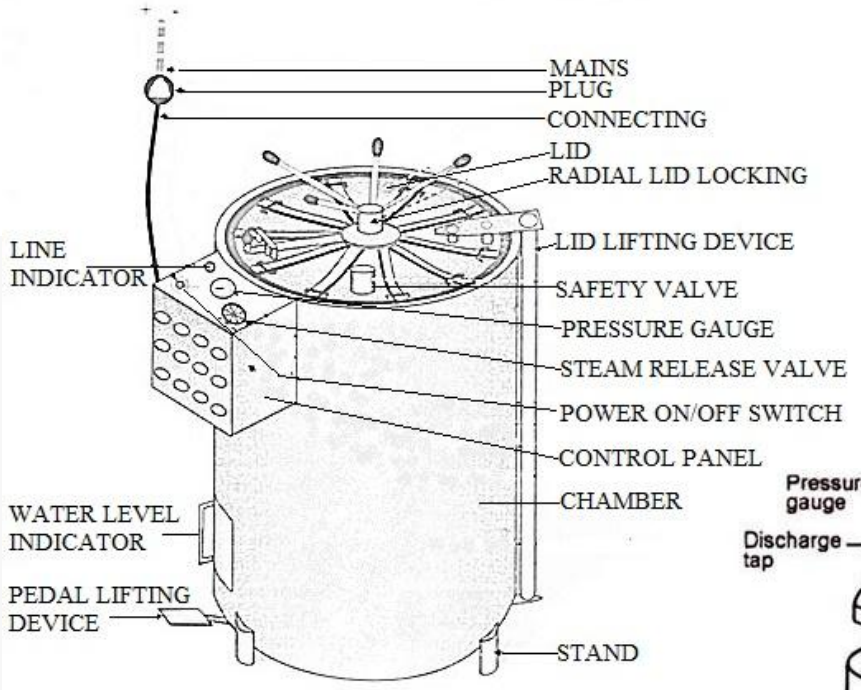
Inoculation chamber is an instrument used for aseptic transfer of culture from one media to another or for sub culturing and isolation. Inoculation chamber is a large wooden cabinet made for short term work. It has four doors, of this two glass doors are on opposite side and they can be used to place the experimental requirements inside the chamber. The front panel has two circular doors which allow the insertion of the hands to operate inside the chamber to conduct inoculation or transfer of culture. The chamber roof is provided with UV light (for sterilizing internal environment) and a fluorescent light (for illumination while working). The switches to operate them are present outside the chamber for easy and safe operation. Inside the chamber the working table or shelves are made up of thick glass sheets.

Working procedure: -

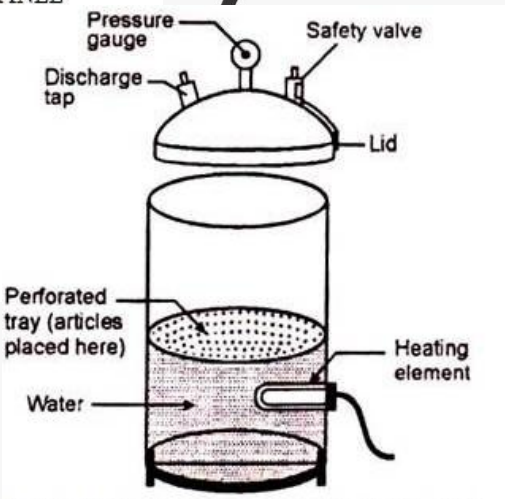
Before commencement of experiment clean the inoculation chamber using a suitable disinfectant like 70% alcohol. Through the doors present on the opposite sides of the chamber, keep the material required for experiment and close the doors. Switch on the UV light for 15 minutes to sterilize the interior of the chamber. Later switch off the UV light and switch on normal light and transfer or inoculation of culture is performed. Bunsen burner or spirit lamp can be used for aseptic transfer, flaming the loop before and after inoculation etc., After completion of experiment clean the working surface and take out all the apparatus and waste.

Inoculation chamber can be used for transferring media into plate or tubes, inoculation, streaking, etc.,

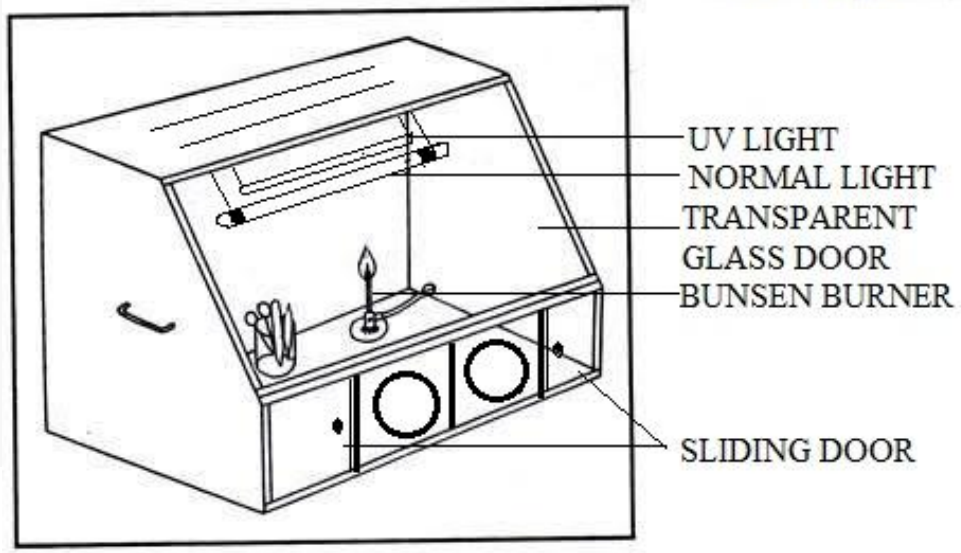
STUDY OF INSTRUMENTS



AUTOCLAVE

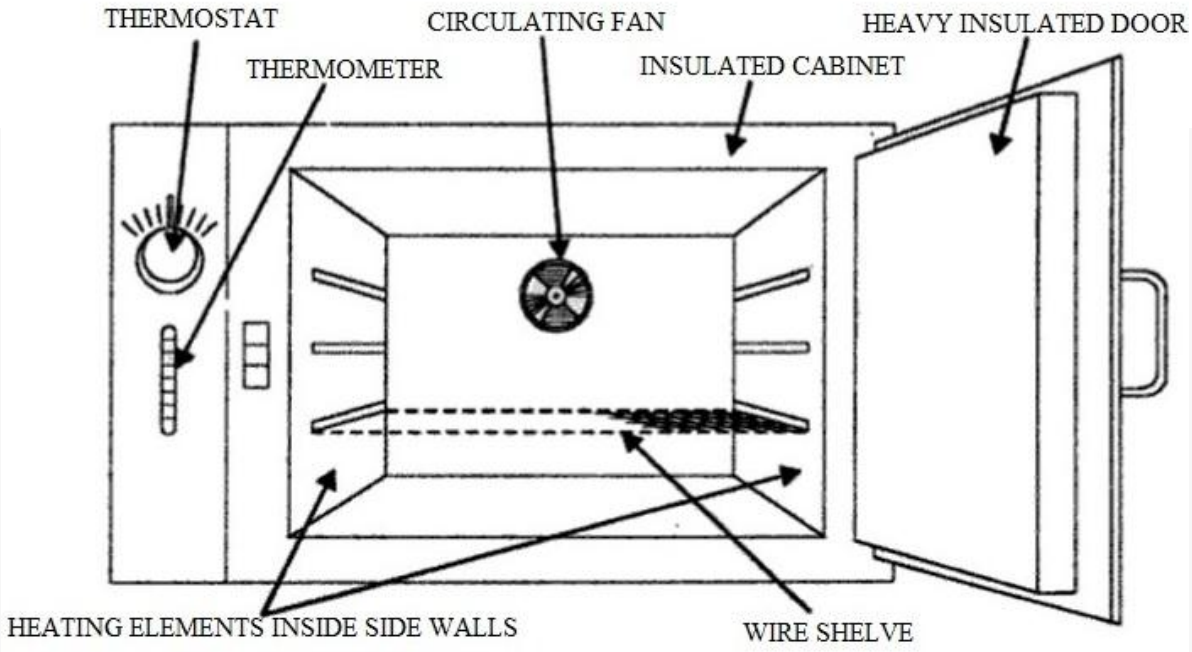


INTERNAL COMPONENTS OF AUTOCLAVE

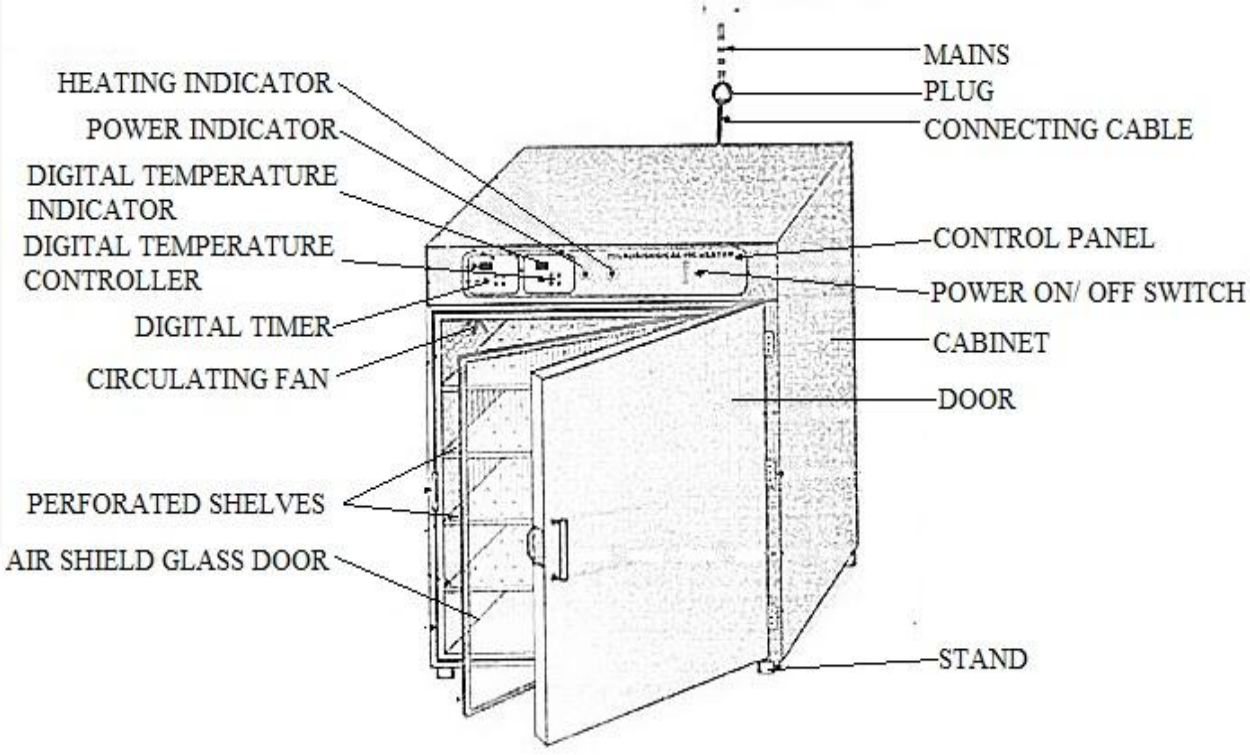


INOCULATION CHAMBER

STUDY OF INSTRUMENTS



A HOT AIR OVEN



INCUBATOR

HOT AIR OVEN

Hot air oven is a sterilization instrument used in science laboratory. It contains a double walled insulated cabinet with outer wall made up of mild steel sheets and an inner wall made up of anodized aluminium or stainless steel. The space between the walls is filled with glass wool to provide thermal insulation. Hot air oven is fitted with an electric heating mechanism and thermostat control, using which the required constant temperature can be obtained by trial and error. A circulating fan is fitted to circulate air within the cabinet. For proper circulation of hot air, the shelves are perforated. It has a single insulated metallic door and control panel containing all the switches and indicators. There is air ventilator on both the sides of cabinet through which moisture laden air escapes out from the oven. Hot air oven sterilizes the object by hot dry air. It kills the microorganisms by dehydrating and oxidizing the cellular constituents. Dry heat is less effective in killing microorganism than moist heat. Higher temperature for longer time period is used with dry air because it has less penetration power.

It removes moisture from microorganisms and thus interferes with coagulation of microbial proteins.

Hot air oven can be used at different temperatures. Operating time depends on the temperatures.

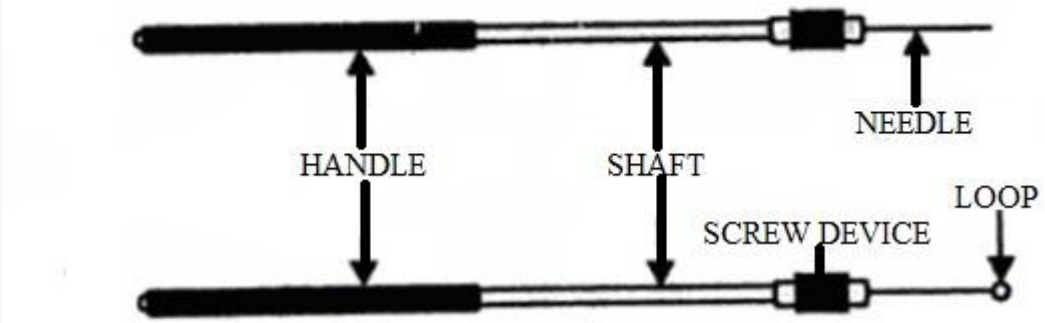
A hot air oven is used to sterilize glass wares such as test tubes, petriplates, corrodible metal instruments, powders, oils etc., which can tolerate prolonged heat exposure but get spoiled by moist heat. However, it is not suitable for sensitive materials like plastic and rubber items. Liquid substances, such as prepared media and saline solutions cannot be sterilized in oven, as they lose water due to evaporation.

INCUBATOR

An incubator is a double walled insulated cabinet with outer wall made up of mild steel sheets and an inner wall made up of anodized aluminium or stainless steel. The space between the walls is filled with glass wool to provide thermal insulation. It is fitted with heating element at the bottom, removable perforated shelves and a circulating fan for uniform distribution of heat. It has two doors - inner glass door to view the content, kept inside the incubator and outer metallic insulated door with a rubber gasket. It has a control panel with on and off switch, thermostat, indicator. Similar to oven, most of the incubators use dry heat for temperature control. Moist environment can be provided by keeping water in a beaker inside the cabinet which also avoids quick drying of media. There is air ventilator on both the sides of cabinet through which moisture produced by the living organisms escapes out from the incubator. Incubation is done in an incubator, which maintains a constant temperature level thermostatically, specifically suitable for the growth of a specific microbe. The usual temperature of incubation is 37°C. The growth of microorganims is altered by the chemical and physical nature of its surrounding. Most important environmental features altering microbial growth are pH, water, oxygen, temperature etc., The cardinal temperature (i.e., minimum, optimum and maximum) for growh can be achieved by using incubator.

INOCULATION LOOP

The inoculation loop is a commonly used tool used for aseptic transfer. An inoculating loop consists of an insulated wooden or plastic handle provide with a screw device at the top which holds a heat resistance nichrome or platinum wire, approximately 3 inches long. It is a straight wire in case of inoculating needle but in inoculating loop, the wire end is bent round to form a loop. It is sterilized before and after use by heating or flaming in the blue (hottest) portion of Bunsen burner until it is red hot followed by cooling either by touching to petridish cover or inner surface of culture tube to avoid killing of microbial cells. The inoculation loop is mainly used to transfer (sub-culture) from liquid culture, the liquid drop being help across the loop area by surface tension.



INOCULATION LOOP

CLEANING OF GLASSWARES

All the glassware used for microbiology work should be perfectly clean and sterilized. New glassware can be washed with detergent, followed by washing with tap water and rinsing with distilled water. The conventional method of washing glassware involves soaking glass in a chromic acid-sulfuric acid bath overnight followed by washing and brushing with a detergent or a special laboratory cleaning product called alconox, teepol, lab wash, lab clean etc. After they have been thoroughly cleaned, they are rinsed with tap water and finally with deionised water. To prepare a chromic acid wash, mix 20 g of sodium or potassium chromate with sufficient distilled water to make a paste of chromate salt. Add 300 ml of concentrated sulfuric acid. Use the mixture until it turns green in color.

Removing dirt: Fill or rinse with an acid solution. Allow to stand for several hours, if necessary. Follow with multiple deionised water rinses.

Cleaning pipettes: Pipettes may be cleaned with a warm solution of detergent or with cleaning solution. Draw in sufficient liquid to fill the bulb to about one-third of its capacity. While holding it nearly horizontal, carefully rotate the pipette so that all interior surfaces are covered. Drain inverted and rinse thoroughly with deionised water.

Cleaning burette: Thoroughly clean the tube with detergent and a long brush. Allow the washings to exit through the burette tip.

Culture Tubes: Culture tubes which have been used previously must be sterilized before cleaning. The best method for sterilizing culture tubes is by autoclaving for 30 minutes at 121°C (15 psi pressure). Media which solidifies on cooling should be poured out while the tubes are hot. After the tubes are emptied, brush with detergent and water, rinse thoroughly with tap water, rinse with distilled water, place in a basket and dry.

Dishes and Culture Bottles: Wrap in heavy paper or place in a petri dish can. Sterilize in the autoclave or dry air sterilizer. After the bottles are emptied, brush with detergent and water, rinse thoroughly with tap water, rinse with distilled water, place in a basket and dry.

Slides and Cover Glass: It is especially important that microscope slides and cover glass used for the preparation of blood films or bacteriologic smears be perfectly clean and free from scratches. Slides should be washed, placed in glacial acetic acid for 10 minutes, rinsed with distilled water and wiped dry with clean paper towels or cloth. Once the slides have been washed, place them in a wide jar of alcohol. As needed, remove from the jar and wipe dry. If the slides are dry stored, wash them with alcohol before use.

Contaminated Glassware: Glassware which is contaminated with blood clots, such as serology tubes, culture media, petri dishes, etc., must be sterilized before cleaning.

STERILIZATION OF GLASSWARES

Sterilization is the process by which all living cells, spores, and acellular entities (e.g., viruses, viroids and prions) are either destroyed or removed from an object or habitat. A sterile object is totally free of viable microorganisms, spores, and other infectious agents.

STERILIZATION WITH SATURATED STEAM (AUTOCLAVE)

Preparation of glasswares for sterilization

Cover the open ends of washed and dried glassware with non-absorbent cotton plugs and wrap in butter paper/ Aluminium foil. Wrap pipettes individually in butter paper.

Cover the petri-plates with the lid and place them in the stainless steel petri-plate containers or wrap four petriplates in butter paper or aluminium foil.

Wrap glass ware/apparatus, which are to be autoclaved with butter paper/Aluminium foil.

The most useful approach for sterilization is autoclaving, in which items are sterilized by exposure to steam at 121°C and 15 psi of pressure for 15 minutes or longer, depending on the nature of the item. Increased pressure in an autoclave increases the boiling point of water and produces steam with higher temperature. Under these conditions, microorganisms, even endospores, will not survive longer than about 12 to 13 minutes. Autoclaving because of the greater penetration capacity kills microbes by hydrolysis and coagulation of cellular proteins. This method is rapid and dependable. Modern autoclaves are designed to ensure that all of the air has been expelled and only steam is present in the autoclave chamber. They are carefully temperature controlled as well.

METHODS FOR STERILIZING IN AUTOCLAVE

1. Open the lid of the autoclave and check that there is sufficient amount of distilled or deionised water in it. If necessary, refill.
2. Place the correctly packaged materials into the chamber of the autoclave. Close the lid of the autoclave.
3. Make sure that the exhaust valve is open. Turn on the heating of the autoclave.
4. If an intense (a thick, milky white) steam outflow can be detected through the outlet tube of the exhaust valve, wait for 4-5 minutes and close the exhaust valve.
5. With the help of a built-in thermometer and manometer, check the temperature and pressure increase inside the chamber of the autoclave.
6. The sterilization time (15 minutes or more) begins only when the temperature equalization (to 121°C) in the chamber has occurred. It is important that the operator stays with the device and controls the process of sterilization from the time it is turned on until the end of the sterilization period.
7. Turn off the power switch of the autoclave when the sterilization cycle/period has ended.
8. Allow the device to cool down to at least 60-70°C.
9. For decompression, slowly open the safety valve. Thereafter, carefully open the lid of the autoclave and remove the sterilized materials, using heat-proof gloves.
10. Mention on the sterilized glassware with date of sterilization and store sterilized glassware/apparatus in a clean dry place.

STERILIZATION WITH DRY HEAT (HOT AIR OVEN)

Preparation of glasswares for sterilization

Appropriate containers for use in a hot air oven are as follows:

Glass pipettes (graduated and Pasteur) with ends plugged to a depth of 20mm with nonabsorbent cotton wool. Dry swabs in glass tubes, Glass petri dishes, Glass test tubes with non-absorbent cotton wool plugged. Bottles with aluminum tops with silicone linings. Glass flasks or cylinders covered with aluminum foil.

Often, dry glassware such as pipettes and petriplates must be sterilized. Steam tends to etch glassware and also leaves it damp. Therefore, such items are generally dry-heat sterilized. The glassware is placed in an electric oven set to operate between 160° and 170°C. Since dry heat is not as effective as wet heat, the glassware must be kept at this temperature for about 2 hours or longer. The oven temperature must not rise above 180°C or any cotton or paper present will char. The contents must not be removed from the oven immediately as a slow cooling period is necessary ideally when the temperature has reduced down to 50°c, but no less. The reason for the gradual cooling period is to avoid the cracking of glassware as well as preventing air (that could potentially contain contaminating organisms) entering the oven. Spores of *Bacillus subtilis* are most heat resistant to dry heat processes, hence this bacterium is used as a biological indicator for evaluating the efficiency of the oven for sterility.

METHODS FOR STERILIZING IN AUTOCLAVE

1. Place the articles in the oven without wrapping. Do not pack tightly. In case of pipettes, plug the wide end of each pipette with cotton and place them in a pipette can (made of aluminium, stainless or copper) with loosely fitting end, test tubes are plugged with cotton and placed in metal baskets.
2. Close the door of the oven.
3. Switch on the heating element.
4. Allow the temperature to rise slowly.
5. Set the oven at the required temperature with the thermostat.
6. Sterilize for the desired length of time (e.g. at 160°C for 2 hours), duration of sterilization has to be considered only when the oven has reached the required temperature.
7. Switch off the oven and allow it to cool slowly.
8. Remove the cooled articles from the oven using heat-proof gloves.
9. Mention the date of sterilization on the sterilized glassware and store sterilized glassware/apparatus in a clean dry place.

PREPARATION OF MEDIA

Microorganisms, like all other living organisms, require basic nutrients, for its survival and growth. The cultivation of microorganisms on an artificial growth medium requires that the medium supply all the nutritional and energy requirements necessary for growth and a favorable growth environment. The food material on which microorganisms are grown in the laboratory is known as a culture medium (pl. media) and the growth itself called a culture. Based on the composition there are three different types of media. (i) Natural or empirical culture media (whose exact chemical composition is not known, ex: milk, juices etc.,) (ii) Semi-synthetic media (Chemical composition is partially known, ex: Nutrient agar, potato dextrose agar) (iii) Chemically defined, or synthetic media (composed of known amounts of pure chemicals ex: Martin's rose Bengal agar, Czapek solution). Such media are often used as a general purpose medium. These are composed of complex materials that are rich in vitamins and nutrients.

Three physical forms are used: liquid, or broth, media; semisolid media; and solid media. The major difference among these media is that solid and semisolid media contain a solidifying agent (usually agar), whereas a liquid medium does not. Liquid media, such as nutrient broth, can be used to propagate large numbers of microorganisms in fermentation studies and for various biochemical tests. Semisolid media can also be used in fermentation studies, in determining bacterial motility, and in promoting anaerobic growth. Solid media, such as nutrient agar, are used (1) for the surface growth of microorganisms in order to observe colony appearance, (2) for pure culture isolations, (3) for storage of cultures, and (4) to observe specific biochemical reactions.

NUTRIENT AGAR (NA) MEDIUM (ALLEN, 1953):

Nutrient media are basic and general purpose culture media used for maintaining, cultivation and enumeration of bacteria. Peptic digest of animal tissue and beef extract provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients necessary for the growth of bacteria. Sodium chloride maintains the osmotic equilibrium of the medium.

Composition of nutrient agar

Peptone	- 5.0 g
Beef extract	- 3.0 g
Agar	- 15.0 g
Sodium chloride	- 5.0 g
Distilled water	- 1000.0 ml
pH	- 7.8

MARTIN'S ROSE BENGAL AGAR (MRBA) MEDIUM (MARTIN, 1950):

Rose Bengal Agar Base is recommended for the selective isolation and enumeration of yeasts and moulds from environmental materials and foodstuffs. Peptone provides the carbon and nitrogen sources required for good growth of a wide variety of organisms. Dextrose is an energy source. Monopotassium phosphate provides buffering capability. Magnesium sulphate provides necessary trace elements. Rose Bengal is a selective agent that inhibits bacterial growth and restricts the size and height of colonies of the more rapidly growing moulds. Rose

Bengal is taken up by yeast and mould colonies, thereby facilitating their recognition and enumeration. Streptomycin inhibit bacteria.

Composition of Martin Rose Bengal agar

Glucose	- 10.0 g
Peptone	- 5.0 g
Potassium di hydrogen phosphate	- l.0 g
Magnesium sulphate	- 0.5 g
Rose Bengal	- 33.0 mg
Streptomycin	- 0.003 g
Agar	- 15.0 g
Distilled water	- 1000 mL

PROCEDURE FOR PREPARATION OF NA/MRBA MEDIUM

- Accurately weigh the chemical ingredients of nutrient agar/ Martin rose Bengal agar and transfer them into a beaker containing 500ml distilled water.
- Gently heat the contents with slight agitation to dissolve the ingredients.
- Add more distilled water to make the volume to 1 litre.
- Measure pH of the media by using pH meter and adjust the pH to desired level (before adding agar and before adding Rose Bengal in case of MRBA) by adding drops of either HCl or NaOH solution.
- Media is dispensed into conical flask and apply prepared cotton plugs to the mouth of the conical flask. Tightly cover the mouth of cotton plugs with aluminium foil or a paper and tie with a rubber band or thread.
- Transfer the conical flask into iron basket and place inside the autoclave or pressure cooker and sterilise at 121°C for 30minutes.
- When temperature cools down take out the conical flask and use when required or store at room temperature for further use.

ISOLATION OF BACTERIA FROM SOIL BY POUR PLATE METHOD

Microorganisms are present everywhere and are abundant in our environment. Typical soil has millions of bacteria in each gram. The microbial population of soil is largest in the top few centimeters and declines rapidly with depth. Since soils vary greatly with respect to their physical features (e.g., pH, general type, temperature, and other related factors), the microorganisms present will also vary. Viruses, mycoplasma, bacteria, fungi, protozoa, algae, cyanobacteria, nematodes, insects and other invertebrates are all important members of the soil community. These contributes to global elemental cycling, fertility and makes nutrients available to plants. Not surprisingly, no single technique is available to count the microbial diversity found in average garden soil.

The serial dilution – agar plating method or viable plate count method is used commonly for isolation of microorganisms. Robert Koch developed the pour plate method. In this method successive dilutions of the known sample are added into sterile petriplates to which molten (45°C) agar is added and thoroughly mixed by rotating the plates, which is then allowed to solidify. The plates are incubated in an inverted position for 48 hours at 37°C and are examined for the presence of colonies of different microorganisms. These can be identified based on colony characteristics.

REQUIREMENTS:

Soil sample

Media: -

a. Nutrient Agar for isolation of Bacteria

b. Martin Rose Bengal Agar for isolation of Fungi (Note: Add streptomycin after autoclaving to avoid growth of bacterial colonies).

Sterile distilled water blanks

a. 1 test tube with 10ml distilled water
b. 6 test tubes with 9ml distilled water

Sterile 1ml pipettes -7

Sterile petriplates – 14

Test tube stand

Bunsen burner or spirit lamp

Marker pen or wax pencil

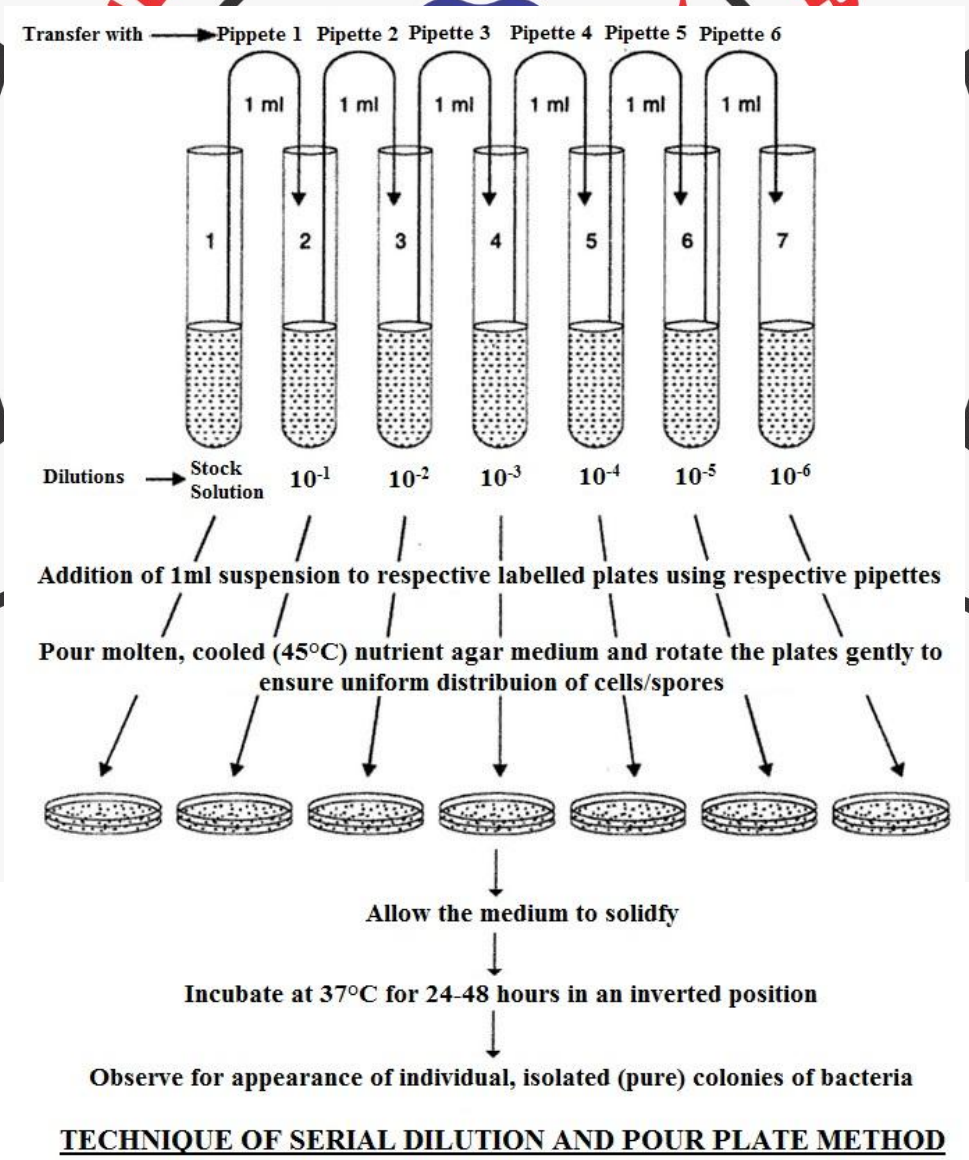
PROCEDURE:

1. Collect the soil samples at random, minimum five, from a field, mix thoroughly to make a composite sample.
2. Follow aseptic conditions and arrange the distilled water blanks in a test tube rack and label them 1-7.
3. Add 1.0g sample of finely ground, air dried soil in to test tube no.1 containing 10ml sterile distilled water, vigorously shake the dilution to obtain uniform suspension of microorganisms. This is the stock solution.
4. Transfer 1ml of stock solution into test tube no.2 containing 9ml of sterile distilled water using a sterile 1ml pipette to make 1:10 (10^{-1}) dilution and return the pipette to stock solution tube. Shake it well.

5. Prepare another dilution 1:100 (10^{-2}) by pipetting 1ml suspension from test tube no.2 to test tube no. 3 containing 9ml sterile distilled water using fresh sterile 1ml pipette and shake it. In the same way prepare dilutions of 10^{-3} to 10^{-7} in different test tubes by using separate 1ml sterile pipettes.
6. Transfer 1ml of stock solution from test tube no.1 to sterile petriplates no.1 using respective pipette, rotate the plate gently to ensure uniform dispersion of microbes in the medium. Allow the medium to solidify. Similarly, prepare other 6 petriplates by inoculating each dilution by sterile pipette and pouring the media.
7. Two sets of the above is done. One is for isolation of bacteria and other is for fungi.
8. Incubate Nutrient Agar plates for 24-48 hours at 37°C in inverted position for the growth of bacterial colonies. But incubate Martin Rose Bengal Agar plates for 3-4 days at 28°C in inverted position for the growth of colonies of Fungi.

OBSERVATION

Observe the plates for number and distribution of colonies of bacteria, fungi and others from each dilution. More concentrated suspension will have more clumps of microbes and colonies will be reducing as on dilutions and least diluted will have well dispersed colonies.



TECHNIQUE OF SERIAL DILUTION AND POUR PLATE METHOD

COLONY CHARACTERISTICS OF BACTERIA

AIM: - To study the cultural characteristics of given bacteria and fungi (for tentative identification).

INTRODUCTION: - The cultural characteristics of an organism pertain to its macroscopic appearance on different kinds of media. For the general description of colonies, nutrient agar or any complex, rich medium is useful for this purpose.

PRINCIPLE:-

In natural habitats, bacteria usually grow together in populations containing a number of species. In order to adequately study and characterize an individual bacterial species, one needs a pure culture. The spread plate technique is an easy, direct way of achieving this result. A colony is a large number of bacterial cells on solid medium, which is visible to the naked eye as a discrete entity. In this procedure, one assumes that a colony is derived from one cell and therefore represents a clone of a pure culture. After incubation, the general form of the colony and the shape of the edge or margin can be determined by looking down at the top of the colony. The nature of the colony elevation is apparent when viewed from the side as the plate is held at eye level. After a well-isolated colony has been identified, it can then be picked up and streaked onto a fresh medium to obtain a pure culture.

REQUIREMENTS: -

24 to 48 hour cultures of Bacteria (*Bacillus, Staphylococcus, Pseudomonas, Escherichia coli, Streptococcus*) and Fungi (*Penicillium, Aspergillus, Fusarium, Cladosporium, Yeast*), Bunsen burner, inoculating loop, 95% ethyl alcohol, L-shaped glass rod, Marker, 500-ml beaker, pipettes.

PROCEDURE:

1. With a marker, label the petriplates with the name of the bacteria or fungi to be inoculated and date.
2. Pipette 0.1 ml of the respective bacterial culture onto the center of a NA/MRBA plate and is spread evenly over the surface with a sterile, L-shaped glass rod.
3. Repeat the procedure to inoculate the remaining petriplates.
4. Invert the plates and incubate for 24 to 48 hours at room temperature or 30°C depending on culture requirement.
5. After incubation, measure some representative colonies and carefully observe their morphology (Shape, colour, elevation, margin, opacity etc.). Record your results.

Colony characters are described in the following manner.

Shape – Circular (Round), irregular, rhizoid, filamentous, filiform, punctiform etc.
Margin – Entire (Smooth), wavy (undulated), lobate, irregular (erose), ciliate, branched etc.
Surface texture – Smooth, rough, wrinkled, concentric, dry, powdery, mucoid, granular etc.
Consistency – Viscous, rubbery etc.
Opacity – Opaque, transparent, fluorescent etc.
Pigmentation – Bluish, green, black, white, yellow, orange, grey, pink, brown etc.
Elevation – Raised, flat, convex, drop-like, umbonate, ingrowing into medium, crateriform etc.

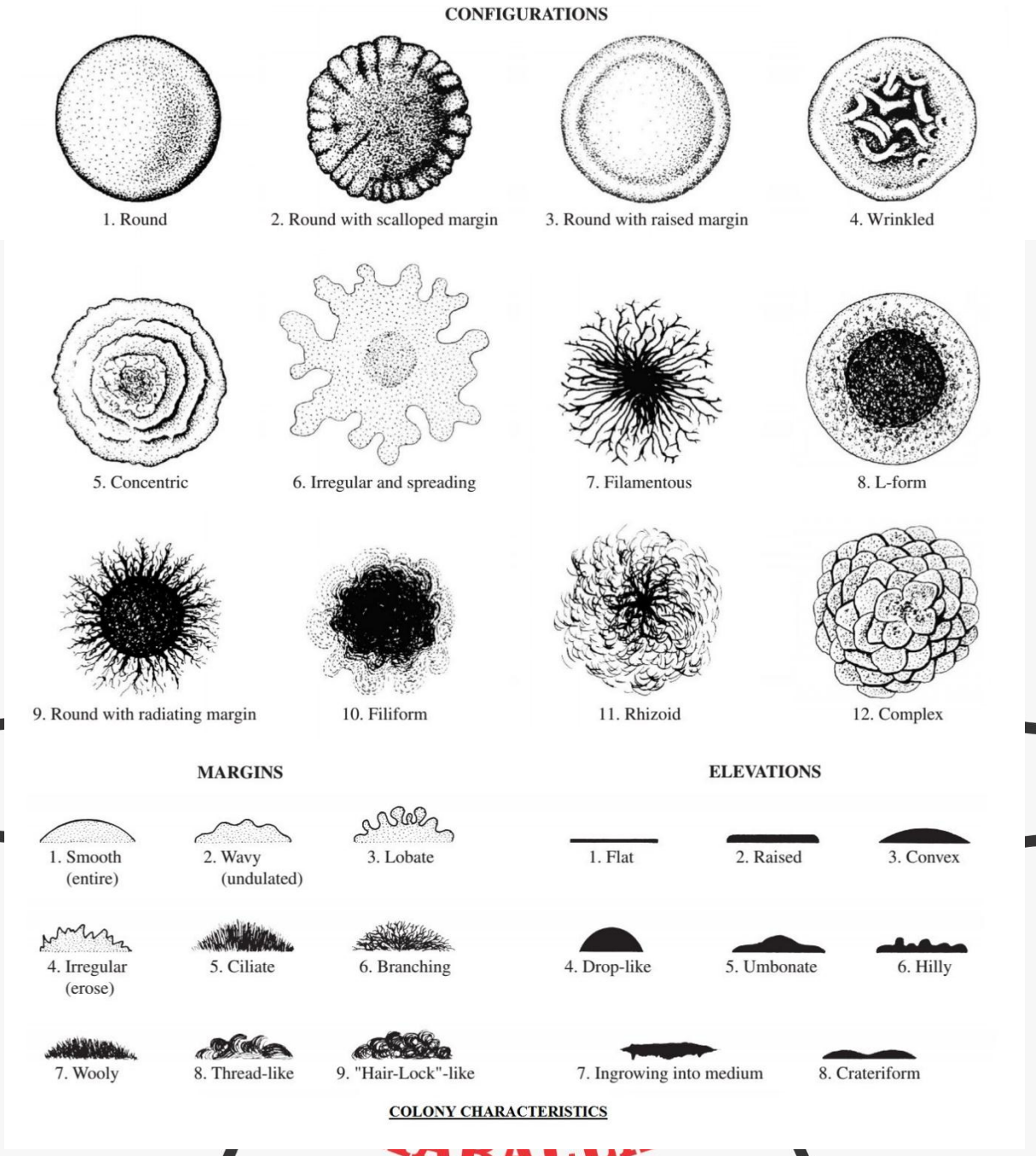
COLONY CHARACTERISTICS: - Bacteria

Sl. no	Shape	Colour	Elevation	Margin	Opacity	Gram's character	Possible organism
1	Circular	Cream	Raised	Entire	Opaque	G +ve Rods	*Bacillus*
2	Punctiform	White or Yellow	Flat	Entire	Opaque	G +ve cocci in clusters	*Staphylococcus*
3	Irregular	Fluorescent green/purple	Flat	Lobate	Translucent	G -ve Rods	*Pseudomonas*
4	Circular	White	Flat	Entire	Translucent	G –ve short Rods	*Escherichia coli*
5	Circular	Light orange	Raised	Entire	Opaque	G +ve cocci in chains	*Streptococci*

COLONY CHARACTERISTICS: - Fungi

Sl. no	Colour		Margin	Surface texture	Possible organism
	Upper surface	Lower surface			
1	Green with white periphery	White	Lobed/filamentous	Powdery with radiating marks	*Penicillium*
2	Black/green/different color	Black	Entire/filamentous	Powdery/granular	*Aspergillus*
3	White	Pink	Filamentous	Cottony	*Fusarium*
4	Green	Black	Finely lobed	Velvet	*Cladosporium*
5	Pink	Pink	Entire	Smooth	*Yeast*

Note: Any three Bacterial and any three Fungal colonies to be studied.



Filiform: characterized by uniform growth along the line of inoculation
Echinulate: margins of growth exhibit toothed appearance
Beaded: separate or semi confluent colonies along the line of inoculation
Effuse: growth is thin, veil-like, unusually spreading
Arborescent: branched, treelike growth
Rhizoid: root like appearance
Crateriform: saucer-shaped liquefaction
Napiform: turnip-like
Infundibular: funnel-like or inverted cone
Saccate: elongate sac, tubular, cylindrical
Stratiform: liquefied to the walls of the tube in the upper region

STUDY OF PLANT DISEASES

BACTERIAL DISEASE – CITRUS CANKER

Introduction: - The tumour like margins surrounding the wound are known as "Cankers".

Host: - Citrus species

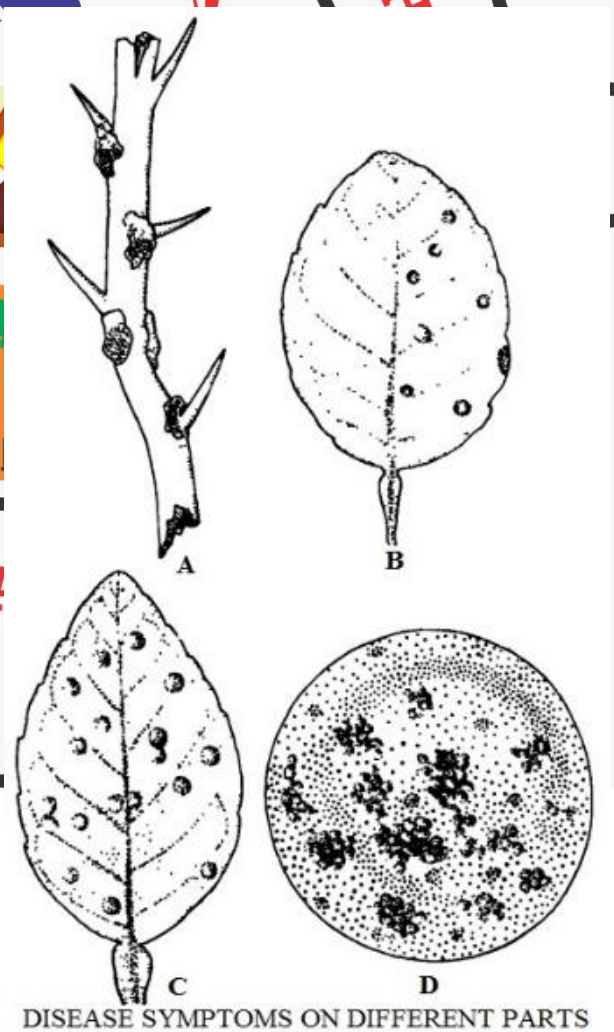
Causal Organism: - The pathogen responsible for citrus canker is *Xanthomonas citri* (*Phytomonas citri*). This organism is a short, rod-shaped bacterium, motile by means of a single polar flagellum.

Symptoms: - The disease symptoms appear on aerial parts such as leaves, twigs, fruits etc. producing crust like lesions or scabby spots and small open wounds or cankers. The cankers first appear as small yellowish brown, watery, translucent raised pimples on the ventral surface of leaves later as the spots became white or grayish and finally rupture exposing a light brown spongy central mass. The older lesions became corky, brown or sometimes pinkish and are surrounded by persistent halo. The lesions on the twigs are usually deformed and unmarketable.

Disease cycle: Disease is transmitted from older lesions to a new locality by wind, rain water and insects (Citrus leaf miners), even humans during transport. Infection occurs through stomata, wounds etc.

Control measures:

1. Destruction of all canker infected trees by burning.
2. Cut, separate and burn the infected parts.
3. Spraying with Bordeaux mixture, lime Sulphur to protect the fruits.
4. Spray neem cake, antibiotics such as streptomycin sulphate, phytomycin.
5. Look for resistant varieties.



DISEASE SYMPTOMS ON DIFFERENT PARTS
A. ON STEM, B,C. ON LEAF D. ON FRUIT

CITRUS CANKER

MYCOPLASMAL DISEASE – SANDAL SPIKE DISEASE

Introduction: Sandal spike disease is one of the important as the sandal tree is one of the most valuable of our forest trees. This was first reported from Frazerpet in coorg (1899). Since then it is rapidly spreading throughout southern Karnataka and adjoining areas of Andhra Pradesh and Tamil Nadu.

Host: *Santalum album*

Causal organism: *Mycoplasma* (Mollicutes) or PPLO (Pleuro Pneumonia like organism) or MLO (*Mycoplasma* like organism).

Symptoms: The characteristic symptoms of disease appear in the aerial parts, especially in the leaves. In sandal spike disease two types of symptoms are seen.

1. Witches broom 2. Pendulous spike

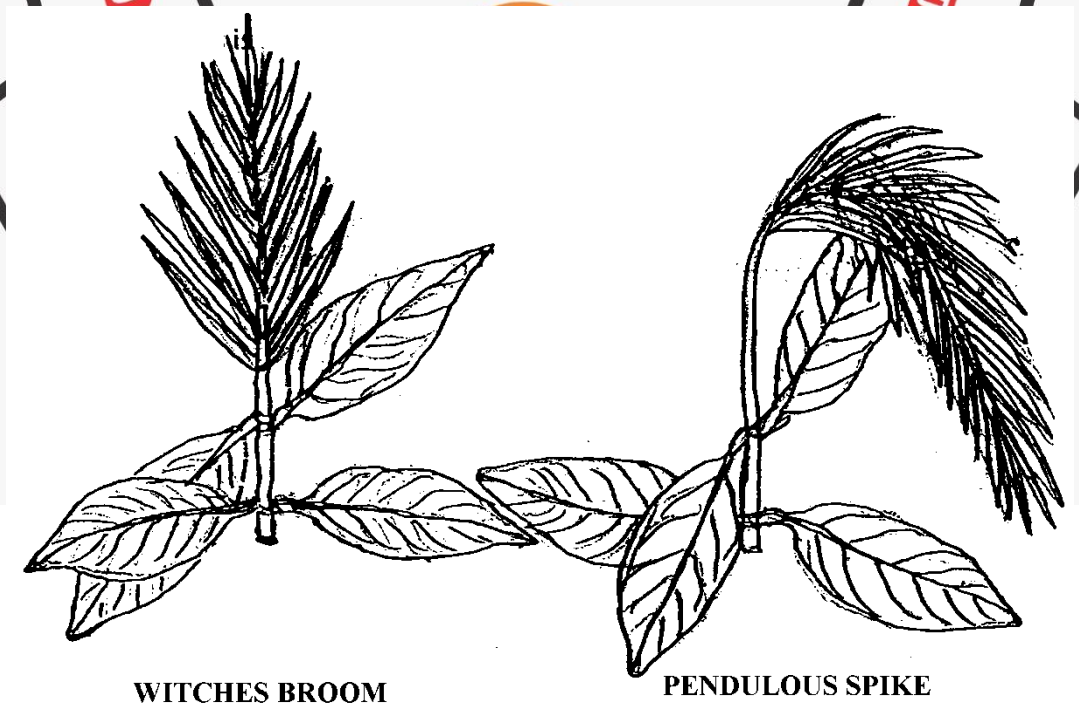
Witches broom – It is characterized by drastic reduction in leaf area and shortening of internodes of infected branches to give a broom like appearance, hence the name is given as witches broom.

Pendulous spike – It is characterized by the marked and unusual activity of the apical bud to the suppression of all the auxiliary buds, as a result there will be less branching. The affected shoot become usually long and droop down hence the name pendulous spike.

Disease Cycle: The causal agent, *Mycoplasma* transmit from infected plant to healthy one through agents like leaf hoppers (Jassid), vector of rice virus, through grafts and parasite like *Cuscuta*.

Control Measures:

1. Cutting and burning of infected plant parts.
2. Fungicide treatments like benlate along with antibiotics in different suitable intervals is superior to individual treatment.



WITCHES BROOM PENDULOUS SPIKE

SANDAL SPIKE DISEASE

VIRAL DISEASES – TOMATO LEAF CURL

Introduction: The disease has been reported from many part of the world. It is fairly common in the winter season in India.

Host: *Lycopersicon esculentum* (Tomato)

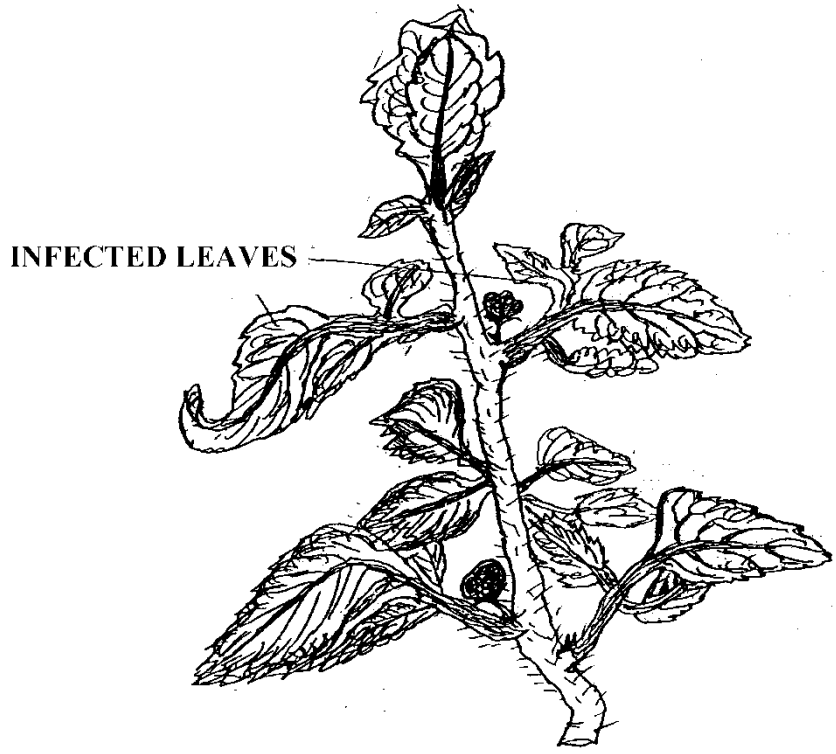
Causal organism: Begomovirus or TYLCV (Tomato yellow leaf curl virus) belong to family Geminiviridae.

Symptoms: The infection appear on leaves and shoots as a result the infected plant show bushy (because of excessive branching), stunted growth and drooping. The upper and lower leaf margins of leaves roll inward or upward along the midrib with yellowish margins. The leaves also show puckering, mottle, vein clearing. The infected leaves are thicker than healthy leaves with leathery texture. The infected leaves turn yellow, cupped and curly. Partial or complete sterility are the characteristic symptoms.

Disease cycle: It is not seed borne, but the seeds of freshly infected fruits may be contaminated. The principal vector is White fly (*Bemisia tabaci*).

Control measures:

1. Using resistant varieties for cultivation.
2. Identify the diseased plant, immediately remove and burn them.
3. Follow alternating or crop rotation by planting plants which are not susceptible to whitefly. Do not plant cotton and tomato together.
4. Spraying with Ekatox (0.02%) and Rogor (0.05%) at regular intervals of ten days helps to reduce the incidence of the disease.
5. Control insect vectors by systemic insecticides like methyl parathion (0.02%) and dimethoate (0.05%)



LEAF CURL OF TOMATO

VIRAL DISEASES – PAPAYA LEAF CURL

Introduction: The leaf curl of papaya is widely spread disease in many states of India especially in Bihar, Delhi, Madhya Pradesh, Uttar Pradesh, Tamil Nadu etc.

Host: *Carica papaya* (Papaya)

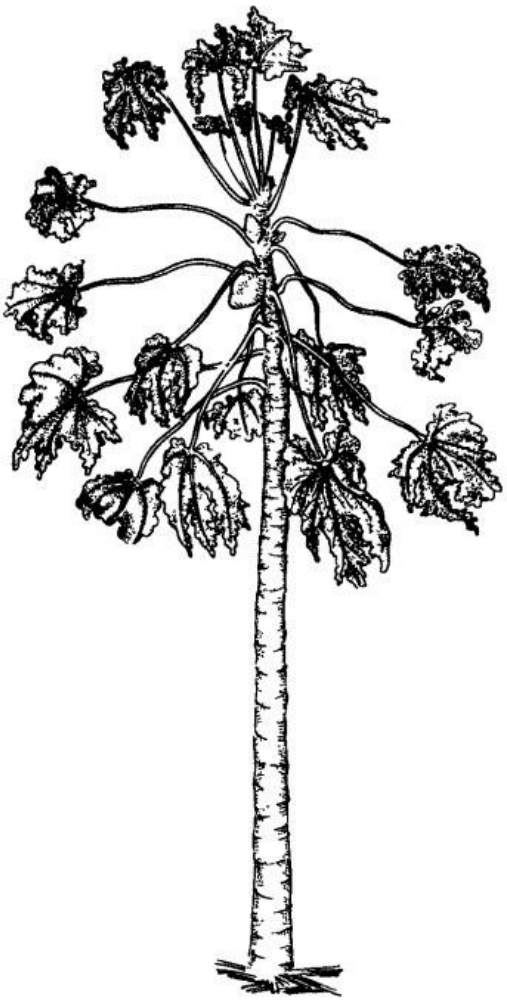
Causal agent: Papaya leaf curl virus (PLCV) or Tobacco leaf curl virus (*Tobacco virus 16* or *Nicotiana virus 10*)

Symptoms: The prominent symptoms are downward and inward rolling of leaves and thickening of veins. The leaves show vein clearing, reduction in size, crinkling, distortion and raised interveinal areas (due to hypertrophy). They become leathery and brittle. The severe infection cause the leaf margins turn downwards and form inverted cup like structures. The petioles are twisted in zigzag manner. The growth is checked (stunt) and fails to flower.

Disease cycle: In nature the virus is transmitted by white fly (*Bemisia tobaci*). The virus can be transmitted by graft or juice inoculation.

Control Measures:

1. As soon as the disease is noticed the plant must be uprooted and destroyed.
2. Avoid growing alternative hosts such as tomato and tobacco.
3. Spray insecticides for vector control.



PLANT OF PAPAYA SHOWING SYMPTOMS
LEAF CURL OF PAPAYA

VIRAL DISEASE: YELLOW MOSAIC OF BEANS

Introduction: BYMV was first reported in the early 1900's infecting garden pea in the Northeastern United States. Today the virus is distributed all over the world. This destructive disease of Urd and Moong can cause total yield losses if infection is early. It also attacks Pigeon pea.

Host: legumes- green beans (*Phaseolus vulgaris*), peanuts (*Arachis hypogaea*), soyabeans (*Glycine max*), Faba beans (*Vicia faba*), etc.

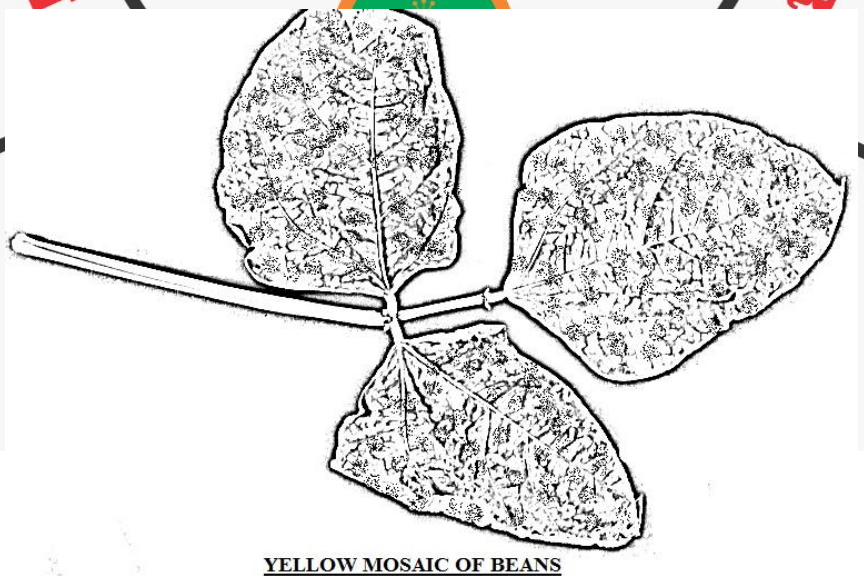
Causal organism: Bean yellow mosaic virus (BYMV) is a plant pathogenic virus [1] in the genus *Potyvirus* and the virus family Potyviridae.

Symptoms: The first sign of the disease is appearance of mosaic (yellow round spots scattered on the leaf lamina), leaf size reduction and leaf mottling. Leaves often become brittle, glossy and cupped downward. BYMV affects older leaves. Stunting may be severe or mild, depending upon the virus strain. Some cultivars may exhibit veinal necrosis and there is reduction in number and size of pods. The pods are deformed and they contain shriveled and undersized seeds.

Disease cycle: The BYMV is not transmitted by sap, seed or soil. The only known vector is *Bemisia tabaci*.

Control measures:

1. Once the plant has either strain of BYMV, there is no treatment and the plant must be destroyed.
2. Aphid control is vital to control BYMV. Check the underside of the leaves for aphids and if found treat immediately with an insecticidal soap or neem oil.
3. A few resistant cultivars have been developed.
4. Spray with metasystox (0.1%) at 10-15 days interval.
5. Rotate the bean crop every year, especially if you have had any infection in the past.



YELLOW MOSAIC OF BEANS

OTHER PLANT DISEASES– LITTLE LEAF OF TOMATO

Introduction: Tomato is one of the most important vegetable crops of the world. Modern cooking without tomato is virtually unknown. The fruits are very rich in vitamins and the pigment lycopene acts an antioxidant.

Host: *Lycopersicon esculentum* (Tomato)

Causal organism: According to Amy Grant, the possible culprits for little leaf disease are :-

a. A bacterium – *Bacillus cereus*
b. A fungus - *Aspergillus wentii*
c. Soil borne fungus - *Macrophomina phaseolina*

Disease Symptoms:

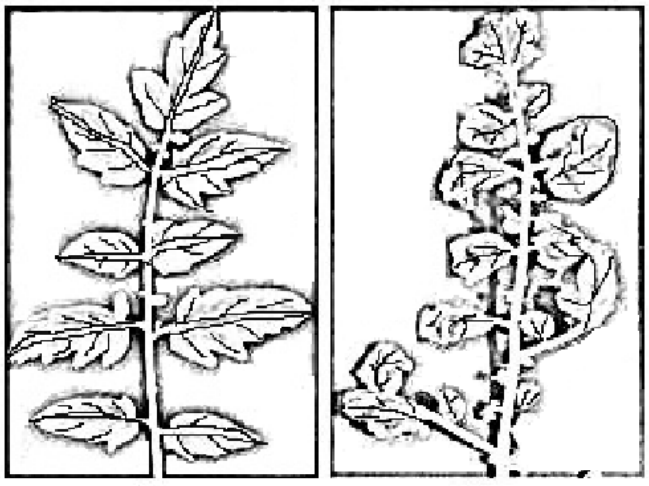
1. The most significant symptom is reduction in size of the leaf. Leaves along midrib do not expand properly.
2. Young leaves exhibit interveinal chlorosis.
3. Buds fail to grow or even if grown distorted.
4. The affected fruit will have practically no seeds. Flattened fruit with radial cracks from calyx to the blossom scar.

The causative agent is unknown, but symptoms are similar to frenching of tobacco where, under certain conditions, a nonparasitic soil microorganism disturbs the protein metabolism of the plant. Little Leaf (frenching) in tobacco is favored by moderate temperatures (70-95°F).

Symptoms are more severe on neutral or alkaline soils, rarely in soil with a pH of 6.3 or less. Symptoms are more prevalent in wet areas. The problem does not carryover, and drying the field will result in normal growth of the plant.

Control measures:

Currently, no commercial cultivars with known resistance to little leaf are available. Because the cause is yet undetermined, there is no chemical control available either. Drying out wet areas of the garden and reducing the soil pH to 6.3 or less with ammonium sulfate worked in around the roots are the only known controls, cultural or otherwise.



HEALTHY LEAF INFECTED LEAF

LITTLE LEAF OF TOMATO

GRAM'S STAINING: -

a) *Rhizobium* from root nodules

b) *Lactobacillus* from curds

Introduction: - Gram staining is a differential staining method for bacteria first developed by Dr. Hans Christian Gram, a Danish physician in 1884. It is a very useful technique for identifying and classifying the bacteria into two groups. 1. The Gram +ve 2. The Gram –ve.

In this staining process, the bacteria are fixed as a smear to the glass slide and subjected to four different reagents namely; Crystal violet (Primary stain), iodine (mordant), ethyl alcohol (decolorizing agent), and safranin (Counter stain). The bacteria which retain dark blue colour of the primary stain are called Gram +ve whereas those which losses the crystal violet and become stained by safranin (counter stain) appear red are called Gram –ve bacteria.

Mechanism of staining:

The difference in staining response is related to the chemical composition and architecture of the cell wall of the bacteria.

1) The cell wall of Gram –ve bacteria is thin, complex and multilayered with relatively high lipid content in addition to protein and mucopeptides. Lipid is dissolved by alcohol resulting in formation of pores in the cell wall that facilitate leakage of crystal violet and iodine complex. Therefore, the bacteria become decolorized and late take counter stain (Safranin) and appear red.
2) The cell wall of Gram +ve bacteria is thick and chemically simple, composed of protein and peptidoglycan when treated with alcohol, it causes dehydration and closure of cell wall pores therefore the crystal violet iodine complex is retained and the cell remains blue.

Requirements: -

Bacterial Sample: root nodules of *Trigonella* plant (Methi) and Whey from curd.

Reagents: Crystal violet, Gram's iodine, Alcohol and Safranin.

Miscellaneous: Watch glass, slides, cover glass, spirit lamp, blotting paper, microscopes, inoculation loop, distilled water wash bottle, dropper.

Procedure for staining *Lactobacillus* from curd

1. Take clean and dried glass slide and place a drop of curd sample and gently spread to make a thin smear with another slide or inoculating loop and is fixed by passing it over the flame of the spirit lamp. .
2. Allow the smear with crystal violet for about 1 minute.
3. Wash the slide with distilled water for few seconds using wash bottle.
4. Cover the smear with Gram's iodine for 1 minute.
5. Wash off the iodine solution with water and add drops of 95% ethyl alcohol to the smear until no more colour flows from the smear.
6. Wash the slide with distilled water and drain.
7. Counter stain with safranin for 1minute.
8. Wash the smear with distilled water and air dry the smear, add a drop of glycerine, put a cover glass on the smear and observe under the different objectives of microscope for Gram's reaction.

Procedure for staining *Rhizobium* from legume root nodules

1. Separate root nodules from *Trigonella* plant, wash thoroughly with tap water and distilled water and surface sterilize it with 0.1% mercuric chloride solution for 1 minute.
2. Select a good root nodule on to a dry watch glass and crush in 2 to 3 drops of distilled water, a milky white suspension of bacteria is formed. Or Place a nodule on a clean glass slide and crush it by pressing another slide over it and smearing.
3. A thin smear from suspension is made on a clean glass slide which is fixed by passing it over the flame of the spirit lamp.
4. Stain the smear with crystal violet for 60 seconds.
5. Wash with distilled water using wash bottle 2-3 times.
6. Cover the smear with iodine solution for 60 seconds.
7. Wash off iodine solution with distilled water.
8. Add ethyl alcohol drop by drop until no more colour flows from the smear.
9. Wash the slide with distilled water and drain.
10. Counter stain with safranin for 1minute.
11. Wash with distilled water 2-3 times and blot dry. Air dry the smear, add a drop of glycerine, put a cover glass on the smear and observe under the different objectives of microscope for Gram's reaction.

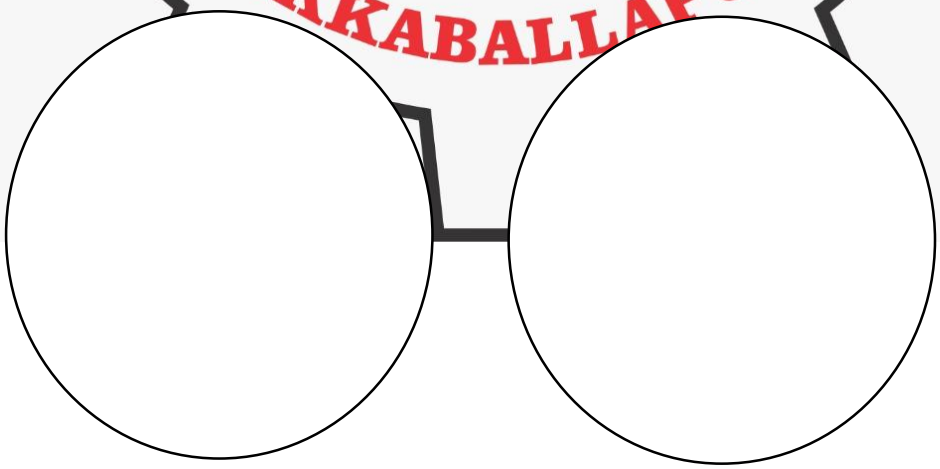
Observation: Bacteria appearing purple colour are referred to as Gram positive bacteria and those appearing pink are called gram negative bacteria.

Result: The bacterial cells observed under microscope were _____

Discussion: Though Gram staining is the most widely used technique. Reaction may vary with pH of the medium. Dead Gram +ve organism are said to stain Gram –ve. The common assumption is that Gram +ve organisms give the strongest reaction when they are very young with a tendency to become negative as grow older. This holds true in case of spherical bacteria.

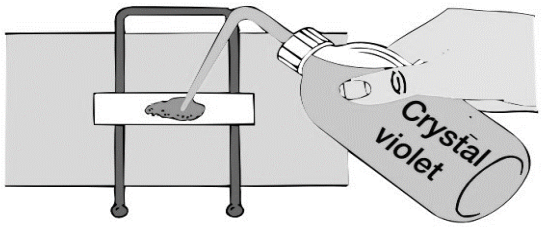
Precautions:

1. Smear should be properly heat fixed.
2. Too concentration or too diluted bacterial suspensions and stains are not to be used.
3. Slide should be completely blotted dry before observation.

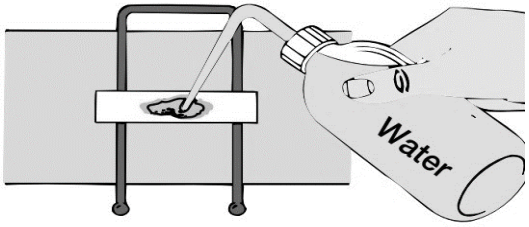


Gram positive bacteria Gram negative bacteria

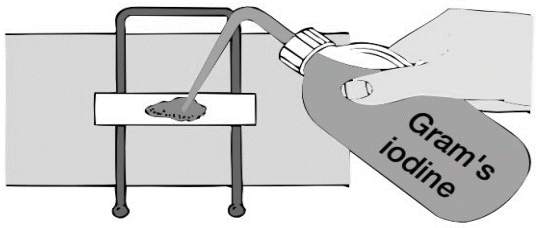
Gram-stain Procedure.



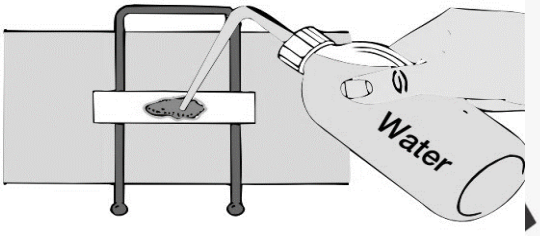
(a) Crystal violet; 30 seconds



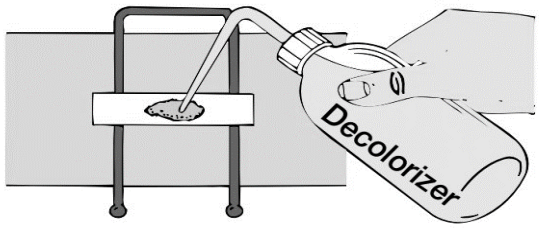
(b) Rinse for 5 seconds



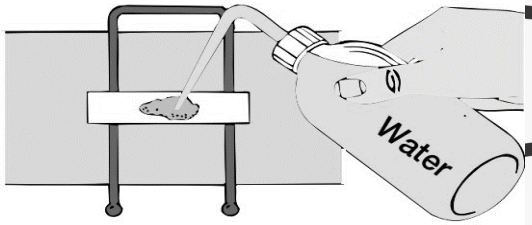
(c) Cover with Gram's iodine for 1 minute



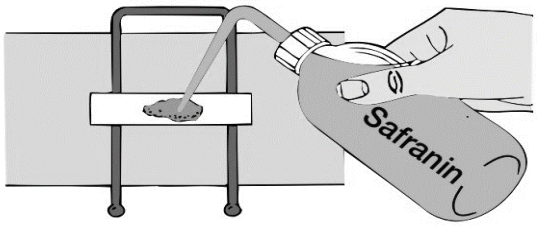
(d) Rinse with water for 5 seconds



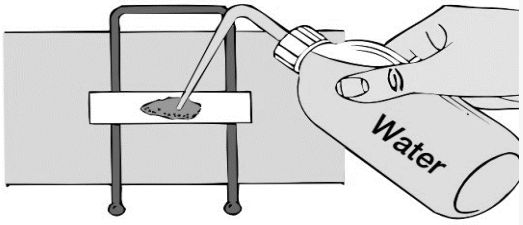
(e) Decolorize for 15–30 seconds



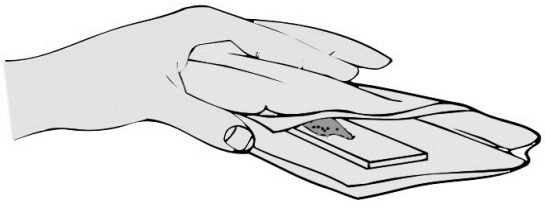
(f) Rinse with water for 5 seconds



(g) Counterstain with safranin for about 60–80 seconds



(h) Rinse for 5 seconds



(i) Blot dry with bibulous paper

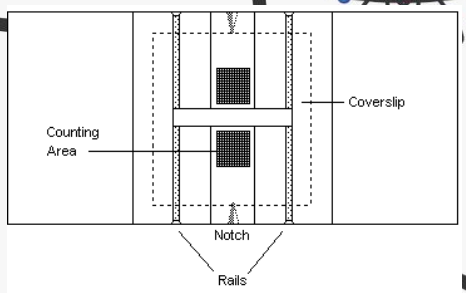
MEASUREMENT OF CELL CONCENTRATION OF FUNGAL SPORES OR YEAST CELLS BY USING HAEMOCYTOMETER (COUNTING CHAMBER).

Introduction:

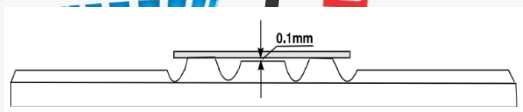
It is used to count cells (blood cells, cells in fluid of the brain and spinal cord, bacteria, sperm, Pollen, fungus spores etc.,) or other particles in suspensions under a microscope.

Construction and Principle of the Haemocytometer:

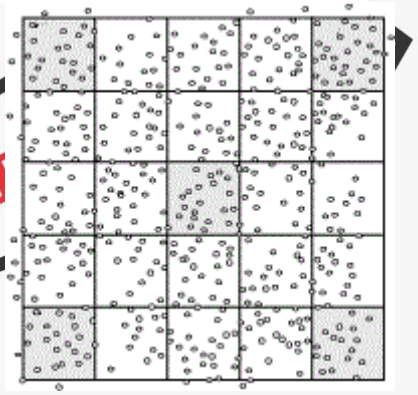
The Haemocytometer was invented by Louis-Charles Malassez and consists of a thick glass microscope slide with a depression in the center that creates a chamber. The device is carefully crafted with laser so that the area bounded by the lines is known, and the depth of the chamber is also known. It is therefore possible to count the number of cells or particles in a specific volume of fluid, and thereby calculate the concentration of cells in the fluid overall. There are four longitudinal grooves in the central third of a glass slide. The two larger external surfaces are unfinished and are used for marking purposes. The central support and the two external supports are ground flat, parallel and polished. The surface of the central support is slightly deeper than the ones of the two external supports. The counting grids are engraved into the central support (chamber base). When a cover glass is placed on to these external supports this forms a capillary gap between the rear side of this cover glass and the central support of the counting chamber. Depth of chamber is 0.1 mm. The center of the depression consists of a specific area divided into 9 squares measuring 1 sq. mm. The large square in the middle is also divided five times into 25 smaller squares with an edge length of 0.2 mm each and an area of 0.04 sq. mm each. These squares in turn are divided into sixteen very small squares each with an area of 0.0025 sq. mm. These smallest squares are used for counting.



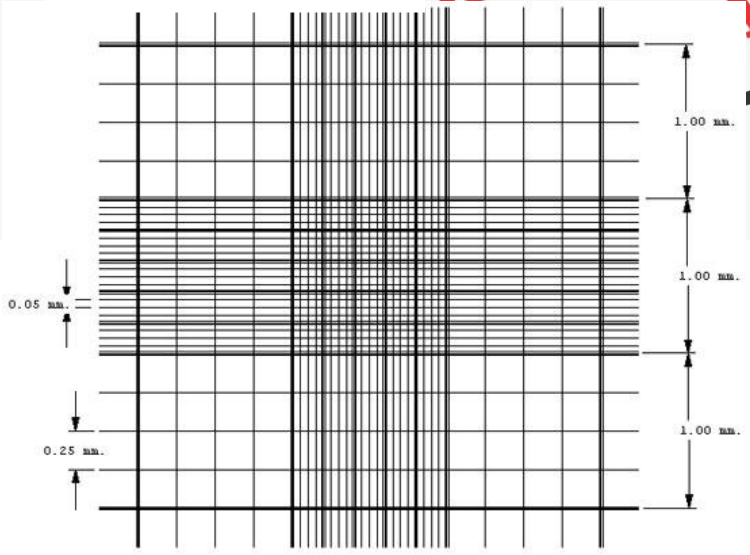
TOP VIEW HAEMOCYTOMETER

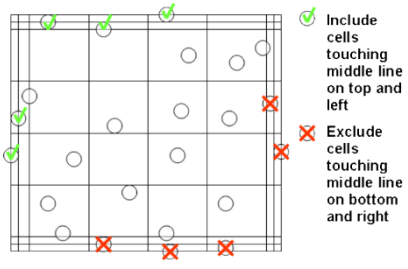


SIDE VIEW HAEMOCYTOMETER



CENTER SINGLE LARGE SQUARE ENLARGED





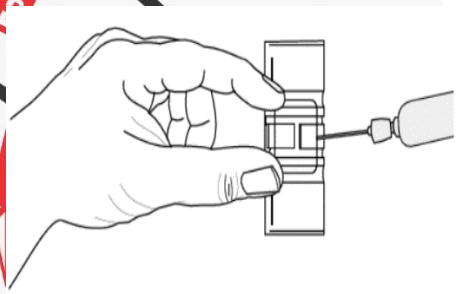
NINE LARGE SQUARES

Procedure:

1. Clean the slide before you load the sample by rinsing the slide and cover slip with 70-95% ethanol. Air dry or gently wipe the slide and cover slip with lens paper or Kim wipes. Never use paper towels or soaps.
2. Using a micropipette, quickly and smoothly without interruption, add 10 µl of your cell suspension (or 1 drop from a transfer pipette) to the v-shaped groove on each side of the haemocytometer.
3. Place the coverslip on top to cover the reflective surfaces.
4. Allow each chamber to fill by capillary action. The chamber should not be overfilled or under filled.
5. View and count the cells in the small squares under a microscope preferably 100x magnification.

Calculation:

The total number of cells per microliter of sample can be calculated from the number of cell counted and area counted. This is because the ruled areas of the chamber contain an exact volume of diluted sample. Since only a small volume of diluted sample is counted, a general formula must be used to convert the count into the number of cells/microliter.



The dilution factor used in the formula is determined by the sample dilution used in the cell count. The depth used in the formula is always 0.1mm. The area counted will vary for each type of cell count and is calculated using the dimensions of the ruled area.

1. The area of the small square = length × breadth
   = 0.05mm × 0.05mm
   = $0.0025mm^2$
2. The volume of small square = Area × depth
   = $0.0025mm^2$ × 0.1mm
   = $0.00025mm^3$

Total number of spores or cells counted in small square = X = _____

Total number of small squares counted = Y = _____

□□=Z

Formula:

$$\frac{Z \times 10.00025 \times d}{}$$

= _____ cells/ml

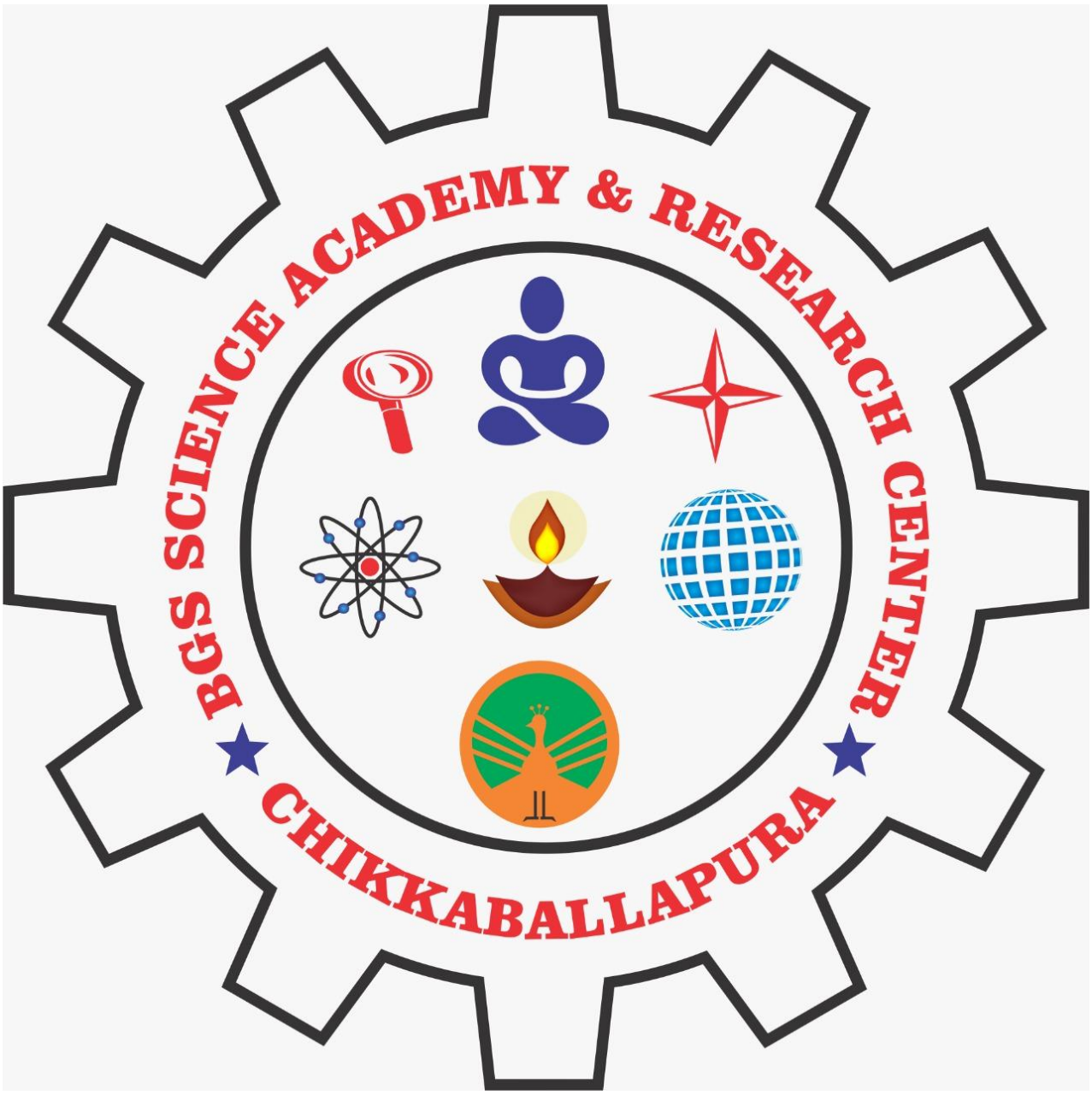
Instruction and precaution for calculation

1. The four corner squares are used for spore and white blood cell (WBC) count. Each square which is divide into 16 small squares with a depth of 0.1mm. The volume of each 116×125×0.1mm×d . These squares can be seen in low power and high power only.
2. The central square is used for red blood cell (RBC), bacteria and small sized fungal spore. This square is made up of 25 small squares, each square is further divided into 16 small 116×125×0.1mm×dilution factor.

3. The cells on the top and left touching the middle line of the perimeter of each square are counted.
4. A minimum of 200-250 spores / cells should be counted to avoid error.

Result:

The total number of cells or spores per ml of the given sample is _____



TYPE STUDY OF CYANOBACTERIA

The members of the class cyanophyceae or myxophyceae are called cyanobacteria or blue green algae (due to the presence of specific pigmentation in their cells). This class contains about 2,500 species placed under 165 genera, which are distributed mainly in fresh water but some are semi-terrestrial and few are marine also.

Ex: *Anabaena*, *Scytonema*, *Spirulina*

Anabaena

Classification:

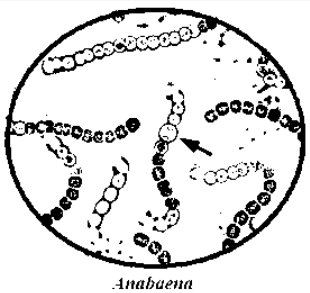
Class – Cyanophyceae
Order – Nostocales
Family – Nostocaceae
Genus – *Anabaena* (Gr: anabaino, I will go up)

Introduction:

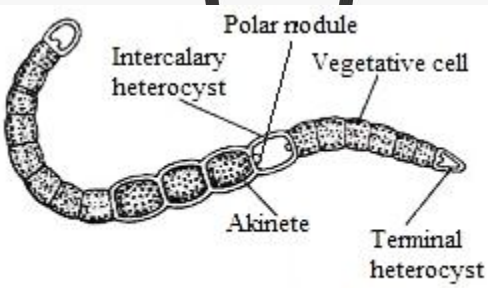
About 25 species of *Anabaena* have been recorded from India. Some of the important species are *Anabaena plantorica*, *A. spiroides*, *A. aphanizomenoides*, *A. sphaerica*, *A. gelatinicola*, *A. iyengarii* etc., They are fresh water free floating forms commonly found in ponds, pools, rice fields and in wet places. Some of them are symbiotic with coralloid roots of *Cycas* (*A. cycadacearum*), leaves of *Azolla* (*A. azollae*) and in the thallus of *Anthoceros*. It also forms water bloom.

General characters

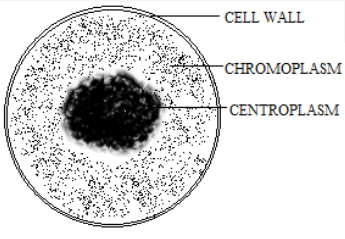
1) The thallus is filamentous (ordinarily straight but they may be circinate or irregular), unbranched, occur singly within a hyaline and watery gelatinous sheath but generally not surrounded by mucilaginous sheath.
2) The filament consists of chain of spherical or barrel or sub cylindrical cells.
3) Several terminal and intercalary heterocysts are present in the trichome. Heterocysts are of same shape as of vegetative cell. The terminal heterocysts are having one polar nodule whereas intercalary heterocysts has two polar nodules.
4) Special type of cell with thick wall and larger in size called akinetes are present either on one side of heterocyst or on both the sides or away from the heterocyst.
5) Each vegetative cell exhibits a typical cyanophycean cell structure with three layers (inner thin cellular layer, medium pectic layer and outer mucilage layer)
6) Protoplasm is composed of peripheral chromoplasm (contains pigment), central nucleus like material called centroplasm or chromatin granules (colourless) and gas vacuoles.
7) No sexual reproduction but asexual reproduction takes place by hormogones, Akinetes and heterocysts.
8) Akinetes and heterocysts play a role in nitrogen fixation.



Anabaena
HABIT UNDER LOW POWER



A Filament enlarged



A SINGLE VEGETATIVE CELL ENLARGED

Anabaena

Scytonema

Classification:

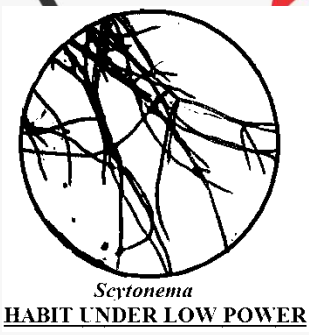
Class – Cyanophyceae
Order – Nostocales
Family – Scytonemataceae
Genus – *Scytonema* (Gr Skytos-leather, nema-thread)

Introduction:

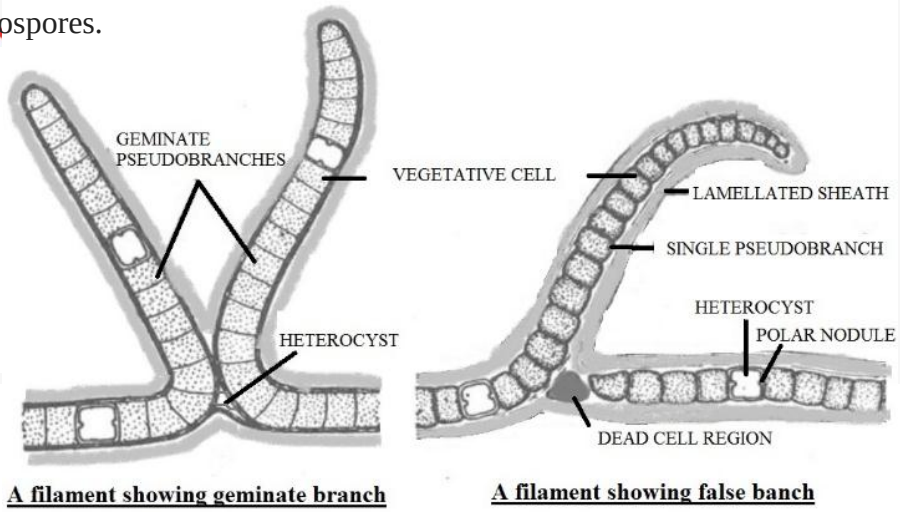
About 40 species of Genus *Scytonema* have been recorded from India. Some common species are *Scytonema ocellatum, S. simplex, S. iyengarii, S, vivilove, S. varuith* etc., Among 40 species 9 are aquatic and 31 are semi-terrestrial found on the bottom of ponds, pools, damp soil, some are in symbiotic association with fungus to form lichens and few are epiphytic.

General characters:

1. The plant body is filamentous, branched*, surrounded by a lamellated mucilage sheath and interwoven to form a felty mass.
2. The branching in *Scytonema* is peculiar with two types of false branching i.e., geminate branch and false branch. The geminate branching takes place at heterocyst cell and form two branches whereas false branching takes place at dead cell region with single branch.
3. Vegetative cells may be discoid or rectangular or cylindrical in shape.
4. The special type of transparent or pale yellow large cells are present in between the vegetative cells called heterocysts, which may be terminal or intercalary with one or two nodules respectively.
5. Each vegetative cell resembles a typical cyanophycean structure with central centroplasm, granular cytoplasm, peripheral chromoplasm and pseudovacuoles.
6. Absence of sexual reproduction but vegetatively reproduce with the help of hormogones. In rare species reproduction may be through pseudohormogonia, Akinetes and exospores.



Scytonema
HABIT UNDER LOW POWER



A filament showing geminate branch

A filament showing false banch

Scytonema

Spirulina

Classification:

Class – Cyanophyceae
Order – Nostocales
Family – Oscillatoriaceae
Genus – *Spirulina* (L. spirula, a small coil)

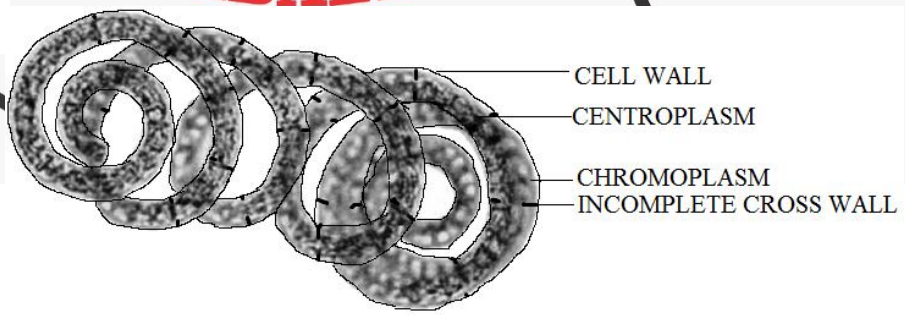
Introduction:

Only few species of *Spirulina* have been discovered and some well-known species are *Spirulina major, S. subsalsa, S. subtilisima, S. platensis, S. princeps, S. fenneri, S. versicolor.* They are free floating (planktonic) found in fresh water ponds, pools, ditches and may also grow in brackish water rich in salts.

General characters:

1. It is unicellular blue green algae.
2. The cell is cylindrical long spirally twist into a helix and it is not surrounded by mucilage sheath.
3. Occasionally cross walls may be found making the multicellular like appearance. Sometimes the cross walls are incomplete.
4. The terminal part of cell is rounded and has no calyptra.
5. Cell wall is multilayered made up of mucopolymer and pectic compounds.
6. The protoplast of the cell is differentiated into peripheral pigmented chromoplasm, central colorless incipient nucleus or centroplasm. It contains pseudo vacuoles or gas vacuoles (buoyancy).
7. No sexual reproduction, asexual reproduction takes place through fragmentation and hormogones formation.

Spirulina
HABIT UNDER LOW POWER



SINGLE CELL ENLARGED

Spirulina

TYPE STUDY OF ALGAE (PHYCOLOGY)

Chlamydomonas

Classification:

Class – Chlorophyceae
Order – Volvocales
Family – Chlamydomonadaceae
Genus – *Chlamydomonas*

Introduction: Genus *Chlamydomonas* is represented by about 500 species. Some important species are *C. reticulata*, *C. halophila*, *C. ehrenbergii*, *C. nivalis*, *C. yellowstonensis*. It is mainly found in fresh water rich in nitrogen salts and organic matter. It is also found in stagnant water of ponds, pools, ditches, water tanks, sewage tanks and in slow running water. Some species are found in salty brackish water and as cryophytes.

Vegetative structure:

Chlamydomonas is simple, unicellular, autotrophic, eukaryotic, planktonic algae. The shape of thallus can be oval, spherical, oblong, ellipsoidal or pyriform. They have narrow anterior end and a broad posterior end. The cell is surrounded by a smooth, thin and firm cell wall made of cellulose. The cell wall at the anterior end is extended to make apical papilla. The cytoplasmic structure includes the nucleus, mitochondria, endoplasmic reticulum, dictyosomes, ribosomes etc. The thallus contains single large, dark nucleus lying inside the cavity of the cup shaped parietal chloroplast. The dictyosomes or Golgi bodies are found near the nucleus. Each cell contains two contractile vacuoles (excretory or osmoregulation in function) located at the base of flagella. The anterior part of thallus bears two flagella. Both the flagella are whiplash. Each flagellum originates from a basal granule or blepharoplast. The pyrenoids are concerned with synthesis of starch. The anterior side of the chloroplast contains a tiny spot of orange or reddish colour called stigma or eyespot (photoreceptive organ).

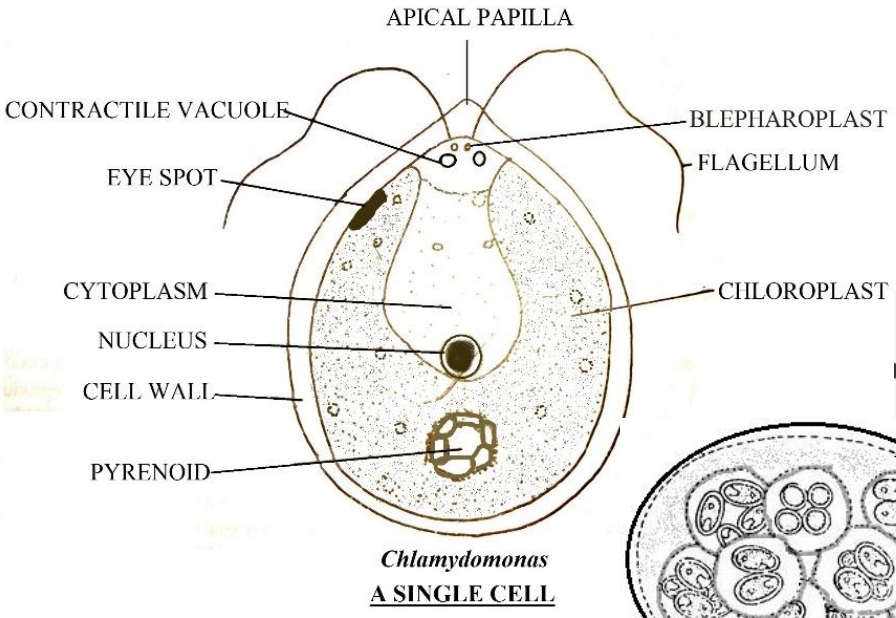
Reproduction:

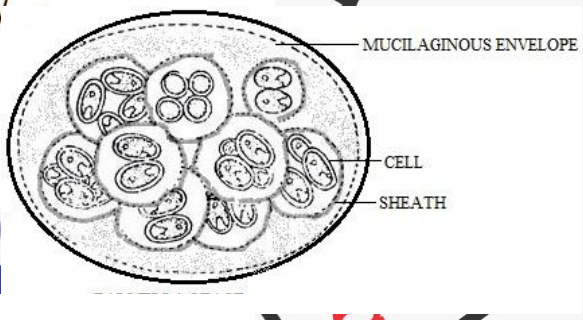
The reproduction in Chlamydomonas is both asexual and sexual.

Asexual Reproduction takes place by Zoospores, Aplanospores, Hypnospores, Palmella Stage.

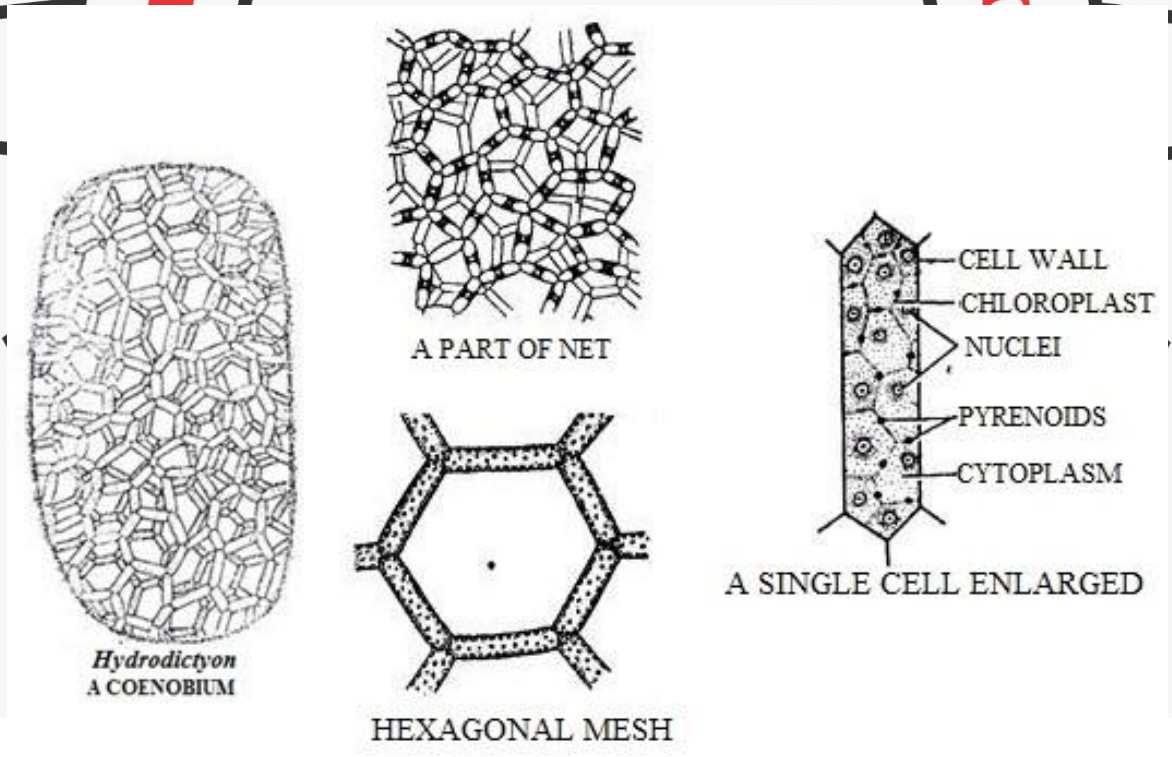
Palmella Stage: The palmella stage is formed under unfavorable conditions as shortage of water, excess of salts etc. The protoplast of parent cell divides to make many daughter protoplasts but they do not form zoospores. The parent cell wall gelatinizes to make mucilaginous sheath around daughter protoplasts. The daughter protoplasts also develop gelatinous wall around themselves but do not develop flagella. These protoplast segments are called palmellospores. The division and red visions of these protoplast ultimately forms amorphous colony with indefinite number of spores and it is called palmella stage. When favourable conditions return the gelatinous wall is dissolved, palmellospores develop flagella, and the spores are released to make new thalli.

The sexual reproduction in Chlamydomonas can be isogamous, anisogamous or oogamous. The thallus can be homothallic i.e., both types of gametes are produced in same thallus or can be heterothallic i.e., gametes come from different parents.





Chlamydomonas



Hydrodictyon

Hydrodictyon

Classification:

Class – Chlorophyceae
Order – Chlorococcales
Family – Hydrodictyaceae
Genus – *Hydrodictyon* (WATER NET)

Introduction: *Hydrodictyon* is represented by 5 species. Only two species are reported from India. *H. reticulatum and H. indicum* are common species. Due to its net like plant body, it is commonly known as **'water net'** sometimes nets assume big size and cover the entire pond. The species are commonly found floating but also lie on the bottom in slow running water or still water of ponds, pools and lakes.

Thallus structure:

A macroscopic, mature, non-motile, coenobium consists of a hollow cylindrical network which is closed at both the ends. It is flat and saucer shaped and its maximum size is generally 20-30 cm. The mature net of coenobium is made up of a few hundred to several thousand cells. These cells are joined at the end and form pentagonal or hexagonal structures. These structures are called meshes. Bach mesh interspace is generally bounded by 5-6 or rarely three cells. At each angle of the net or mesh meet three cells.

Cell Structure of *Hydrodictyon*:

Each cell is long, cylindrical or ovoid in shape. Its internal structure can be differentiated into two parts: cell wall and protoplasm.

Cell wall is two layered and is made up of cellulose. It encloses protoplasm. When young, the cells are uninucleate, but at maturity they become multinucleate (coenocytic).

Cells contain reticulate chloroplast with many pyrenoids. All the typical structures of green algae like ribosomes, mitochondria, dictyosomes are also present. As the cell matures, a central vacuole appears and the protoplasm becomes peripheral.

Reproduction:

It is of three types:

Vegetative, asexual and sexual.

- Vegetative Reproduction takes place by fragmentation.
- Asexual Reproduction takes place by the formation of auto colonies or daughter colonies. These colonies are formed by the biflagellate, uninucleate zoospores.
- Sexual Reproduction is isogamous.

Oedogonium

Classification:

Class – Chlorophyceae
Order – Chlorococcales
Family – Hydrodictyaceae
Genus – *Oedogonium* (Gr. oedos, swelling; gonos, reproductive bodies)

Introduction: Genus *Oedogonium* includes about 400 species more than 200 have been reported from India. Common species are *O. americanum, O. reliate, O. intermedium.* They are exclusively fresh water alga commonly found in pools, ponds, lakes etc. The filamentous plant body may get attached with the stone, wood, aquatic plants, small branches of dead plant remain in water etc. Some species are terrestrial.

Thallus structure:

The thalloid plant body is multicellular, filamentous, unbranched and cells of each filament are attached end to end and form uniseriate row. The filament is differentiated into 3 types of cells: 1. Basal cell, 2. Apical cell and 3. Middle (Intercalary) cells.

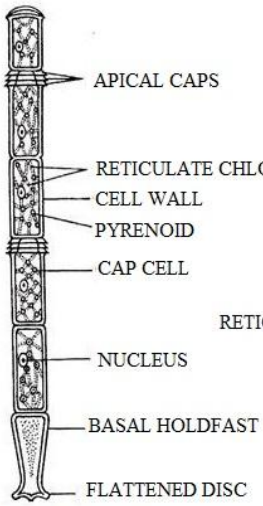
1. Basal Cell is the lowermost cell of the filament. The cell is long, gradually narrowed and towards the basal end it expands to form simple, disc like, multilobed or finger shaped structure. The cell is generally colourless and performs the function of fixation to the substratum and called holdfast.
2. Apical Cell is the topmost cell of the filament. The cell is usually rounded towards apical side.
3. Middle Cells are present between basal and apical cells and are alike. The cells are rectangular in shape.
4. The cells are surrounded by thick and rigid cell wall with three layers - an outer chitin, middle pectin and innermost cellulosic. Just interior to the wall, cell membrane is present, which encloses the protoplast. The protoplast consists of cytoplasm, chloroplast and nucleus. The cells contain many small or single large vacuoles situated in the center and remain filled with cell sap.
5. The Chloroplast is single, large and reticulate, which remains embedded in the cytoplasm and contains pyrenoids. Cells are uninucleate and nucleus is generally present in the center of the cell within the cytoplasm or it may be eccentric. Towards the upper end of some cells a ring like structure is present known as cap or apical cap. The cell with cap is called cap cell.

Reproduction:

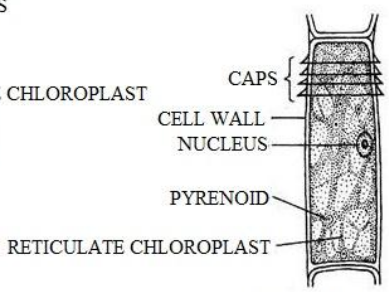
Oedogonium reproduces by all the three means: vegetative, asexual and sexual. vegetative by fragmentation and akinete formation. Asexual reproduction takes place by means of zoospores. The sexual reproduction in Oedogonium is an advanced oogamous type. The male gametes or antherozoides are produced in antheridium and the female gamete or egg is produced in oogonium. Based on the development of sex organs, species of *Oedogonium* are divided into two groups: i) Macrandrous and ii) Nannandrous type.

In macrandrous type the antheridia develop into the filament of normal size. These may be monoecious (In this type antheridia and oogonia are borne on the same filament) or dioecious (In this type the antheridia and oogonia are borne on the different filaments).

The nannandrous species are always dioecious (heterothallic) i.e., antheridia and oogonia are borne on different filaments. In nannandrous type the antheridia develop on small and thin male filament, the dwarf male or nannandrium (remain attached with the oogonium wall or its lower cell, the supporting cell), develop on germination of androspore.

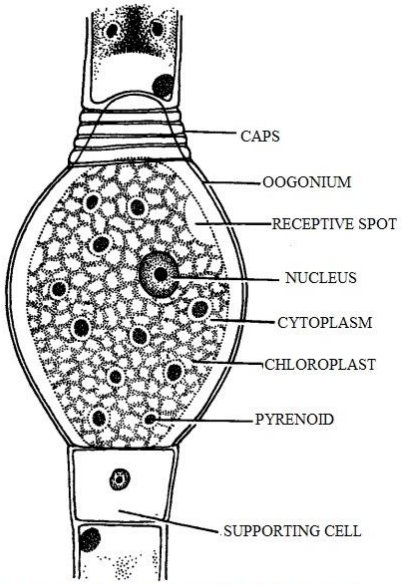


A VEGETATIVE THALLUS

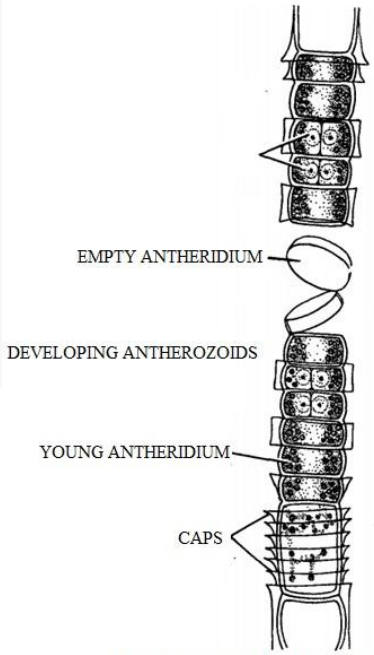


A CELL ENLARGED

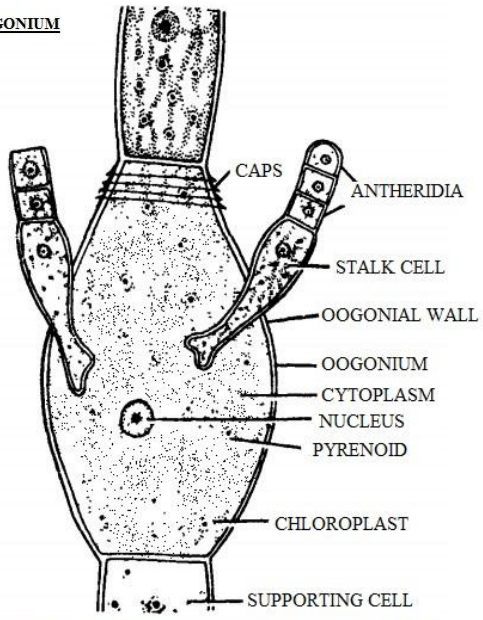
Oedogonium



MACRANDROUS SPECIES SHOWING OOGONIUM

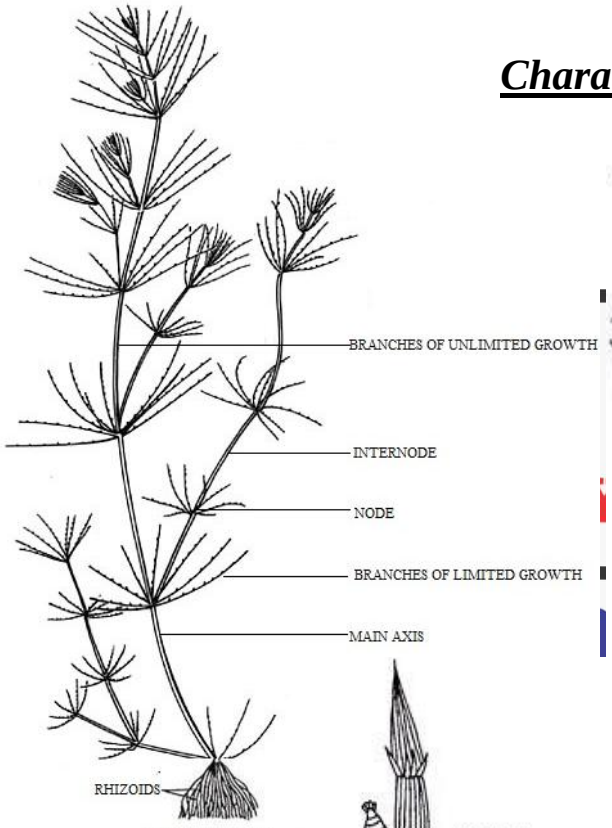


MACRANDROUS SPECIES SHOWING ANTHERIDIA

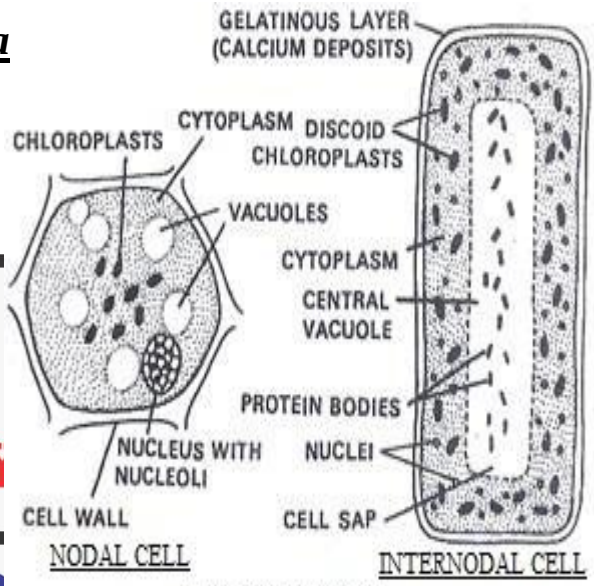


A NANNADROUS SPECIES SHOWING NANNANDRIA

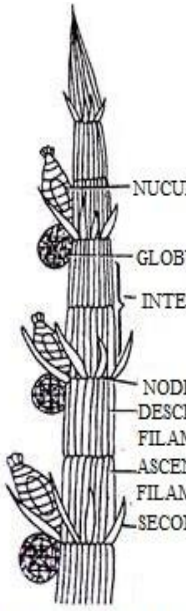
Chara



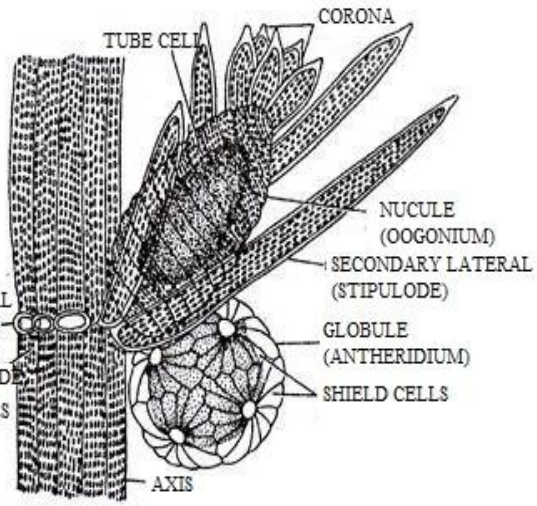
HABIT OF *Chara*



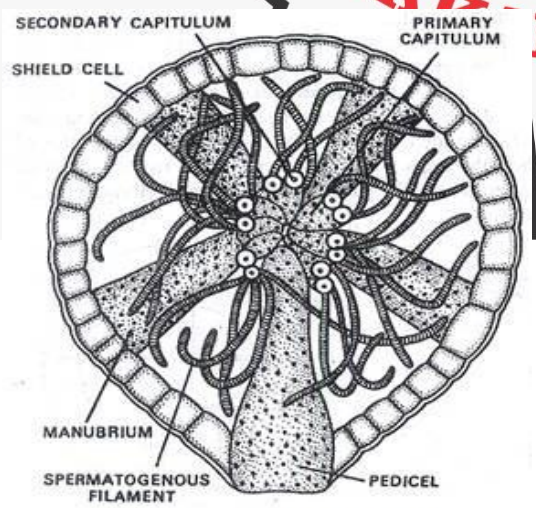
CELL STRUCTURE



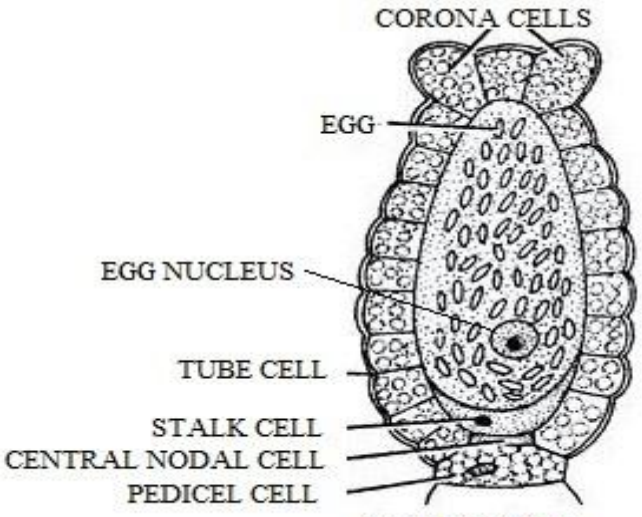
A PRIMARY LATERAL



SEX ORGANS OF *Chara*



L.S THROUGH GLOBULE



L/S OF NUCULE

Chara

Classification:

Class – Chlorophyceae
Order – Charales
Family – Characeae
Genus – *Chara*

Introduction: There are about 90 species in this genus. In India Chara is represented by about 30 species. The well-known species are *Chara baltica, C. zeylanica, C. wallichii, C. roxallina, C. braunii, C. gracilis, C. hatei,* etc. They are freshwater and grow submerged attached to the muddy bottom of the pools, ponds of clear water, wet soil, rice field etc.

Structure of the plant body: Chara plants are called stonewort (encrusted with calcium carbonate) or aquatic horsetail (resemble Equisetum). The thallus of Chara is branched, multicellular and macroscopic. The thallus is mainly differentiated into rhizoids and main axis. The rhizoids are white, thread like, multicellular, uniseriate and branched structures help in attachment of plant to substratum. The main axis is erect, long, branched and differentiated into nodes and internodes. The internode consists of single, much elongated or oblong cell. The internodal cells in some species may be surrounded by one celled thick layer called cortex and such species are called as corticate species. The species in which cortical layer is absent
are called ecorticate species.

On nodes develop these following types of appendages:

(i) Branches of limited growth or branchlets or primary laterals or leaves - branches of first order, grow in whorls from the nodes of the axis. The stipulodes and reproductive structures are formed on the node of these branches.

(ii) The branches of unlimited growth arise from the axils of the branches of limited growth hence these are also called auxiliary branches or long laterals. differentiated into nodes and internodes. At nodes they bear primary laterals and these branches look like the main axis.

(iii) The basal node of the branches of limited growth develops short, oval, pointed single cell outgrowths called stipulodes.

The main axis of Chara consists of mainly two types of cells:

(i) Nodal cells (ii) Internodal cells.

The nodal cells are smaller in size and isodiametric. The cells are dense cytoplasmic, uninucleate with few small ellipsoidal chloroplasts. Many small vacuoles are present. The internodal cells are much elongated with a cytoplasm present around a large central vacuole. The cells are multinucleate and contain many discoid chloroplasts. In both, cytoplasm is also differentiated into outer exoplasm and inner endoplasm. The endoplasm shows streaming movements. The cell wall consists of homogeneous cellulose, outer gelatinous layer and deposition of calcium.

Reproduction:

The reproduction takes place by vegetative and sexual methods. Asexual reproduction is not found. The vegetative reproduction takes place by (a) tubers or Bulbils (b) Amorphous bulbils (b) amylum stars and (c) secondary protonema.

The sexual reproduction in Chara is of highly advanced oogamous type. The sex organs are macroscopic and complex in organization.

The male sex organs are called antheridium or globule and the female oogonium or nucule. Most of the species are homothallic (monoecious) and few are heterothallic (dioecious). The sex organs arise on the branches of limited growth or primary laterals, the nucule above the globule. The development of globule and nucule takes place simultaneously but species globule matures before nucule.

The mature globule is large, spherical, red or yellow structure with a long pedicel and made up of 8 curved shield cells, 8 elongated manubrial cells, 8 centrally located primary capitulum cells and 48 secondary capitulum cells. The secondary capitulum cells give rise to many antheridial filaments which give rise to sperm mother cells. Each one forms a single bi-flagellated antherozoid.

The nucule of Chara is large, green, oval structure with short stalk. The nucule is surrounded by five tube cells. The tips of tube cells form corona at the top of nucule. The oogonial cell possesses a single large egg or ovum.

Sargassum

Classification:

Class : Phaeophyceae
Order : Fucales
Family : Sargassaceae
Genus : *Sargassum*

Introduction:

The genus Sargassum is represented by about 150 species. The genus is widely distributed; specially in warmer regions mainly in tropical and subtropical seas of the southern hemisphere. In India *Sargassum* is represented by about 16 species. Some common Indian species are: *S. carpophylla S. christifolium, S. cinereum, S. duplicatum, S. ilicifolium, S. myriocystwn, S. plagiophyllum and S. wightii*. The alga grows attached to the rocks in little bushes in the intertidal zone or in the shallow puddles of the zone.

Thallus Structure

The thallus of *Sargassum* is diploid and sporophytic. The thallus is differentiated into holdfast and the main axis. The attaching disc or holdfast is discoid or warty structure; it helps in attachment of thallus to substratum. In some species the hold fast is stolon like and in some free floating forms the holdfast is absent.

The main axis or stipe or **'stem'** is erect, elongated, cylindrical or flat upto cm in length. The main axis bears large number of primary laterals or branches in spiral phyllotaxy of 2/5 or the primary laterals are arranged on two sides of the main axis. The branching is always monopodial. The main axis and primary laterals bear flat leaf-like branches known as secondary laterals or **"leaves"**. The leaf-like laterals are flat and simple with blade, veins and petiole like structure. The branch system arises from the base of a **'leaf'** like lateral. The little branched laterals which arise from the base of **'leaves'** are variously modified, some into air bladders. These are globular or spherical, air filled structures. They help in floating of plants by increasing buoyancy. Another modification of these laterals is in the form of highly branched or swollen structures bearing reproductive bodies called receptacles. The receptacles bear reproductive structure in special flask shaped cavities called as conceptacles.

Internal Structures:

Main Axis:

1. Axis is differentiated into meristoderm, cortex and medulla.
2. Meristoderm is the outermost layer made up of closely-packed, small cells. The cells are meristematic in nature and filled with chromatophores. It is also covered by a layer of cuticle. This layer is also known as epidermis, and due to the presence of chromatophores its function is photosynthetic.
3. Cortex is made up of many parenchymatous cells. Cells are polygonal in shape and bear intercellular spaces. Cells are filled with reserve food.
4. Centre of the axis is occupied by a thick-walled region or medulla. Cells of the medulla are narrow and elongated. Conduction of water and other nutrients is supposed to be the function of medulla.

T.S. of Leaf:

1. All the structures in leaf are essentially similar to that of axis, i.e., it is also divided into epidermis, cortex and medulla.

2. Leaf is more thick in the midrib region and the wings are narrow, long and thin .
3. Epidermis is made up of small cells having chromatophores. Medulla is present only in the midrib region.
5. Many flask-shaped cryptostomata or sterile conceptacles are also present in the leaf. They contain many sterile hairs or paraphyses.
6. Cryptostomata open by a pore known as ostiole.
7. Epidermis is photosynthetic in function, cortex acts as storage region, and medulla serves the function of conduction.

T.S. of Air Bladder:

1. The outermost layer is meristoderm.
2. Inner to the meristoderm is present many-celled thick parenchymatous cortex.
3. In the centre of the air bladder is present a hollow cavity filled with air and gases.
4. Air bladders help in floatation.

Reproduction:

1. Asexual reproduction is absent.
2. Sexual reproduction is oogamous. Male and female sex organs are known as antheridia and oogonia, respectively.
3. Sex organs are present in flask-shaped fertile conceptacles, restricted only in specialized branches called receptacles.
4. Plants are monoecious but few species are dioecious. In monoecious plants also, the male and female sex organs are generally present in different conceptacles. So, conceptacles are generally unisexual.

T.S. of Receptacular Branch:

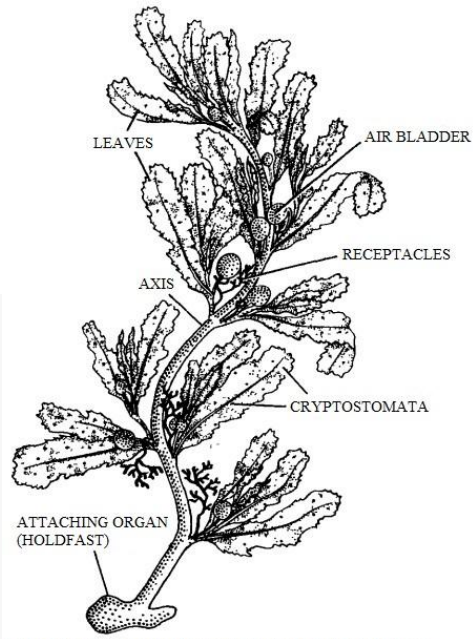
1. Receptacle contains many flask-shaped fertile conceptacles.
2. Each conceptacle is in the form of a cavity opening outside by an ostiole.
3. Cells of conceptacle wall are filled with many chromatophores.
4. Wall of the conceptacle gives rise to many multicellular, branched or unbranched, hair-like paraphyses.
5. Each conceptacle contains either antheridia or oogonia, and known as male or female conceptacle, respectively.

T.S. Through Male Conceptacle:

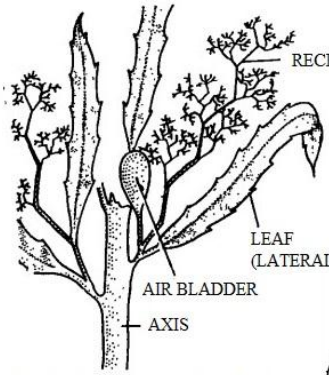
1. In the male conceptacle are present many antheridia.
2. Each antheridium is a small, ovoid and stalked structure present generally on lower branches of paraphyses.
3. Each antheridium contains many antherozoids.
5. Each antherozoid is unicellular, uninucleate and pear- shaped structure, with two laterally attached flagella.

T.S. Through Female Conceptacle:

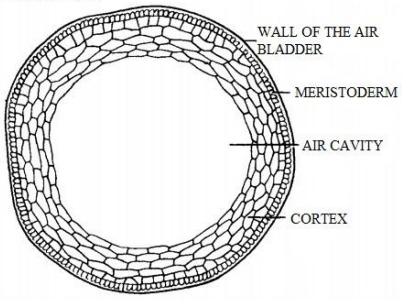
1. Many oogonia are present in the female conceptacle.
3. Each oogonium is rounded, and sessile when young, but contains long gelatinous stalk at maturity. Oogonium remains surrounded by three-layered wall, i.e., exochite, mesochite and endochite.
5. Each oogonium contains a single uninucleate egg.
6. Fertilization is internal and zygote germinates without any resting period.



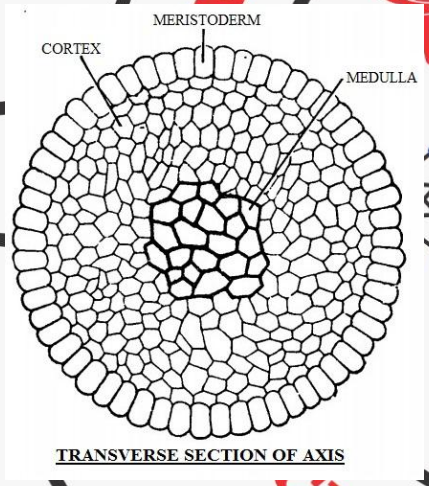
THALLUS TO SHOW EXTERNAL FEATURES



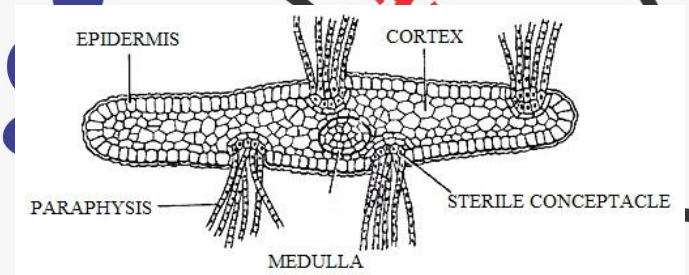
A PORTION OF THALLUS



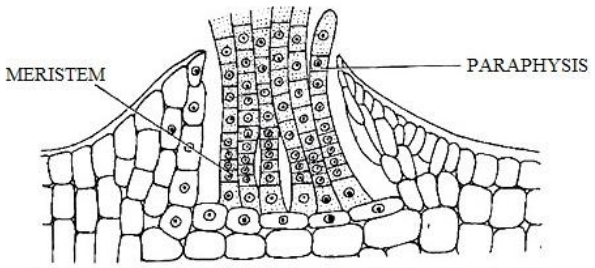
TRANSVERSE SECTION THROUGH AIR BLADDER



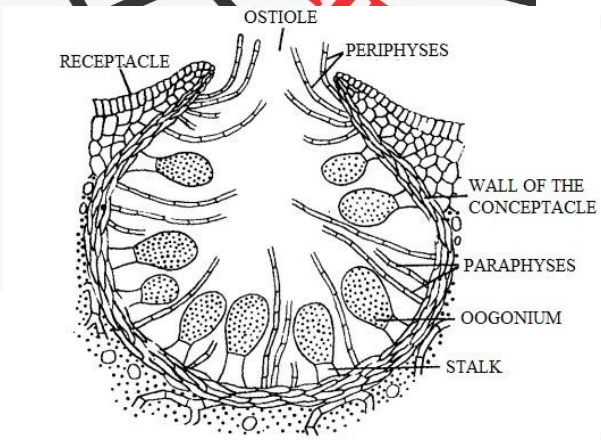
TRANSVERSE SECTION OF AXIS



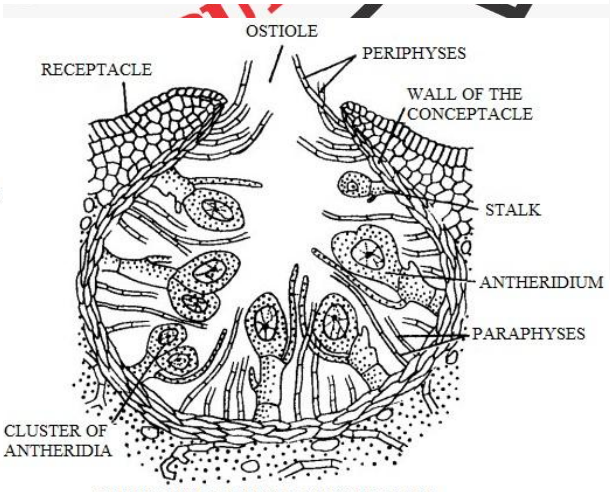
TRANSVERSE SECTION OF LEAF



TRANSVERSE SECTION OF STERILE CONCEPTACLE



STRUCTURE OF FEMALE MATURED CONCEPTACLE



STRUCTURE OF MALE CONCEPTACLE

Sargassum

Polysiphonia

Classification:

Class – Rhodophyceae
Subclass - Florideae
Order – Ceramiales
Family – Rhodomelaceae
Genus – *Polysiphonia*

Introduction: *Polysiphonia* is a large genus with about 200 species. The genus is represented in India by about 16 species. Common species are *P. platicarpa, P. ferulacea, P. urceolata, P. fastigiata* and *P. variegata*. Most of the species are lithophytes i.e., found growing on rocks. Some species are epiphytic, found growing on other plants and algae. Some species are semi parasitic.

Thallus Structure of *Polysiphonia*:

The thallus is filamentous, red or purple red in colour. The thallus is multiaxial and all cells are connected by pit connections hence, the name given is *Polysiphonia*. Due to continuous branching and rebranching the thallus has feathery appearance. The thallus is heterotrichous and is differentiated into a basal prostrate system and erect aerial system. The prostrate system creeps over the substratum. Its functions are attachment of the thallus to the substratum by unicellular richly branched rhizoids arises from multiaxial prostrate system or erect system and in perennation. The erect aerial system arises from the prostrate system. It is made of multiaxial branched filaments. These are made of a central large filament or central siphon of cylindrical cells. The central siphon is surrounded by a number of pericentral cells. The length of central and pericentral siphons is equal hence, the filaments appear to be divided into nodes and internodes like. The thallus of *Polysiphonia* bears two types of branches (a) Short branches (b) Long branches. The branches are lateral and monopodial.

The short branches or trichoblasts are branches of limited growth. These are uniaxial in structure. The long lateral branches are branches of unlimited growth are polysiphonous at the base and monosiphonous in terminal parts. The cortical cells arise from outer pericentral cells by pericentral division.

Cell Structure of *Polysiphonia*:

The cells of central and pericentral siphons are cylindrical and elongated. The cell wall is differentiated into outer pectic and inner cellulosic layer. The cell contains a large central vacuole with tonoplast. The cytoplasm is present between the cell wall and the central vacuole. The cell contains a number of red discoid chromatophores which lack pyrenoids.

Reproduction:

In life cycle of *Polysiphonia* both asexual and sexual reproduction takes place. Sexual reproduction is oogamous type and plants are dioecious i.e., male and female sex organs are produced on fertile trichoblasts of different male and female gametophytic plants.

Structure of spermatangia: The male sex organ is spermatangia or antheridia. The mature spermatangium is a globular or oblong, unicellular structure. Its cell wall is differentiated into three layers, inner refractive, middle gelatinous and outer thick layer. The uninucleate protoplast of spermatangium forms a male gamete or spermatium. The spermatium is non-motile and is released through an apical pore in the spermatangium.

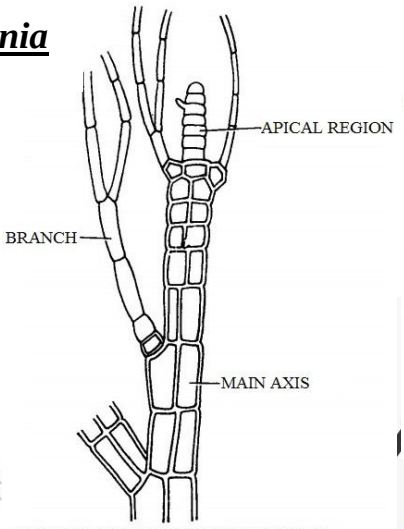
Structure of carpogonia: The female sex organ of *Polysiphonia* is called as carpogonium. The basal swollen flask shaped cell of the carpogonial branch functions as carpogonium or egg cell and the upper tubular elongated part is called trichogyne. The sterile sheath around carpogonium is called pericarp.

Carposporophyte: This is diploid sporophytic phase of *Polysiphonia* and is dependent upon the gametophyte. It is urn shaped structure. The carposporophyte or cystocarp or gonimocarp is made of many gonimoblast filaments attached on the placental cell which remain covered by sterile pericarp. Gonimoblast filaments form carposporangium which again forms diploid carpospores which are liberated through the ostiole of carposporophyte. The germination of diploid carpospore results in the formation of diploid tetrasporophytic plant.

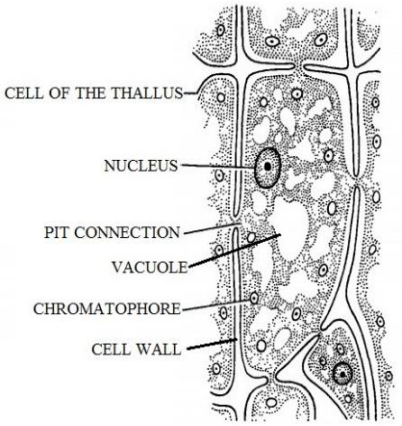
Tetrasporophyte: The tetrasporophytes are free living diploid plants in the life cycle of *Polysiphonia*. Morphologically these plants are similar to haploid gametophytic. It reproduces asexually by means of spherical tetrasporangia borne on pericentral cells of tetrasporophytic plant. The branches bearing tetra sporangia become twisted and swollen and are called stichidia. Each tetrasporangium produces four haploid tetra spores or meiospores which are arranged tetrahedrally. The tetra spores on maturity are liberated by splitting of sporangial wall accompanied by lifting of the cover cell. Two of the four tetra spores germinate to make haploid male gametophytic plant and the two make haploid female gametophytic plants.

Polysiphonia

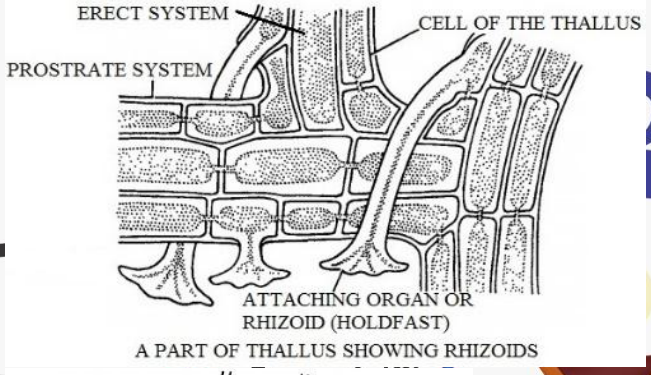
HABIT



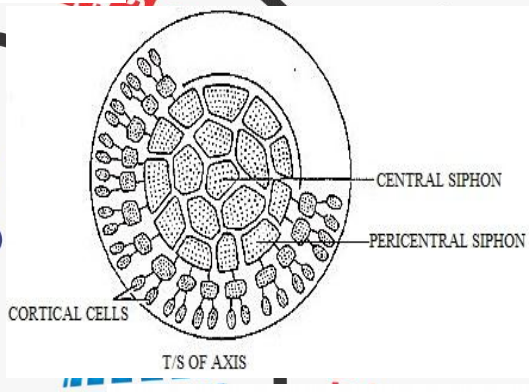
A PART OF THALLUS WITH BRANCHES



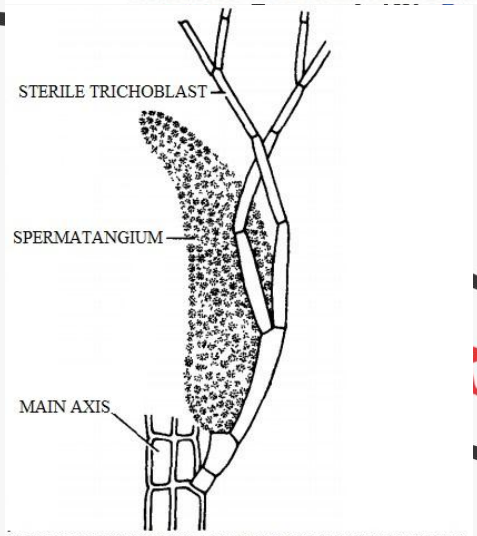
SINGLE CELL ENLARGED



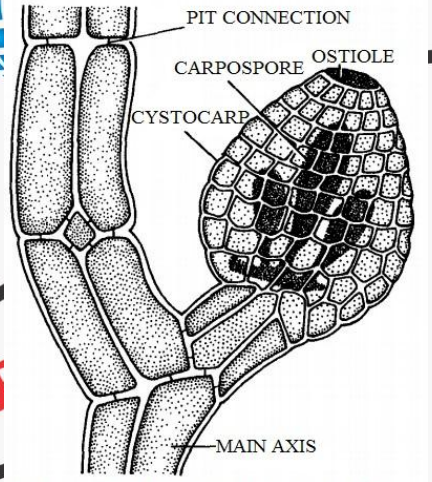
A PART OF THALLUS SHOWING RHIZOIDS



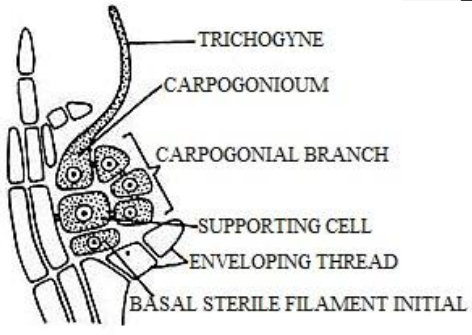
T/S OF AXIS



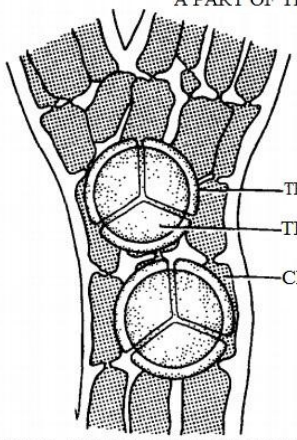
FERTILE BRANCH BEARING SPERMATANGIUM



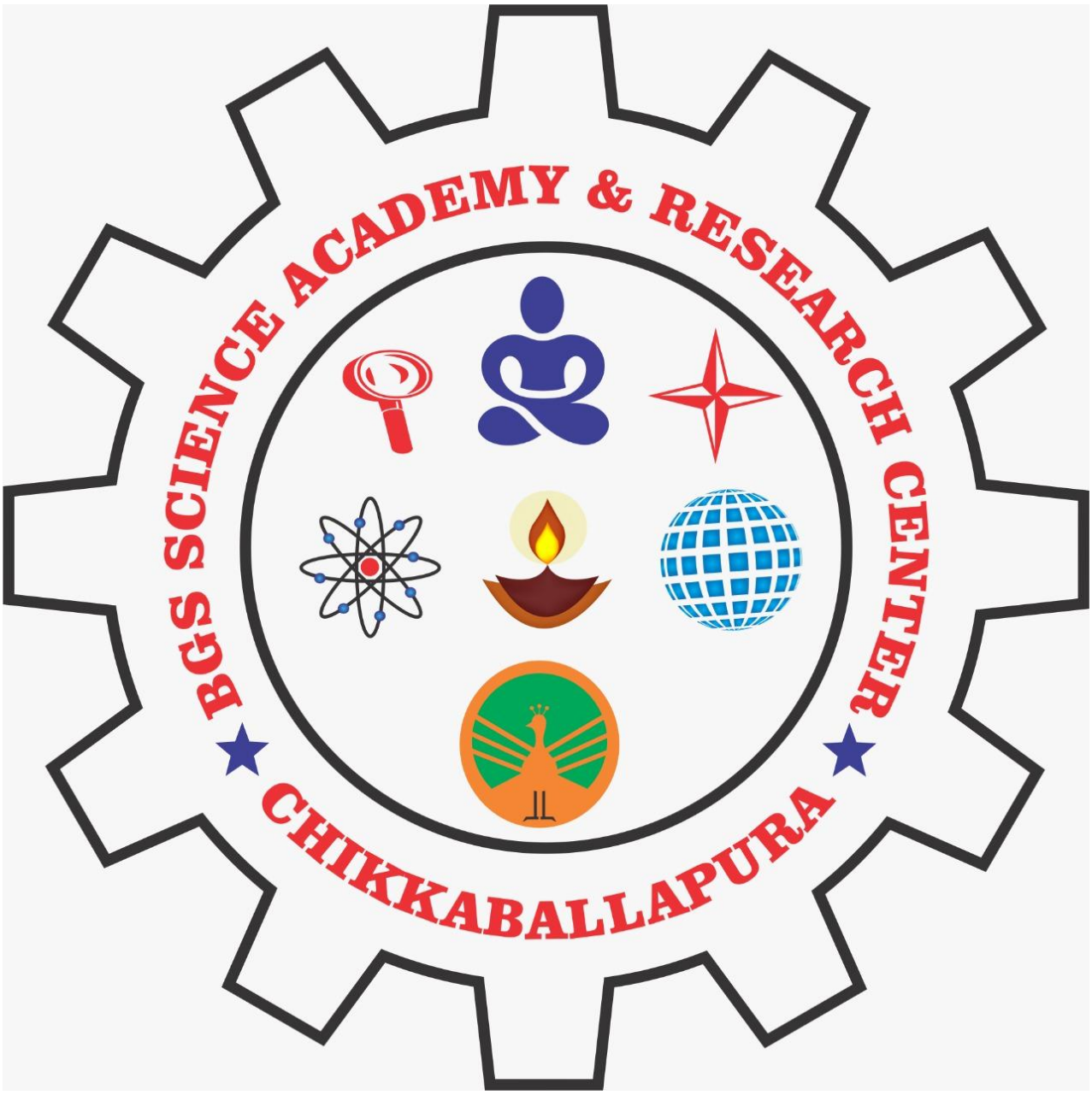
A PART OF THALLUS WITH CYSTOCARP



A PART OF FEMALE PLANT BEARING CARPOSRORANGIUM



A PART OF THALLUS WITH TETRASPORANGIA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