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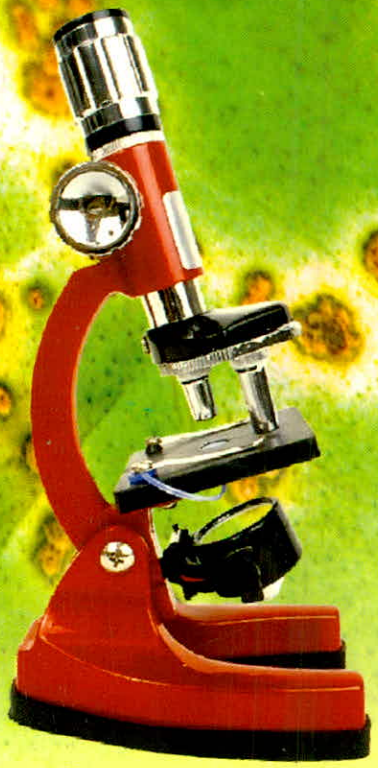
PRACTICAL MANUAL OF PLANT PATHOLOGY



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DEPARTMENT PLANT PATHOLOGY

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इंदिरा गांधी कृषि विश्वविद्यालय

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Dr. C.R. Hazra
Vice Chancellor

FOREWORD

Agriculture being an applied education, there is ever increasing need in strengthening the practical oriented teaching. Subject like Plant Pathology, which deals with different diseases, about their detailed knowledge and most importantly their economic management, are the needs of the day. Most of the graduates are straight away opting the professional careers and the competency of such graduates much depends on their practical knowledge about the subject. Therefore, I strongly feel that a practical manual like the present one is having an immense importance in imparting the knowledge and skill while studying different microbes of agricultural importance.

The authors have made all the efforts in meeting the requirement of the students for a practical manual for consultation at one place. I strongly believe that this manual will greatly help the students in understanding the practical aspects of the subject. I congratulate the authors for their sincere effort.

Raipur
Dated : 28.12.2005

(C.R. Hazra)

FOREWORD

Unlike other sciences, the teaching in agricultural sciences need to be more practical oriented and the students should be well trained in basic concepts of agriculture. Especially in disciplines like plant pathology, practical training in the laboratory is as important as that in the field. Looking into the importance of the practical knowledge to the agricultural graduates, the Indian Council of Agricultural Research (ICAR), New Delhi is providing funds for developing good text books as well as practical manuals in different disciplines of agricultural sciences.

The efforts made by the teachers of the Department of Plant Pathology Drs. N. Khare, N. Lakpale and V.S. Thrimurty are appreciable. This practical manual encompasses the laboratory protocols, handling the procedures, latest equipment, identification and detailing of important pathogens with disease symptoms etc. This is very good and handy practical manual for the students of both undergraduate courses. It is also very useful to the young and newly inducted teachers, extension workers as well as to progressive and educated farmers.

I, on behalf of the University, compliment the authors for bringing out such a useful practical manual in plant pathology, which is not only simple but also exhaustive in its way. I hope this manual paves a new way for the teaching of agricultural sciences in general and plant pathology in particular.

(A.S.R.A.S. Sastri)
Dean Faculty of Agriculture

Raipur
Dated : 28.12.2005

PREFACE

Practical competences are very much important for the agricultural sciences understanding and application. Undergraduate programme especially forms the foundation for developing such competences. Therefore, this manual was prepared with a view to impart the guidance for the practicals at undergraduate level for agricultural students and allied sciences. While preparing this manual latest courses recommended for plant pathology by the apex committees for undergraduate programme were thoroughly taken care.

While preparing this manual standard texts of eminent authors were consulted for the authenticity. Authors are extremely thankful to the University authorities for extending financial and administrative support for the publication of this manual.

We also acknowledge the assistance of Dr. M.N. Naugraiya, Senior Scientist and Co-ordinator, Technical Cell, I.G.A.U., Raipur (C.G.) for editing and Shri Puran Lal Patle for formatting of this manual.

Though all necessary care was taken during the preparation, some mistakes might have been eluded from our sight. Any such mistakes, suggestions etc. brought to the knowledge will be highly appreciated.

Authors

Raipur
Dated : 28.12.2005

CONTENTS

CHAPTER - I

LABORATORY SAFETY: GENERAL RULES AND REGULATIONS

1. Keep doors and windows closed during the laboratory session to prevent contamination through air currents.

2. Place coats, books and other paraphernalia in specified locations of laboratory - never on bench tops.

3. At the beginning and termination of each laboratory session, wipe the bench tops with a disinfectant solution.

4. Do not smoke, eat, or drink in the laboratory. These activities are absolutely prohibited.

5. Wear a lab coat or apron while working in the laboratory to protect clothing from contamination or accidental discoloration by staining solutions and from other hazards.

6. Never remove media, equipment and especially the bacterial cultures as such from the laboratory. Doing so is absolutely prohibited.

7. Do not place contaminated instruments, such as inoculating loops, needles, and pipettes, on bench tops. Loops and needles should be disposed of in designated receptacles.

8. On completion of the laboratory session, place all cultures and materials in the disposal area as designated by the instructor.

Observe following regulations to prevent accidental injury and infection:

1. Wash the hands with liquid detergent and dry with paper towels upon entering and prior to leaving the laboratory.

2. Carry cultures in a test-tube rack when moving around the laboratory. Likewise, keep cultures in a test-tube rack on the bench tops when not in use. This serves a dual purpose: to prevent accidents and avoid contamination of yourself and the environment.

3. Immediately cover spilled cultures or broken culture tubes with paper towels and then saturate them with disinfectant solution. Following 15 minutes of reaction time, remove the towels and dispose of them in a manner indicated by the instructor.

4. Report accidental cuts or burns to the instructor immediately.

5. Never do mouth pipetting of broth cultures or chemical reagents. Doing so is strictly prohibited. Pipetting is to be carried out with the aid of a mechanical pipetting device.

6. Use only self-stick labels for the identification of experimental cultures.

CHAPTER - II

COMMON LABORATORY EQUIPMENTS/INSTRUMENTS, TOOLS AND GLASSWARES

During practical classes, different types of laboratory equipments/ instruments, tools and glass wares are used by students as per the need of the exercise. So, students must know, at least general information or basics of that particular article. In general, the following articles are used in common during laboratory exercises.

A. EQUIPMENTS/INSTRUMENTS

1. **Autoclave -** To sterilize the media and other liquid materials at 15 pounds/ inch^2 pressure for 15-20 minutes.
2. **Hot air oven -** To sterilize the glasswares at 180°C for 1 hours.
3. **BOD incubator -** To incubate the cultures of the microorganisms at desirable temperatures (generally at 25-30°C).
4. **Laminar air flow -** For isolation and inoculation of microbes under aseptic (sterilized air) conditions.
5. **Inoculation chamber (Hood) -** Used for isolation and inoculation of microbes under aseptic conditions (Fig.1).
6. **Water bath -** For melting of solidified media and general boiling purpose.
7. **Hot plate -** For general heating purpose where direct (flame) heat is not required.
8. **Microtome -** For section cutting with the help of paraffin block. Types- (a) Sledge (b) Rotary (c) Rocking (d) Freezing.

1. **Shaker** - For liquid culture studies.

2. **Refrigerator** - For storage of pure cultures of microbes under low temperature for limited period of time.

3. **Deep freezer** - For storage of pure cultures of microbes at -4 to $-30^{0}C$ for fairly long period.

4. **Centrifuge** - Used in various ways as per requirement of study. Types- (a) Low speed (3000-5000 rpm) (b) High speed (cooling centrifuge) (5000-30000 rpm)

5. **Spectrophotometer** - Used in microbial growth studies by using different paths of wavelengths and photoelectric cell.

6. **Microscopes** - To see the minute objects in magnified form with the help of combination of lenses.

7. **Top pan balance** - Used for weighing of chemicals etc. up to 0.001 gm.

B. TOOLS

1. **Spirit lamp** - Used for slide drying and in isolation and inoculation process [Fig.2 (i)].

2. **Inoculation needle** - Used in isolation and inoculation purpose, loop made up of nichrome wire [Fig.2 (ii)].

3. **Forceps** - Used in isolation and inoculation process and in seed pathology [Fig.2 (iii)].

4. **Scissors** - Used in general cutting of disease specimen and in isolation process [Fig.2 (iv)].

5. **Needle** - To tease out the disease symptoms during microscopic observation [Fig.2 (v)].

6. **Cork borer** - To cut the microbial disc of different diameter for easy transfer from on site to other [Fig.2 (vi)].

7. **Sieves** - Used in separation of nematodes from soil, available in different mesh sizes.

8. **Mortar and pestle** - Used in grinding of leaf tissues for various purposes [Fig.2 (vii)].

C. GLASS WARES

1. **Conical flasks** - Made up of heat resistant glass, available in different capacities from 50 - 2000 ml, graduated and ungraduated, flasks have short cylindrical open neck, a conical body and flat bottom, used for keeping culture media, water etc. [Fig.3 (i)].

2. **Beakers** - Made up of heat resistant glass, used in preparation of solutions and pouring of liquid material into conical flasks and culture tubes etc. available in various capacities from 25 -2000 ml [Fig. 3 (ii)].

3. **Culture tubes** - Made up of heat resistant glass, used for slant culturing, serial dilutions etc. [Fig.3 (iii)], available in different sizes *viz*; 12 x 75, 12 x 100, 15 x 125, 15 x 150, 18 x 150, 25 x 100, 25 x 150, 25 x 200, 32x200 and 38 x 200 (Diameter x Height in mm).

4. **Petridishes** - Made up of heat resistant glass, used in isolation, purification, and other cultural and seed pathological studies. A pair consists of two circular shallow dishes, which fit in together [Fig.3 (iv)], available in various sizes viz. 50 x 12, 80 x 15, 100 x 15, 150 x 25, 200 x 30 (Diameter x Height in mm).

5. **Volumetric flasks** - Used for making and keeping standard solutions. These are pear shaped bulb having a graduated narrow and long neck with a stopper (glass) and are available in different capacities from 5 - 2000 ml [Fig.3 (v)].

6. **Distillation apparatus** - Used for distillation of water, alcohol etc. [Fig.3 (vi)].

7. **Micro-slides** - Prepared from optically flat glass sheet and are used in microscopic work. The standard size is 75 mm x 25 mm but thickness may be 1.00, 1.10, 1.25, 1.35 or 1.45 mm [Fig.3 (vii)].

8. **Cavity slides** - Sometimes these slides are also used, slides have one, two or three con-cavities on one surface. Microbes kept in these cavities and examine microscopically for spore germination, motility etc. Available in following specification: size - 75 mm long x 25 mm wide x 1.45 mm thick, polished spherical con-cavities are 15 mm in diameter x 0.5 mm deep [Fig.3 (viii)].

9. **Cover slips** - These are optically flat and free from waviness, may be circular, rectangular or square in outline, used for mounting the object in a suitable mounting medium kept on glass slides. These are essential for microscopic observation of plant material under high magnification. These helps in keeping the object in one optical plan, thus facilitates the clear visibility of object, available in various sizes. Circular - 18, 19, 22 and 24 mm diameter, Square - 18 x 18 mm, 22 x 22 mm, 24 x 24 mm, Rectangular - 22 x 25 mm, 22 x 30 mm, 22 x 40 mm, 22 x 50 mm, Thickness - No. 0-0.08-0.13 mm, No. 1 - 0.13-0.16 mm [Fig.3 (ix)].

10. **Pipettes** - These are glass tube open on both ends, one end is jet like slightly narrower than other end, used for volumetric work. Available in different capacities viz. 0.1-50 ml. Three types (a) bulb pipettes (b) cylindrical pipettes (c) micro-pipettes [Fig.3(x)].

11. **Watch glasses** - These are shallow - pan shaped bowl like container used for keeping the specimen to be examined, separation of thin section and other purposes, available in 7.5 - 15 cm diameter [Fig.3 (xi)].

12. **Funnels** - A funnel has two parts, upper part is wide and cone shaped and lower is long narrow neck. The diameter of cone shaped part varies from 5-

12 cm, used for pouring the liquids from one container to other and filtering the solution with the aid of filter paper placed in cone shaped upper part [Fig.3(xii)].

13. **Specimen jars** - Used to preserve the plant material in different types of preservatives for longer period. Sometimes, specimens are mounted on glass plate, also kept in specimen jars filled with preservative [Fig.3 (xiii)]. Two types: -

    (a) Round shaped - These have screw caps or knobbed glass stopper for making jars air tight. Vary in size from 10 x 5 cm to 30 x 10 cm (Height x Diameter)

    (b) Rectangular shaped - A glass sheet is provided with each jars which are fixed to mouth of the jar with grease for making jar air tight. Sizes are 10 x 5 x 6 cm to 30 x 17 x 9 cm (Height x Length x Width).

14. **Measuring cylinders** - These are used in measuring the liquid in desired volume and dispense the volume of solutions to other container, available in various capacities from 1 to 2000 ml [Fig.3 (xiv)].

15. **Coplin jars** - These are used in staining during preparation of permanent slides with microtome section. There are 12 or 20 divisions inside the jar, a lid and slides are arranged back to back and inserted in each divisions. Jars are filled with sufficient stain and slides are kept for desired period of time, depending upon the material to be stained and type of stain [Fig.3 (xv)].

16. **Desiccators** - Used in maintaining different relative humidities by different H_2SO_4: H_2O combination, which are used for relative humidity related studies of microorganisms [Fig.3 (xvi)].

D. MISCELLANEOUS ARTICLES

1. **Wash bottle** - Made up of plastic, provided with a long tube and a nozzle at outside end, available in 500 ml capacity and used to wash laboratory articles.

2. **Dropping bottles** - Made up of plastic with arrangement for dropping the stains drop by drop and are available in 100 ml capacity.

3. **Wire/PVC basket** - Used for keeping the culture tubes in up-right position during sterilization, incubation and storage.

4. **Trays** - Made up of iron, coated with enamel or PVC, used to transport glasswares from one place to another and other general purposes.

5. **Blotting paper** - Used in seed pathological work for making moist chamber, general filtration etc.

6. **Aluminium foil** - Used in wrapping the culture tube or Petridishes having microbial cultures before keeping in freeze or deep freezer to avoid entry of moisture and contaminants in the material.

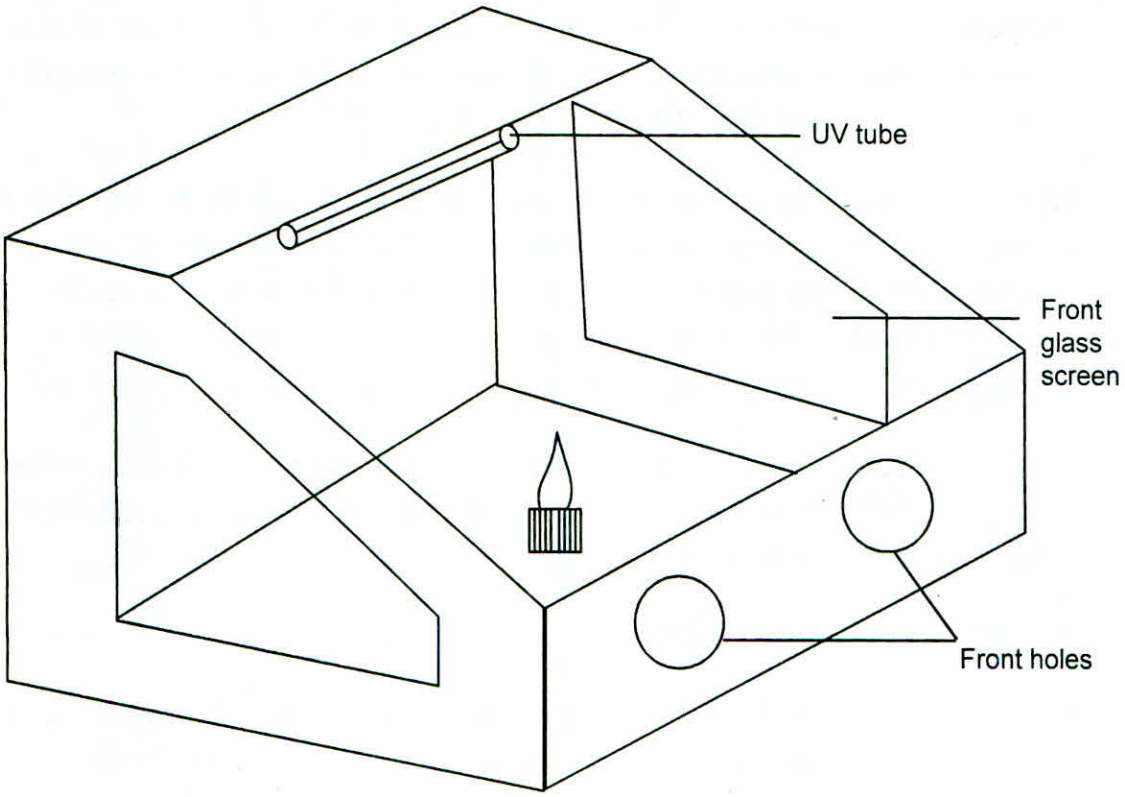


Fig. 1: Inoculation chamber (hood)

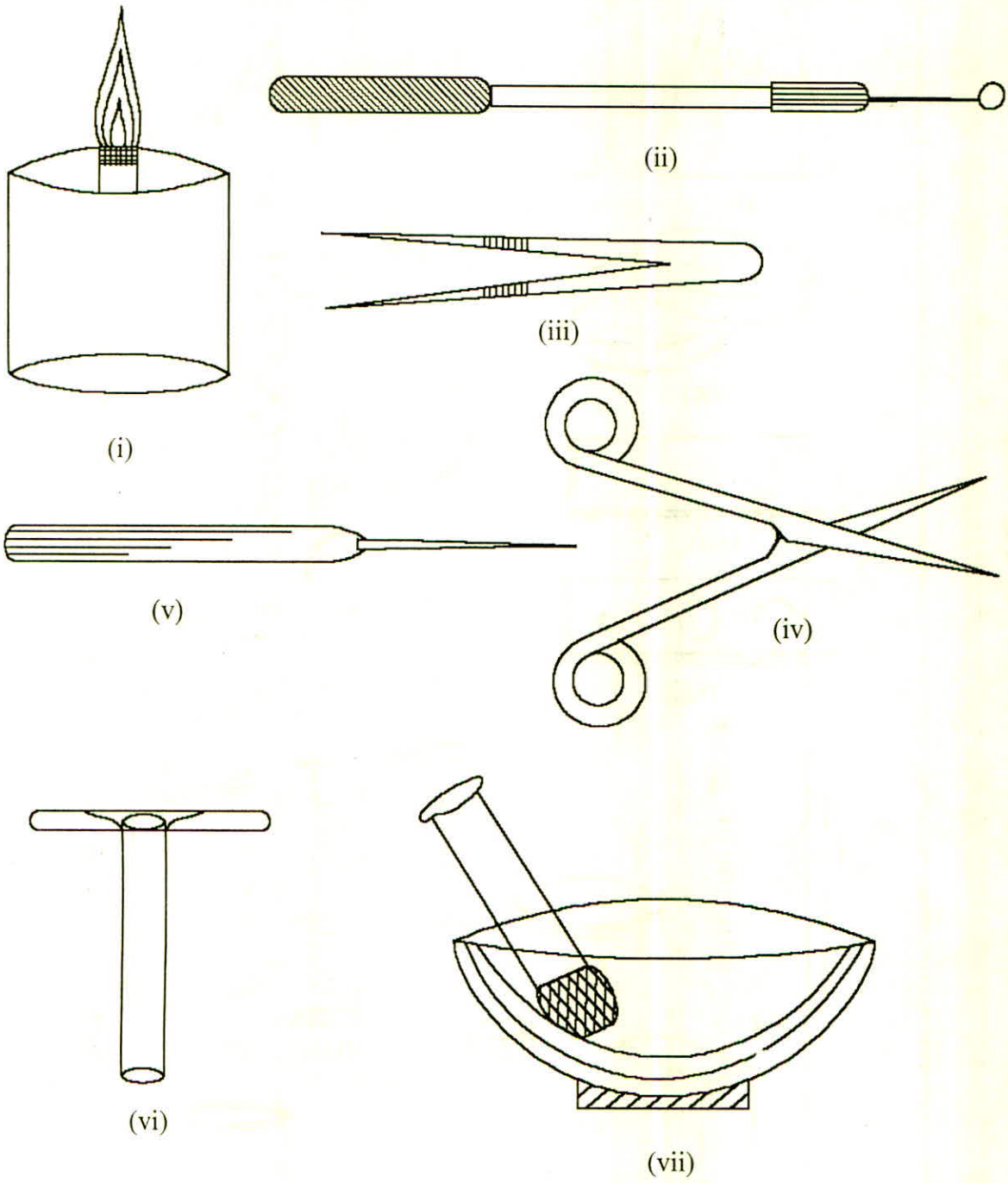


Fig. 2: Common laboratory tools

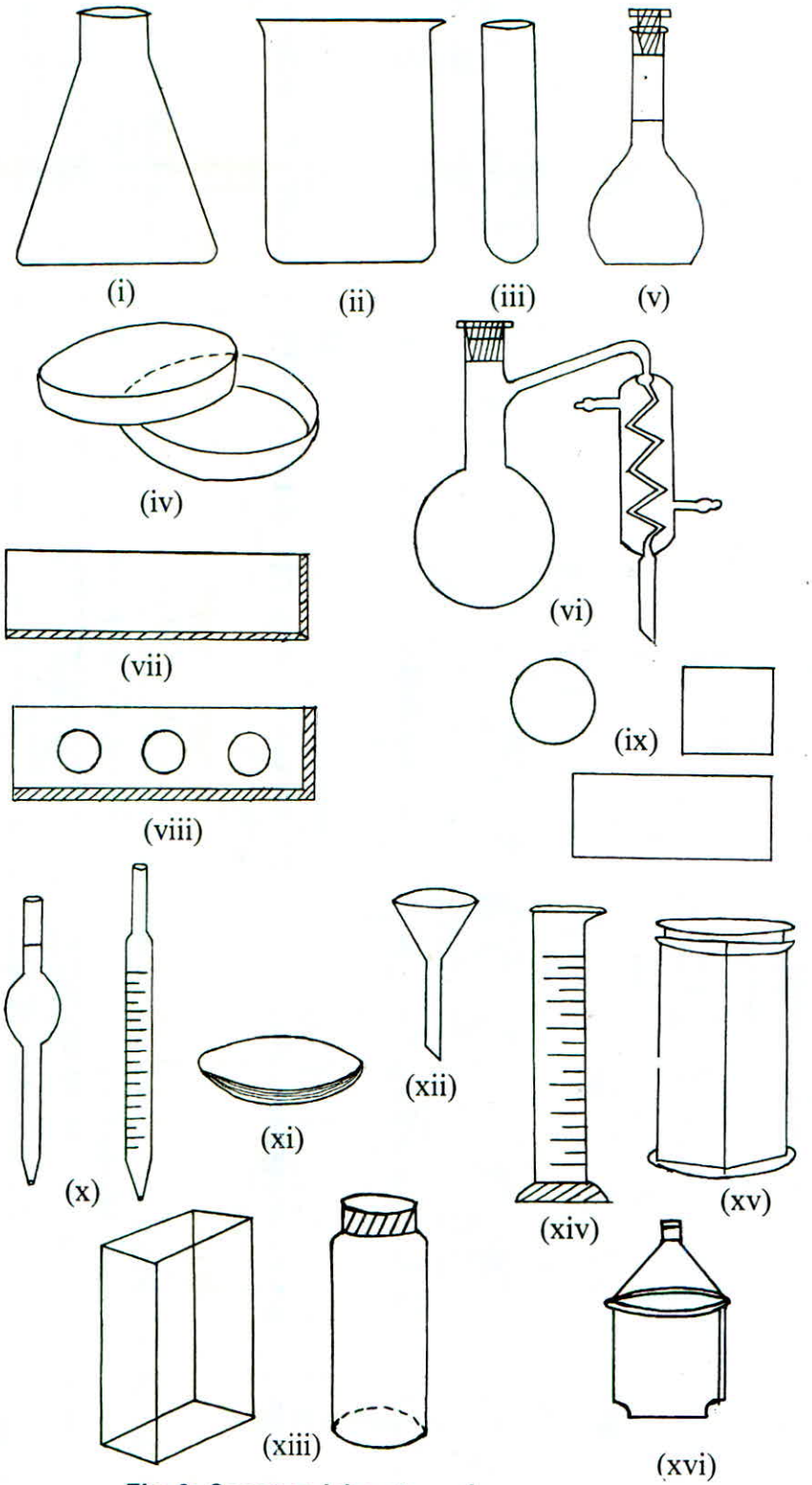


Fig. 3: Common laboratory glass wares

CHAPTER - III

MICROSCOPY AND RELATED FEATURES

To see the plant and animal cells and cell organelles, microscopes are used because these smaller cells can not be seen through naked human eyes due to limited resolving power (capacity to detect) and the object smaller than 100 μ (0.1 mm). With the help of microscopes, which are consisted of simple lens or combination of lenses, the resolving power is increased. The minute objects gets several times magnified and thereby easily seen.

A microscope can be defined as an optical instrument, consisting of lens, or combination of lenses for making enlarged or magnified images of minute objects.

Several types of microscopes are available right from general purpose to very specific one. They are:

1. Simple microscope
2. Compound microscope
3. Phase contrast microscope
4. Dark field microscope
5. Polaroid microscope
6. UV microscope
7. X-ray microscope
8. Stereoscopic binocular microscope
9. Fluorescence microscope
10. Electron microscope

1. SIMPLE MICROSCOPE

Invented by Antony van Leewenhock in 1676. This microscope consists of only one lens (magnifying glass) held in a frame, usually adjustable, and often

provided with a stand for conveniently holding the object to be viewed and a mirror for reflecting the light. With this microscope, the minute object may be magnified from 4-40 times.

2. COMPOUND MICROSCOPE (STUDENT MICROSCOPE)

Invented by Robert Hooke in 1820. This microscope have two sets of lenses, one is known as objective and other as eye piece (ocular) mounted in a holder, commonly known as body tube (Fig. 4). The lens system nearest to the specimen, called the nose piece (objective), magnifies the object to a definite number of times (4x, 10x, 40/45x, 100x). The second lens system, called as the eye piece, further magnifies (10x, 15x) the image formed by the objective. The image seen by the eye has a magnification equal to the product of magnification of two lens system. i.e.

$$\text{Total magnification} = \underset{\text{Magnification}}{\text{Power of eye piece}} \times \underset{\text{Magnification}}{\text{Power of objective}}$$

(A) Objective – Function of objective lens is utilization of a large angular cone of light coming from the specimen, unite the light in a point of the image and magnify the image. Objectives are of three types :

(a) Achromatic (b) Apochromatic (c) Fluorite.

(B) Eye piece (Occular) – Magnification of the real image of the object followed with minor corrections is the function of the eye piece. It also consists of other built in components such as image cross hairs and scales etc. for appropriate usage. Eye pieces are of three types:

(a) Huygenian eye piece (b) Hyperplane eye piece (c) Compensating eye piece.

(C) Condenser – Condenser consists of series of lenses with a purpose to illuminate the object under study with the transmitted light. It is situated between the mirror and objective. A condenser is necessary for the examination of on object with an oil-immersion objective, to obtain adequate illumination. Con-

densers are of three types: (a) Abbe condenser (b) Variable focus condenser (c) Achromatic condenser.

(D) **Mirror** – This consist of metallic frame to which a set of convex and concave mirrors are fitted. The beam of light is reflected by mirror upwards passing through the diaphragm and the central hole provided in the stage.

(E) **Body tube** – It is a hollow, cylindrical, metallic frame and about 16 cm long with eye piece and nose piece (objectives) fitted at upper and lower end respectively.

(F) **Coarse adjustment screw** – It is fitted with the body tube and work on the rack and pinion principle. By this, body tube moves up and down during focus of an object by keeping proper distance.

(G) **Fine adjustment screw** – It is located just below the coarse adjustment screw and work on same principle and purpose. After focusing, a little rotation of this screw brings a sharp image of an object under high power magnification.

(H) **Stage** – A rectangular metallic piece provided with central hole to pass beam of light, and have a pair of clip to hold glass slide firmly. Sometimes slide moving mechanisms are also provided on this stage.

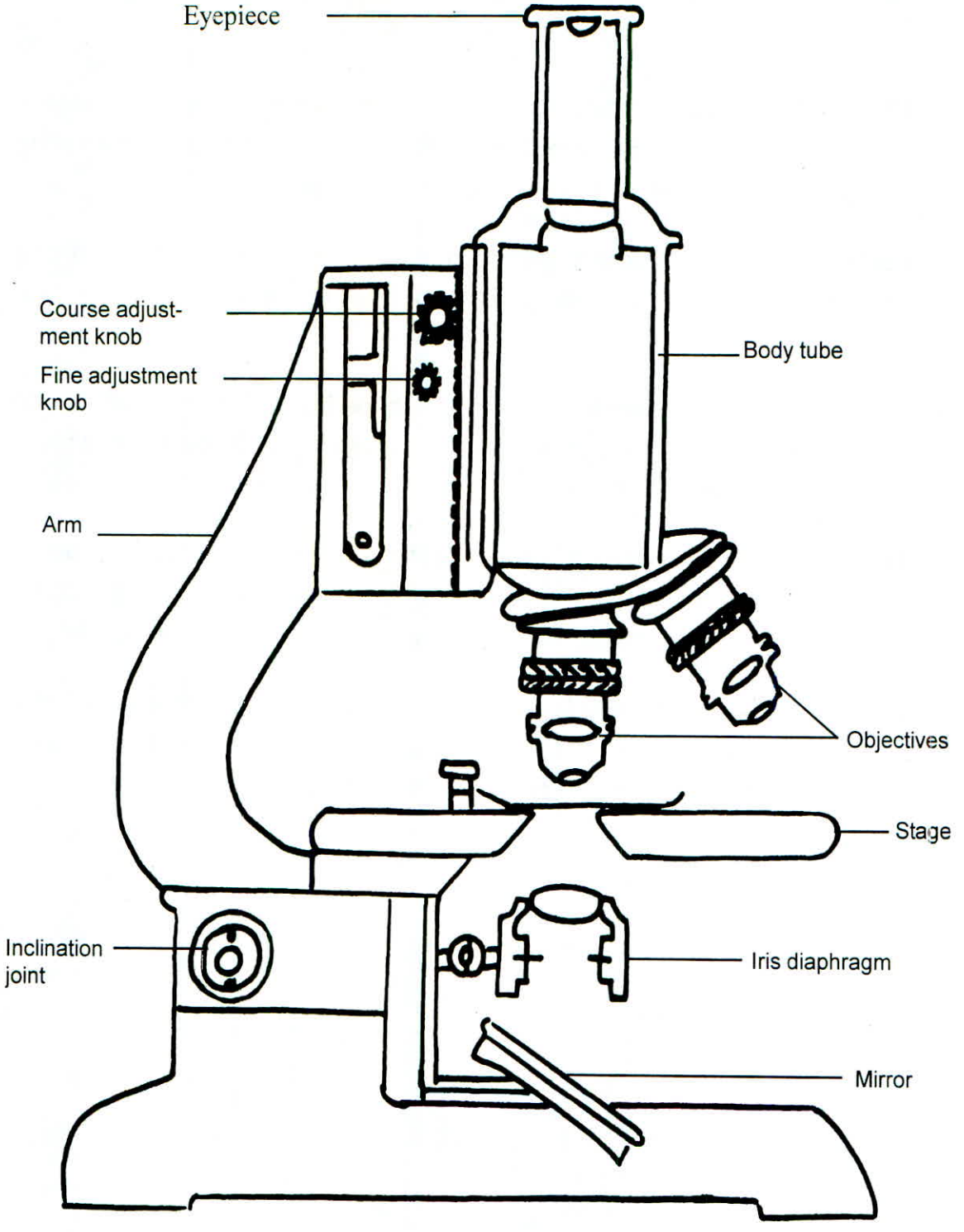


Fig. 4: A Compound Microscope

Exercise No. 1: Draw the symptoms of disease samples through simple microscope and write the Identifying characters

A. Symptom figure

Name of disease _____

Causal organism _____

Symptoms _____

_____

_____

Identifying characters _____

_____

_____

B. Symptom figure

Name of disease _____

Causal organism _____

Symptoms _____

_____

_____

Identifying characters _____

_____

_____

Exercise No. 2: Draw the symptoms of disease samples through simple microscope and write the Identifying characters

A. Symptom figure

Name of disease _____

Causal organism _____

Symptoms _____

_____

_____

Identifying characters _____

_____

_____

B. Section figure

Name of disease _____

Causal organism _____

Symptoms _____

_____

_____

Identifying characters _____

_____

_____

Oil immersion objective

When a path of light passes from one to another medium of different densities (air and glass), the rays of light get refracted. The lens of objective being very small and the rays of light getting reflected, a large amount of light is lost and only small proportion of light enters into objective, resulting in less brighten image of the object. This problem has been over come by using oil immersion objective, which is designed in such a manner as to receive the maximum unrefracted rays of light. The oil immersion required to provide an optically homogenous path of light to travel between the slide and the front lens of the objective. For this, a drop of non-drying immersion oil (cedar wood oil) is put over the cover glass just above the object. This oil has the same refractive index as that of the glass slide. The oil immersion objective is gradually lowered down so as to touch the oil. There is no gap between object and objective lens. After using oil immersion objective, it should be cleaned with tissue paper soaked in xylene or carbon tetrachloride. The magnification power of this objective is 100x.

3. ELECTRON MICROSCOPE

Invented by Knoll and Ruska in 1932. It uses a beam of high speed electrons having an equivalent wavelength of x-rays (about $0.05^{o}A$). This extremely short wavelength gives the electron microscope its fundamental superiority over the light microscope. A close analogy exists between the action of a magnetic or electric field of rotational symmetry or an electron beam and the action of a glass lens on a light beam. After reaching the high velocity, the electron penetrate the specially prepared specimen and than focused by means of magnetic fields to form an enlarge image on a fluorescent screen or a photographic plate. The resolving power of electron microscope is 10^{5} times greater than that of an optical microscope in which path of light is used for illumination.

Camera lucida (Mirror type)

Camera lucida is a drawing prism instrument designed to help in drawing the microscopic object focused, true to their dimensions as viewed directly under

microscopic observations. It has a small beam splitting cube fixed in the region of exit-pupil of eye piece and can be screwed around the body tube of microscope in such a manner as to bring the cube attachment just above the eye piece of microscope. This has a well polished plane mirror fitted in a metal frame (Fig.5).

For drawing, camera lucida is fitted with the microscope is such a manner as to keep the mirror towards the right hand side. A drawing sheet is kept on the table below the mirror. The mirror is adjusted in a position as to face the drawing sheet as well as the beam splitting cube attachment. The object to be sketched is focused by looking it through the eyepiece of microscope. The beam splitting cube is placed over the eye piece and the object is viewed through it. The other type of camera lucida (prism type) is also available for same purpose.

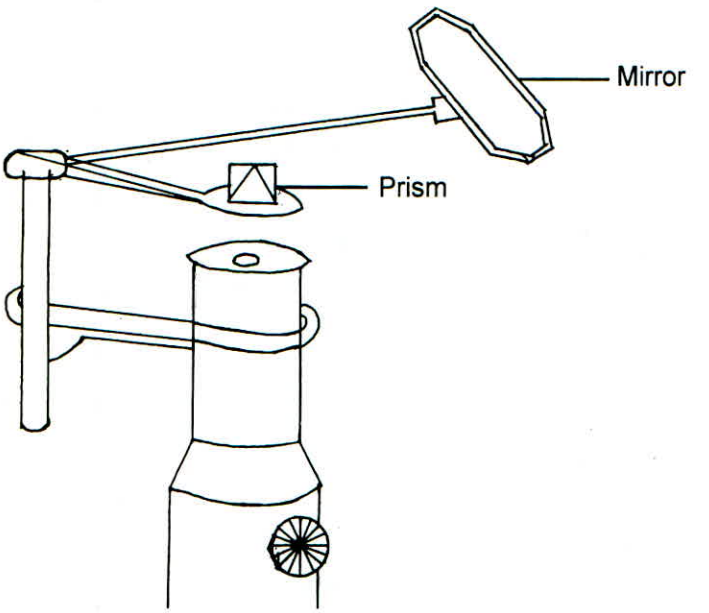


Fig. 5: Camera lucida (Mirror type)

Micrometery

Shape, size and structure of the microorganisms are important for identification and for taxonomic studies of microbes such as spores, fruiting bodies, conidiophores etc. are recorded in microscope by using micrometers. Therefore, micrometry is used for the measurement of the microbes or its parts using ocular and stage micrometer. Ocular micrometer is a glass disc with graduated scale in the middle and marked from 1-10 and sub divided in 10 small divisions between two consecutive numbers (Fig.6). This micrometer is well fitted in huygenian eye piece. Stage micrometer is a glass slide, graduated at the middle with a scale of 1 mm divided into 100 equal divisions, means each division is 0.01 mm (Fig. 7).

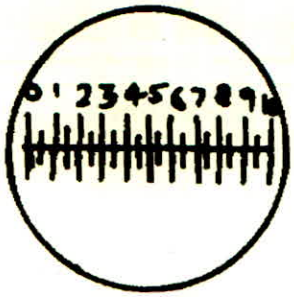


Fig. 6: Ocular micrometer

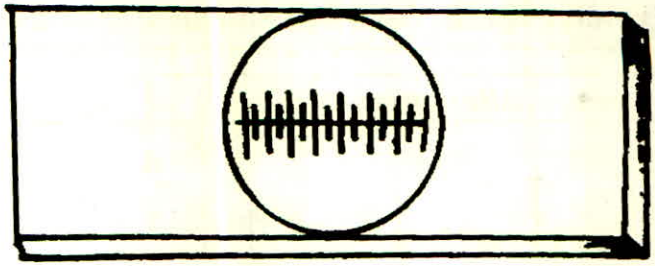


Fig. 7: Stage micrometer

Calibration

The calibration is done by focusing the stage micrometer scale with eye piece containing the ocular micrometer. By doing so, both the scales get super imposed and when 0 of the ocular micrometer coincides with 0 of the stage micrometer keenly count the number of unit of ocular micrometer, which coincide with particular unit of stage micrometer. The value of one unit of ocular micrometer is calculated by the following formula:

$$1 \text{ unit of ocular Micrometer} = \frac{\text{No. of units of stage micrometer x 0.01 mm}}{\text{No. of units of ocular micrometer}}$$

After calibration and during measurement, stage micrometer is removed and object slide is kept for measurement of different parts and numerical value is expressed in microns (μ).

Exercise No. 3: Draw the Fungal spores through camera lucida

Alternaria	*Fusarium*
Puccinia	***Erysiphe***

Exercise No. 4 : Measure the dimensions of micro organisms

S. No.	Pathogen	Length (μ)	Width (μ)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			

CHAPTER - IV

PREPARATION OF TEMPORARY SLIDES AND STAINING

For any microscopic examination, the basic requirement is slide preparation with object (specimen, fungal growth and symptoms part sections etc.) and staining. Slides may be temporary or permanent, but for routine class work, temporary slides are prepared. Preparation of permanent slides takes a long time with more complex procedure.

For the preparation of temporary slide, take a clean, grease free slide, put one drop of water in the centre and place the very thin section of plant diseased material or very little amount of pathogen growth or its part with the help of tip of dissecting needle. Place a cover slip over the object by touching one edge of cover slip to water drop and gently lower down the other edge with the help of tip of needle. Press cover slip slightly to align the object as well as cover slip in one optical plane. The above steps are necessary for preparing air bubbles free slide, which is a common mistake committed by students.

This slide then focused under low power of compound microscope and followed by high power for more enlargements. During preparation of temporary slide, different stains according to purpose are applied e.g. cotton blue for fungus. When pure cultures of microbes are studied, glycerin jelly is also used for mounting purpose. Glycerin jelly has the following ingredients:

Glycerin	-	7.0 ml
Gelatin	-	1.0 g
Water	-	6.0 ml
Phenol	-	1%

Dye

Dye is colouring agent used for general purpose.

Steps of Gram's staining technique

Preparation of bacterial smear on grease free slide

↓

Fixing of bacterial cells on the slide

↓

Put a drop of Crystal violet on smear and keep for 1 minute

↓

Wash slide with water and air dried

↓

Put a drop of Iodine solution on smear and keep for 1 minute

↓

Wash slide with tap water and air dried

↓

Decolourize with Alcohol (95%) till violet colour ceases to come out and air dried

↓

Put a drop of Safranine solution on smear and keep for 1 minute

↓

Wash slide with water and air-dried

↓

Examined under oil immersion objective of compound microscope

↓

Result (Reaction)

If bacterial cells are stained in violet colour, they are Gram positive.

If bacterial cells are stained in red/pink colour, they are Gram negative.

This happens due to differences in the bacterial cell wall composition and the stains used are react differentially with cell walls. The composition of stains used in this technique are given below:

Stains

Stains may be defined as a specific colouring agent (containing chromophore and auxochrome groups linked to benzene ring) used for biological objects and are manufactured with great care under more rigid specifications.

The stains are used -

1. To render microscopic and semitransparent object visible.
2. To reveal their shape and size.
3. To show the various external and internal structures.
4. To produce specific physical or chemical reactions.
5. To differentiate between the host tissue and the microorganism.

After staining, the biological object gets coloured due to chemical reaction between stain and object because both are made up of chemicals / biochemicals. Some parts of a cell are acidic in nature whereas others are basic. The acidic constituents of the cell (Nuclei, chromatin) react with basic stains and basic constituents (cytoplasm) react with acidic stains and finally object is stained.

In general, cotton blue is used to stain fungi,. The constituents are:

Lactic acid	-	20 g
Phenol crystals	-	20 g
Glycerin	-	40 ml
Water	-	20 ml
Cotton blue powder	-	traces (0.5%)

For bacteria, Grams staining technique or differential staining is followed. In this, two stains are used - Crystal violet (primary stain) and Safranine (secondary or counter stain) along with Iodine as mordant. This staining technique was given by Christian Gram in 1884 to differentiate the bacterial population on the basis of staining reaction as Gram positive and/or Gram negative.

i. Crystal violet

Solution A

Crystal violet (90 percent dye content)	- 2 g
Ethyl alcohol (95 percent)	- 20 ml

Solution B

Ammonium oxalate (95 percent)	- 0.8 g
Distilled water	- 80 ml

Mix solution A and B

ii. Iodine solution

Iodine	- 1 g
Potassium iodide	- 2 g
Distilled water	- 300 ml

iii. Safranine solution

Safranine (25 percent solution in 95 percent Ethyl alcohol)	- 10 ml
Distilled water	- 100 ml

Mordant

A mordant can be defined as any substance which is capable of forming insoluble compounds with stains and which enhances their fixation to bacterial cell. eg. Iodine and Tannic acid.

Exercise No. 5 : Differentiate Gram positive and negative bacteria by Gram staining technique

S. No.	Bacteria	Gram positive	Gram negative
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			

CHAPTER - V

STERILIZATION

Sterilization means making free from micro-organism or killing of micro-organism or freeing of an article from all living organisms, including viruses, bacteria, fungi and their spores, both pathogenic and non pathogenic. The method is used in preparation of culture media, suspending fluids, reagents, containers and equipments used in microbiological techniques. To ensure the possibility of perfect pure culture, the micro-organisms present in the media or in/on the container as contaminants, are killed. Sterilization can be done either by physical or chemical means.

METHODS OF STERILIZATION

A. Physical methods

1. Wet heat or moist heat
2. Dry heat
3. Filtration
4. Irradiation

B. Chemical method

1. Strong disinfectants - Formaldehyde, Ethylene oxide.
2. Mild disinfectants - Ethyl alcohol, Iodine, Phenolics.

A. PHYSICAL METHODS

1. Wet or moist heat sterilization

This is most popular way of killing microorganism. Moist heat is most effective and efficient. The principle of moist heat sterilization is mainly based on the holding period at a particular temperature exclusively warming up and cooling down. At high temperature, the activity of cytoplasm ceases i.e. coagulating and denatur-

ing of enzymes and proteins, thus killing the micro-organism. It may also disrupts the cell membrane.

At normal atmospheric pressure water boils and produces steam at 100°C. This temperature is sufficient to kill all non spore forming organisms. In order to destroy the bacterial endospores, sterilization must be done at temperature above 100°C and this is achieved with the use of saturated steam under pressure. Steam under pressure is produced in pressure cooker or in autoclave (developed by Chamberland in 1884). An autoclave is of two types - simple and steam jacketed.

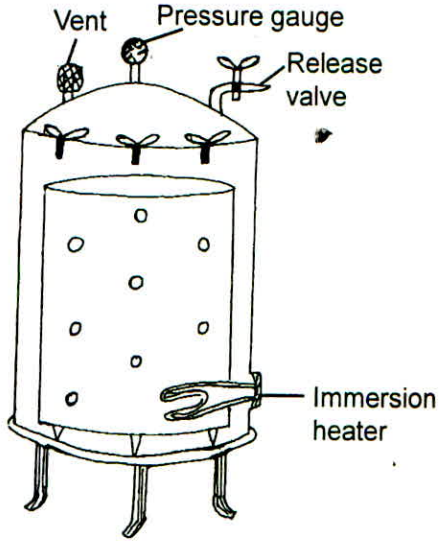


Fig. 8: Autoclave

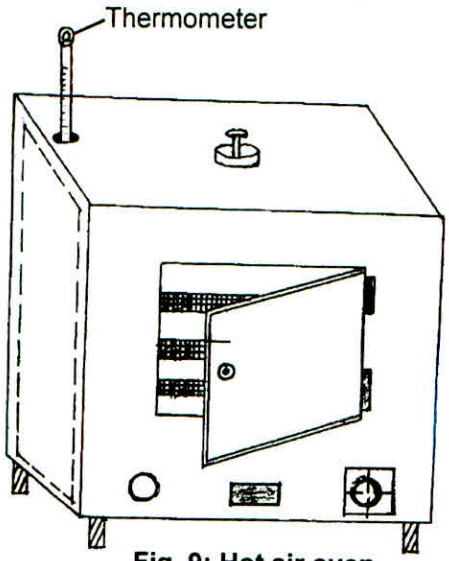


Fig. 9: Hot air oven

The body of an autoclave is made up of gun metal, cylindrical shaped and closed at one end by hinged door. A gasket is provided between door and cylinder. A perforated metal basket is provided within the barrel, used for keeping the articles, to be sterilized. Electric heater boils the water present below the perforated basket. A pressure gauge, safety valve and outlet for steam is also provided on the upper surface of hinged door (Fig. 8). Water is boiled to produce steam, which moves inside the perforated basket and articles freely. The air initially present inside the barrel is forced out until the barrel is filled with saturated steam and outlet is closed. The production of steam continued until the desired temperature and pressure reaches in the barrel, which is normally 121.6°C at 15 pounds/inch^2 (or 1.2 kg/cm^2). At this temperature and pressure, all the vegetative cells and endospores

are killed within 15-20 minutes. Care must be taken that the desired temperature reaches at a certain pressure, depends upon the amount of air mixed with the steam. Sole steam will produce maximum temperature at certain pressure. This method is very useful in sterilizing culture media, water and other articles containing moisture.

2. Dry heat sterilization

Dry heat does not corrode glass wares and metallic instruments as the moist heat does. In spite of this merit, heat sterilization is slow and not suitable for materials like rubber and plastic items, which is heat sensitive. This method destroys the microbial cells by oxidation of cell constituents and denaturation of proteins. Dry heat is useful in sterilization of points of forcep, cork borer, inoculation needle, scissor, razor blade etc. by dipping them in spirit and flamed over spirit lamp or gas burner. The neck and mouth of culture tubes, conical flasks etc. also heated over flame until they become free from microbes.

Glass petri-dishes, pipettes, flasks, culture tubes etc. sterilized in hot air oven (Fig. 9). It is a device heated electrically and provided with a thermostat and a fan to circulate the air inside the oven. In general, 180°C temperature for 1 hour is sufficient to sterilize the materials. The holding time is recorded from the moment the predetermined temperature is achieved. Dry heat sterilized all those articles which are impermeable to moisture.

3. Filter sterilization

Sterilization by filtration is very useful in sterilizing solutions of heat sensitive materials (toxins, antibiotics, serum etc.). By filtration, microbes are simply removed rather than direct killing. Bacteria

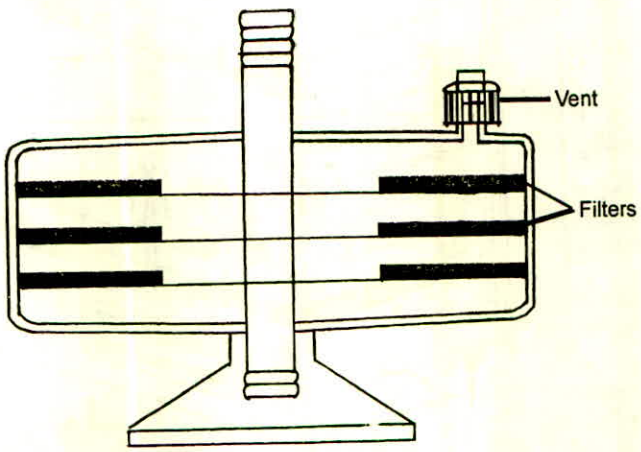


Fig. 10: Membrane filter

can be filtered with the pore size of filter less than 0.75µm. But doing this, the solution is not sterile because viruses and mycoplasma can pass through. Smaller pore sized filters are used for viruses. These filter are also useful in separation of a particular microbes present in the extract/solution, which may used for cultivation in the laboratory.

Types of filters

1. **Sintered glass filters** - made up of finely ground glass.
2. **Asbestos and asbestos-paper disks (Seitz)**- Asbestos pad fitted in between upper cylinder and lower funnel (Fig. 10).
3. **Microfilters** - It can be membrane or asbestos centrifuge or syringe filter used to filter small amount of fluids.
4. **Depth filters** - Made up of either diatomaceous earth (Berkefield) or unglazed porcelain (Chamberlain filters).
5. **Cellulose membrane filters** - These filters can be used for separation of viruses. They are made up of either cellulose nitrate (Gradocol membrane) or cellulose acetate.

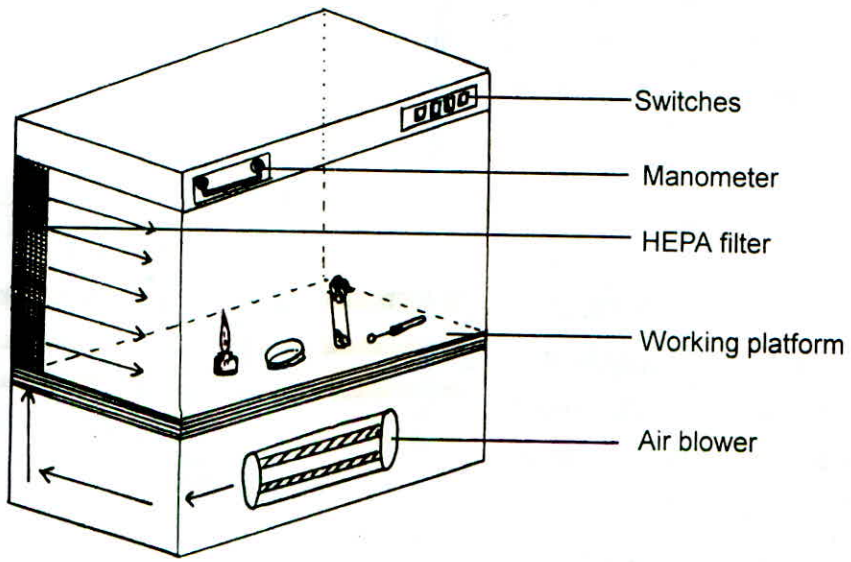


Fig. 11: Laminar Air Flow

Air can also be sterilized by filtration - eg. surgical masks and cotton plugs on culture tubes etc., that permits air in and stop microbes outside. Based on the above principle, Laminar air flow cabinets are available having high efficiency particulate air (HEPA) filters which remove 99.97% of 0.3 μm sized particle (Fig. 11). In this equipment, air forced through HEPA filters and sterile air available across the cabinet opening. This is very useful in précised microbiological work and protects the workers from dangerous micro-organisms.

4. Irradiation sterilization

Ultra-violet rays (UV) have been made in the irradiation of air with the wavelength of 254 nm, which is microbicidal but not too irritating. UV rays acts by inducing thymine diamers in DNA, thus interferes with the replication of DNA in organisms.

An UV ray does not penetrate glass, dirt films, water and other substances very effectively, so this can be used in laminar flow cabinets to sterilized air and any exposed surfaces. UV radiations are harmful to human beings because it burns skin and damages eyes. Therefore, care must be taken during practical use of UV rays.

B. CHEMICAL METHODS

This method is quick in sterilizing glass wares, metallic instruments, working surfaces, leaf surfaces and other articles used in cultural techniques. The chemicals used for sterilization (disinfect) acts by denaturing or altering proteins or lipids in the cytoplasmic membrane of microbial cells and interfere with formation of ATP.

The chemicals used for this purpose must be effective against a wide variety of infectious agents at high dilutions and in presence of organic matter. It should be stable upon storage, odorless or with a pleasant odor, soluble in water and lipids for penetration into microbial cells, have low surface tension, relatively inexpensive and it should not be toxic to mankind or corrosive for common materials. These chemicals are classified in two types.

1. **Strong disinfectants** - These are suitable for application on articles other than skin or tissues - Formaldehyde and Ethylene oxide.

2. **Mild disinfectants** - Ethyl alcohol, Mercuric chloride (0.1% solution for leaf surface disinfection), Sodium hypochlorite, Lysol or Cresol (3%).

PASTEURIZATION

When liquid substances are treated with controlled heating at temperatures well below boiling point, is known as pasteurization. This term has given in the honour of Louis Pasteur, a French Scientist in 1860s. He discovered a brief heating of wine at 55-60°C, destroy the lactic acid and acetic acid fermenting microbes and save the wine from spoilage for longer period.

In the year 1886, two German Chemists V.H. and F. Soxhler used this technique for milk preservation. Now a days, flash pasteurization or high temperature short terms (HTST) pasteurization is practiced for milk processing which consists of quick heating to about 72°C for 15 seconds and then rapid cooling.

TYNDALLIZATION OR FRACTIONAL STERILIZATION

Some times microbes are not completely destroyed at length, mean vegetative cells are killed but not the spores. In such instances, this particular technique is used, in which first steaming kill the vegetative cells of organisms and then spores germinating the following day, are killed by subsequent steaming. This technique is also useful for heat sensitive materials like sugars and gelatin. These can be heat sterilized by exposure at 100°C at intermittent periods eg. 20-45 minutes for 3 successive days. This technique has been named in the honour of its developer John Tyndall, an English Physicist (1820-93).

CHAPTER - VI

CULTIVATION OF MICRO-ORGANISMS

When micro-organisms are studied in the natural habitat, they are intermingled with several other forms of life. For detail study, they are isolated and cultivated on artificial medium under laboratory or *in vitro* conditions. Since all the microorganisms are not culturable on artificial media eg. fungi causing rust, smut, powdery mildew, downy mildew and viruses, as they are obligate in nature, which require living tissues for their growth and multiplication. Majority of microbes are isolated and cultured on artificial media by providing them suitable nutrients in *in vitro* conditions. The medium used for the culture depends upon the nature of pathogen.

Types of Media: Media are of three types:

1. **Natural** – Composition of natural media is not well known. Generally plant products are used @ 20% and added with agar in a liter of water. eg. Carrot juice agar medium.
2. **Artificial media** – Synthetic media are of two types
   a. **Semi synthetic** – When composition is partially known and not purely chemicals. eg. Potato Dextrose Agar

Peeled potato	-	200.0 gm
Dextrose	-	20.0 gm
Agar	-	15.0 gm
Distilled water	-	1000.0 ml

   b. **Synthetic** – When composition is well known and purely chemicals. eg. Richard's Medium

Potassium nitrate	-	10.0 g
Potassium dihydrogen phosphate	-	5.0 g
Magnesium sulphate	-	2.5 g
Ferric chloride	-	0.02 g
Sucrose	-	50.0 g
Agar	-	15.0 g
Distilled water	-	1000 ml

A. ISOLATION OF FUNGI FROM PLANT LEAF

For this purpose, all the materials required i.e. glass wares, nutrient medium and others must be sterilized before start of isolation work, because the purpose is to isolate only one organism of our interest from plant surface without contamination (free from other microbes). All the activities regarding isolation, must be performed under aseptic condition (microbe free) i.e. inside the hood of laminar air flow or in the inoculation chamber.

The pathogen must be isolated from freshly infected leaf or young lesions. The lesions along with some healthy portion are cut into small pieces and surface sterilized by dipping in 0.1% $HgCl_2$ (Mercuric chloride) solution for 1 minute [The surface sterilization can also be done with Sodium hypochlorite solution (2%) or Hydrogen peroxide solution (50%)], then washed in sterile distilled water 3-4 times to remove the disinfectant. The surface sterilized infected leaf piece are placed in the center of previously poured PDA petri-plates (to avoid bacterial contamination, pinch of streptocycline is added to each melted PDA conical flask before pouring the medium in the sterile petri-plate with the help of sterile forcep). These petri-plates are incubated at suitable temperature (usually 22-25°C), preferably in BOD incubator for 48-72 hrs. The hyphal strands are creeping from the margin of infected portion and tips of these hyphal strands are transferred to fresh PDA petri-plates or PDA slants after confirmation of desirable pathogen. This activity is called purification of pathogen and these petri-plates or slants are incubated in BOD incubator at 22-25°C.

In some cases, the infected leaves are incubated in moist chamber and fungal growth from infected portion is transferred directly to culture media.

Preparation of Potato Dextrose Agar (PDA)

Slices of peeled potato (200g) are cooked in 500 ml of distilled water and extract is filtered off. Dextrose (20g) and Agar-Agar (15g) are added to this extract and finally volume made up to 1 lt. Boil this medium once and pour into conical flasks or test tubes, plug them with non-absorbent cotton and sterilized in an auto-clave at 15 pounds/inch^2 (1.2 kg/cm^2) pressure for 20 minutes. Now, the PDA is ready for use.

B. ISOLATION OF FUNGI FROM SOIL

Fungi from soil are isolated by using serial dilution agar plate method. Soil suspension is prepared by taking 1 g of soil suspended in 10 ml sterilized distilled water. Shake well and allow to stand for 15 minutes at room temperature (Stock suspension). The ten fold dilutions are prepared with 1ml supernatant of stock suspension (10^3-10^5). Diluted suspension @ 0.1 ml/petri-plate is spread over previously poured Rose Bengal agar medium or PDA with 500 ppm of streptocycline. This method is called spread plate isolation. After incubation at optimum temperature (25-30°C) for 48-72 hrs or more, colonies of several fungi appear and desired one colony is picked after confirmation and transferred to petri-plates or slants containing fresh medium.

Rose Bengal Agar medium-composition

Dextrose	-	10.0 g
Peptone	-	5.0 g
Potassium dihydrogen phosphate	-	1.0 g
Magnesium sulphate	-	0.5 g
Rose Bengal (1 part in 30,000 parts of medium)		
Agar	-	15.0 g
Distilled water	-	1000 ml

C. ISOLATION OF BACTERIA FROM INFECTED LEAF

Isolation and other activities as described in case of fungi (A), are same for isolation of bacteria except artificial medium. In general, nutrient agar medium is used in place of PDA. After incubation, mucous like substance containing bacterial cells comes out from the margin of infected leaf piece, the colour may vary according to genus of bacteria. This substance then purified by streaking on fresh nutrient agar medium and colony showing identical characters described for particular bacterial pathogen, picked up and inoculated on fresh nutrient agar slants and incubated.

Plant pathogenic bacteria are also isolated by preparing bacterial suspension from infected tissues and streaking on nutrient agar medium. The identical colonies are isolated and subcultured on fresh nutrient agar slants.

Now a days, specific or selective medium is available for isolation and cultivation of specific fungus or bacterium according to their specific nutritional requirements.

D. ISOLATION OF BACTERIA FROM SOIL

For this also, the procedure described earlier for fungi (B), is the same except nutrient agar medium in place of PDA. Occasionally, when soil suspension is mixed with melted nutrient agar medium and allowed to solidify, the method is called pour plate isolation.

Nutrient agar medium composition:

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

Pathogenicity confirmation test

After isolation and purification, the organism should be tested for its pathogenic ability to cause disease on same species of plant. For this purpose, Robert Koch in 1884, laid down a series of conditions which must be fulfilled before the organism can be confirmed as the cause of the disease. This conditions are universally known as Koch's postulates which are as follows:

1. The micro-organism must be consistently associated with the symptom of the disease (Presence of microbes in the diseased part).
2. The micro-organism must be isolated and grown in pure culture, free from all other micro-organisms (Isolation and purification of microbe).
3. The micro-organism from pure culture must be inoculated on to healthy plants of the same species from which they were originally isolated and must reproduce the same disease as was originally observed (pathogenicity).
4. The micro-organism must be re-isolated from the artificially infected host (step 3) and its characteristics must be exactly identical like those observed in step 2 (Re-isolation of microbe).

Agar-Agar and its uses

It is an organic compound and is produced commercially from various genera of red algae like *Ahnfeltia, Grucilaria* and *Gelidium* found in sea. It helps in the solidification of the medium. There are two methods of extracting agar-agar from such algae: a. Freeze thawing method b. Diffusion-vacuum method

Agar-Agar is used in pharmaceuticals, cookery and therapeutic nutrition. It is also used for the preparation of dry, solid and semi-solid culture media for growing micro-organism in laboratory. It is variously used for stabilization of serum, emulsions, cellophane, tightening of paper and leather, production of photo-emulsions etc. Molecular sieves have been prepared from agar-agar and agarose, which are used for separating polymers of different molecular weights. Agarose is the most valuable component of agar-agar.

Exercise No. 6 : Cultivation of micro-organism (Diagrammatic representation)

1. Preparation of media

2. Isolation

3. Purification

4. Pathogenicity test

CHAPTER - VII

ISOLATION OF NEMATODES

Nematodes are small, elongated, worm like in appearance, triploblastic, more or less transparent, marked with striations and other markings and presence of stylet in plant parasitic nematodes.

A. FROM SOIL

1. **Baermann funnel method-** Baermann funnel consists of a fairly large glass funnel (12-15 cm dia) to which a piece of rubber tubing is attached, with a clamp placed on the tubing. The funnel is placed on a stand and filled with water. The soil sample (100-300 cm^3) is placed in the funnel on porous, wet strength paper, sometimes supported by a 5-6 cm circular piece of screen, or in a beaker over which a piece of cloth is fastened with a rubber band. The beaker is then inverted in the funnel, with the cloth and all the soil being below the surface of the water, and allows standing over night or for several hours. The live nematodes move actively and migrate through the cloth or porous paper into the water and sink to the bottom of rubber tubing just above the clamp. More than 90 per cent of the live nematodes are recovered in the first 5-8 ml of water drawn from the rubber tubing and examined under microscope.

2. **Sieving method -** This method is based on the fact that when small soil sample mixed with considerably more water, the nematodes floats in the water and can be collected on sieves with pores of different sizes. The soil (300 cm^3)-water (2 lt) mixture is stirred and then allowed to stand for 2 minutes. The liquid is poured through a 20 mesh (20 holes $inch^2$) sieve which holds large debris but allow the nematodes to pass into container. The liquid containing the nematode is then poured through a 60-mesh sieve, which holds the larger nematodes and some debris but lets the smaller ones pass though into another container. This is

followed by 200 mesh sieve. Both the 60 and the 200 mesh sieves are washed two or three times to remove as much of the debris as possible, and the nematodes are then washed into shallow dishes for direct examination and further isolation.

For further clearing nematodes are subjected to a combination of centrifugal flotation in a sugar solution. The steps are –

1. After following the steps of either method, place contents of beaker or container into centrifuge tubes, spin at 3000 rpm for 4 min., discard supernatant.
2. Fill tubes (1/2) with sugar solution (50%), shake well to suspend the pellet. Centrifuge at 3000 rpm for 1 min.
3. Nematodes decanted into fine sieve.
4. Clean nematodes by flushing into new container for counting and other observations.

B. FROM PLANT MATERIAL

Cut the infested plant material into small pieces or blend for few seconds. The tissues are than placed in the Baermann funnel and rest of the procedure is same as described earlier in isolation from soil.

Exercise No. 7 : Draw the microscopic diagram of nematodes

Diagram :

CHAPTER - VIII

COLLECTION AND PRESERVATION OF DISEASED SPECIMEN

Diseased plant material showing typical symptom should be collected in the morning hours because of sufficient humidity available, which protects the specimen from rapid drying. Also, the visibility of symptoms is better in morning hours than late hours.

Specimens along with some healthy portion are collected in polythene bags (mouth closed) or in vasculum (metallic cane) and pressed within the folded sheets of blotting paper or news paper, by putting them in the plant press consists of a pair of wooden planks of size 30 x 45 cm, tow bolts, 20 cm long, 2 cm in diameter are vertically fitted at both side of lower plank. The upper plank has two holes on either side, of the size of bolts. After placing the upper plank over the lower plank and allowing bolts to pass through the holes, the planks are tied with the help of winged nuts.

Note: (a) A good plant press is that which has ability to hold the plant materials under a constant and firm pressure. It should also help specimens to dry to a stage of crispness in such a manner as to retain the original colours of specimens as far as possible.

(b) Good quality blotter papers should be used for drying of specimens which not only absorbs moisture quickly from the plant specimen pressed in it, but can also be dried more quickly for further use.

After placing the specimens in plant press, it is locked for 24 hrs. This is called 'Sweating period'. After sweating period is over, press is unlocked, blotters are removed and specimens are rearranged as per need. Now, the specimens become flaccid. After rearranging, specimens are kept in between two fresh blot-

ters and tied again in the plant press for next 24 or 36 hrs. A third change follows and specimens are tied up for next 48 hrs. Now, the specimen become ready to mount in a glass frame with cotton base or on the hard sheets or kept in a paper bags with proper labeling. A label must contain following informations :

1. Name of the disease	_____
2. Causal organism	_____
3. Local name of plant material	_____
4. Botanical name of plant material	_____
5. Place of collection (Locality)	_____
6. Date of collection	_____
7. Collected & preserved by	_____

Sometimes, if there is no adequate arrangement for drying, specimens are liable to be spoiled and/or decomposed. To avoid this, specimens are pressed for 24 hrs and then dipped in a solution containing two parts of 40% formaldehyde and three parts of water. After few seconds of dipping, specimens are kept again in the blotting papers between two planks. Later specimens are removed and dried as per convenience. In other method, the above solution is replaced with 1 part of 40% formaldehyde and two parts of 70% alcohol. This solution should be applied to the specimen by flat brush instead of dipping. The solution penetrated inside the specimens and found to be better control from decomposition and/or spoilage.

Wet preservation diseased of specimens

Wet preservation of diseased specimen is done by keeping the material in 4% formaldehyde solution in airtight glass jars. In other method, diseased specimen, specially infected leaves are boiled in open vessel with saturated solution of Acetic acid (50%) and Cupric acetate diluted by water 3-4 times. As the boiling progresses,

first green colour of leaves disappear and then reappear due to saturated solution's colour. This replacement of colour is permanent and remain as such till the specimen physically exists. After boiling is over, specimens are washed thoroughly with tape water twice to remove excess of saturated solution. These specimens are then kept in 4% formaldehyde solution in airtight specimen jars. In other method, specimens are kept in Keep's solution for 2-10 days. The original green colour of specimens is permanently replaced by dark green colour of this solution and then preserved in 4% formaldehyde solution. Composition of Keep's solution:

50% Ethyl alcohol	-	90.0 ml
Glycerin	-	2.5 ml
Formaldehyde	-	5.0 ml
Copper chloride	-	10.0 g
Urenium nitrate	-	1.5 g

By the wet preservation, the shelf life of the specimen increases by many fold without spoilage.

Sometimes, red pigmented specimens are also preserved with original colour by following solution:

Zinc chloride	-	50.0 gm
40% formaldehyde	-	25.0 ml
Glycerin	-	25.0 ml
Water	-	1000.0 ml

Dissolve Zinc chloride in hot water, filter it and add other ingredients. By keeping the red-pigmented specimens in this solution, red colour will not be changed.

CHAPTER - IX

PHANEROGAMIC PLANT PARASITES

More than 2500 species of higher plants are known to live parasitically on other plants. These parasitic plants produce flowers and seeds. They vary greatly in their dependence on their host plant. Some are devoid of chlorophyll and depend entirely upon their host for food supply while others have chlorophyll and obtained only the mineral constituents of food from the host. Relatively few of the known parasitic higher plants cause significant loss to agricultural crops or forest trees. The most common and serious parasites belongs to the following families and genera:

Family	Genus	Nature
Cuscutaceae	*Cuscuta* (dodder, amarbel)	- Stem parasite
Viscaceae	*Arceuthobium, Phoradendron* (Loranthus, Banda, Dendrophthae, Giant mistletoes), *Viscum*	- Semi-stem parasite
Orobanchaceae	*Orobanche* (Broom rapes)	- Root parasite
Scrophulariaceae	*Striga* (witchwood)	- Semi root parasite

Dodder

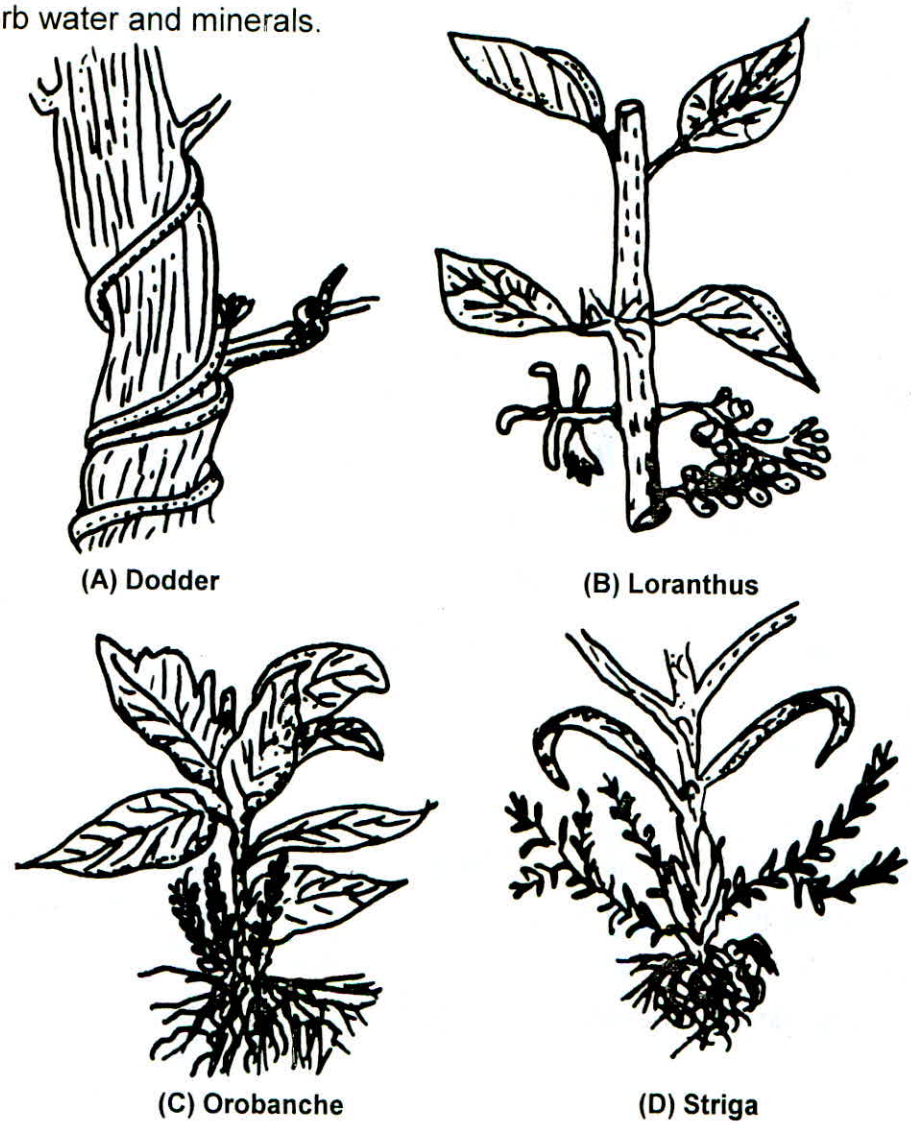
It is slender, achlorophyllous twining plant, the stem is tough, curling, thread-like and leafless, bearing only minute scales in place of leaves. The stem is usually yellowish or orange, sometimes almost white or tinged with red or purple. Clusters of tiny flowers occur on the stem and from them gray to brown seeds are produced (Fig. 12A).

Dodder stems in contact with susceptible host encircle the host plant, send

haustoria into it, and begin to climb the plant. The haustoria penetrate the stem or leaf and reach into the vascular tissues from which they absorb nutrients and water.

Loranthus

These are parasitic evergreens that have well developed leaves and stems (Fig. 12B]. It produces typical green leaves that can carry on photosynthesis usually small, dioecious flowers and berrylike fruits containing single seed. It produces haustorial sinkers, rather than roots, which grow in branches and stems of trees and absorb water and minerals.



(A) Dodder (B) Loranthus

(C) Orobanche (D) Striga

Fig. 12: Phanerogamic plant parasites

Orobanche

It is a whitish to yellowish annual plant, 15-50cm tall, has a fleshy stem, scale like leaves and produces numerous pretty, white, yellow-white or slightly purple, snapdragon like flowers arising singly along the stem (Fig. 12C]. The parasite produce seed pods about 5 cm long, each containing several hundred minute seeds. A large number of stems may be seen breaking the soil around host plant.

On germination, the seed produces a radicle, which grow towards the root of the host plant, become attached to it, and produces a shallow cup-like appressorium that surrounds the root. From the appressorium, a mass of undifferentiated cells penetrate the host, extend to end, occasionally into xylem and absorb nutrients and water from it.

Witchwood or striga

Seeds of striga germinate close to host roots, germinate and grow toward these roots (Fig. 12D). As soon as the rootlet comes in contact with the host root, its tip swells into a bulb shaped haustorium which dissolves and penetrates the host roots and finally its leading cells-tracheids, reach the vessels of the host roots. The tracheids force their way into the vessel, from which they absorb water and nutrients.

CHAPTER - X

NON-PARASITIC DISEASES

1. Black heart of potato: Large tubers are more susceptible to black heart than small ones. The typical symptoms are :

a. Dark gray to purplish or inky black discolouration occurs in the central tissues of the potato tuber (Fig. 13A).

b. Later, the affected tissues may dry out and separate, thus forming cavities.

c. The discolouration may extend to the surface also.

A set of three distinct environmental conditions can cause this disease.

i. Poor ventilation in storage.

ii. High temperature during transit.

iii. High temperature of soil during growth and maturity of tubers in the field.

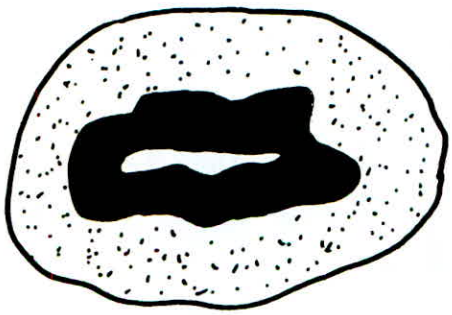


Fig. 13 A: Black heart of potato

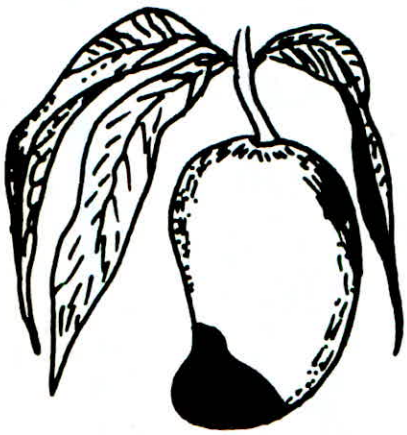


Fig. 13 B: Black tip of mango

2. Black tip of mango: This disease commonly occurs when the fruits are about 6-8 weeks old or when they reach to maturity. The typical symptoms are :

1. Small etiolated area at the distal end which gradually spreads, turns nearly black and covers the tip completely.
2. The tip is flattened with the outer skin turning hard and sunken (Fig. 13B).
3. The inner portion is soft and gives dark brown liquid due to rotting.

The disease is most common in orchards in the vicinity of brick kilns. The smoke of kilns pollutes the air with toxic gases like sulphur dioxide (SO_2) which cause necrosis of fruit tissues.

3. Khaira disease of rice: The disease usually appears 10-15 days after transplanting due to deficiency of zinc. The typical symptoms are :

1. Leaves of the diseased plant show chlorosis at the base.
2. Large number of small brown or bronze spots appear on the leaf lamina surface.
3. These spots coalesce to form bigger spots and ultimately the entire leaf turns bronze coloured and dries.
4. The growth of diseased plant is stunted.
5. Root growth is also restricted and usually the main roots turns brown.
6. The fine roots are destroyed.

CHAPTER - XI

MYCELIUM, SPORES AND FRUITING BODIES OF FUNGI

Fungus – It means mushroom. It is define as eukaryotic, spore bearing, achlorophyllous organism that generally reproduce sexually and asexually, and whose usually filamentous, branched somatic structures are typically surrounded by cell wall containing chitin or cellulose, or both of these substances, together with many other complex molecules.

Hypha - A threadlike, tubular filament of fungus (Pl. hyphae).

Mycelium - Network or mass of hyphae constituting the body of fungus is called mycelium.

Mycelium is of two types-

1. **Aseptate/nonseptate (Coenocytic)** – Mycelium without cross walls (Septa) or compartment just like hollow vessel. The nuclei lie in a common matrix (Fig.14A).
2. **Septate** – Mycelium divided with cross walls (septa) or compartment at regular intervals. These septa have minute pores through which protoplasmic continu-

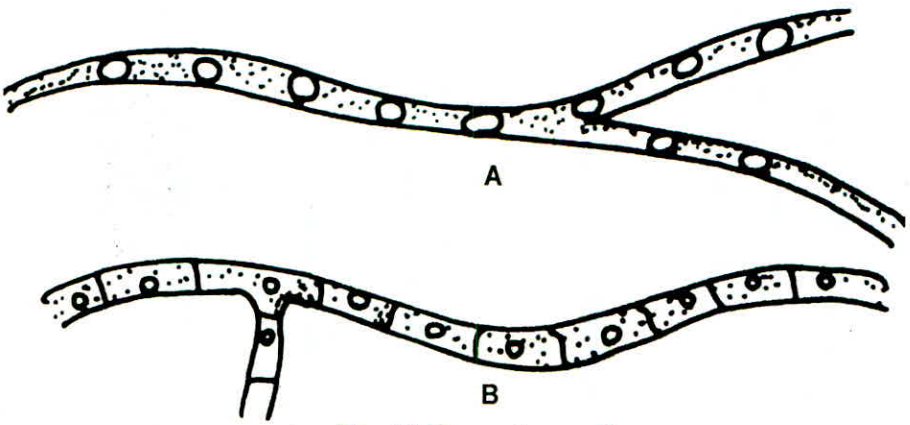


Fig. 14. Type of mycelium

ity is maintained throughout the length of mycelium (Fig.14B). The pores are micro, simple and dolipore

Spore – It is define as a minute propogative unit functioning as a seed, but differing from it in that a spore does not contain a preformed embryo.

Types of spores

A. Asexual

Asexual spore are formed without involvement of any sex organs i.e. they are formed vegetatively (Fig. 15). They are:

1. **Conidia** – Non motile spores formed at the tip or side of sporogenous cell (conidiophore). They may be spherical, ovoid, elongated, cylindrical, thread like, spirally curved or star shaped; unicellular or multicellular (muriform); produced singly or in chains (catenulate) either acropetal or basipetal.

2. **Chlamydospores** – The cells of hyphae become thick walled, either singly or in chains after accumulation of food material in them. They may be terminal or inter-calary in position. They are usually impregnated with hydropholic material and generally functions as resting spores.

3. **Oidia (Arthrospores)** – Thin walled, free hyphal cell derived from the fragmentation of somatic hypha into its component cells, born in chains of basipetal succesion, unicellular, barrel shaped or nearly spherical.

4. **Blastospores** – Spores produced by the process of budding, single celled, in chains but later separated from each other.

5. **Aplanospores** – Spherical, non motile, unicellular, aflagellated.

6. **Zoospores** – Motile by one or two flagellum (whiplash and/or tinsel), pyriform (pear shaped) or reniform (kidney shaped), produced in sporangia, single celled.

7. **Stylospores** – Filiform, elongated, aseptate, bent at the top like a walking stick and single celled.

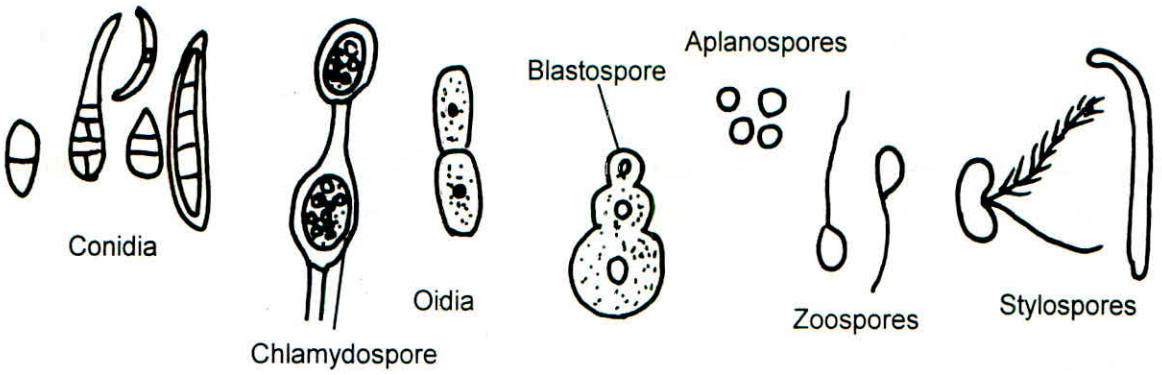


Fig. 15: Asexual fungal spores

B. Sexual spores: Produced by differentiated sex organs i.e. gametangia (Fig. 16).

1. **Oospores** – Formed by gametangial contact (oogamy), generally spherical, thick walled, smooth surface (sometimes warty/ornamented), plerotic or aplerotic.

2. **Zygospores** – Thick walled, heavily pigmented, yellow or black in colour, walls may be rough with peaks or smooth and punctate, provided with suspensors, formed by gametangial copulation.

3. **Ascospores** – Meiospores formed in asci in definite 8 numbers by somatogamy/ spermatization, minute to big, globose to thread-like, colourless to black, unicellular to multicellular.

4. **Basidiospores** – Produced on basidium in definite 4 numbers on tips of sterigmata, unicellular, uninucleate, globose or oval or elongated or sausage-shaped, colourless or pigmented (green, yellow, orange, brown or black), posses natural out growth – 'the hilum'.

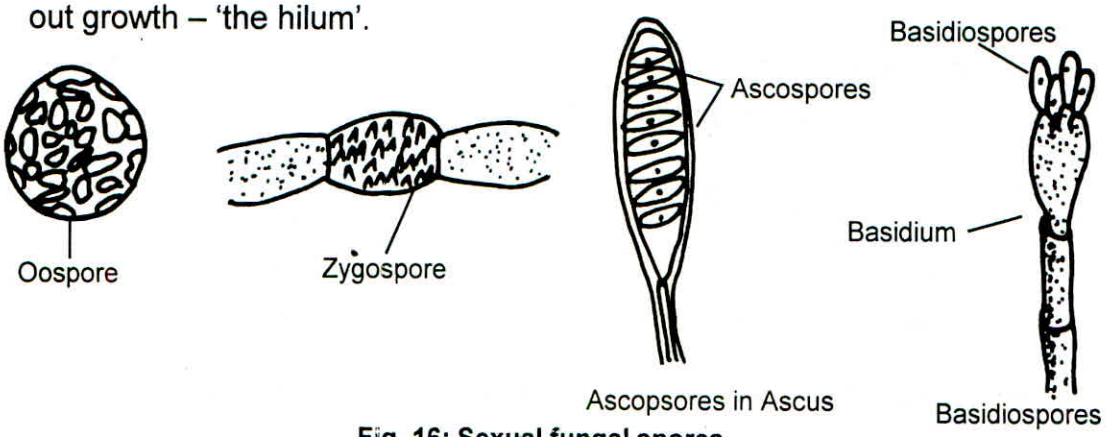


Fig. 16: Sexual fungal spores

Fruiting bodies

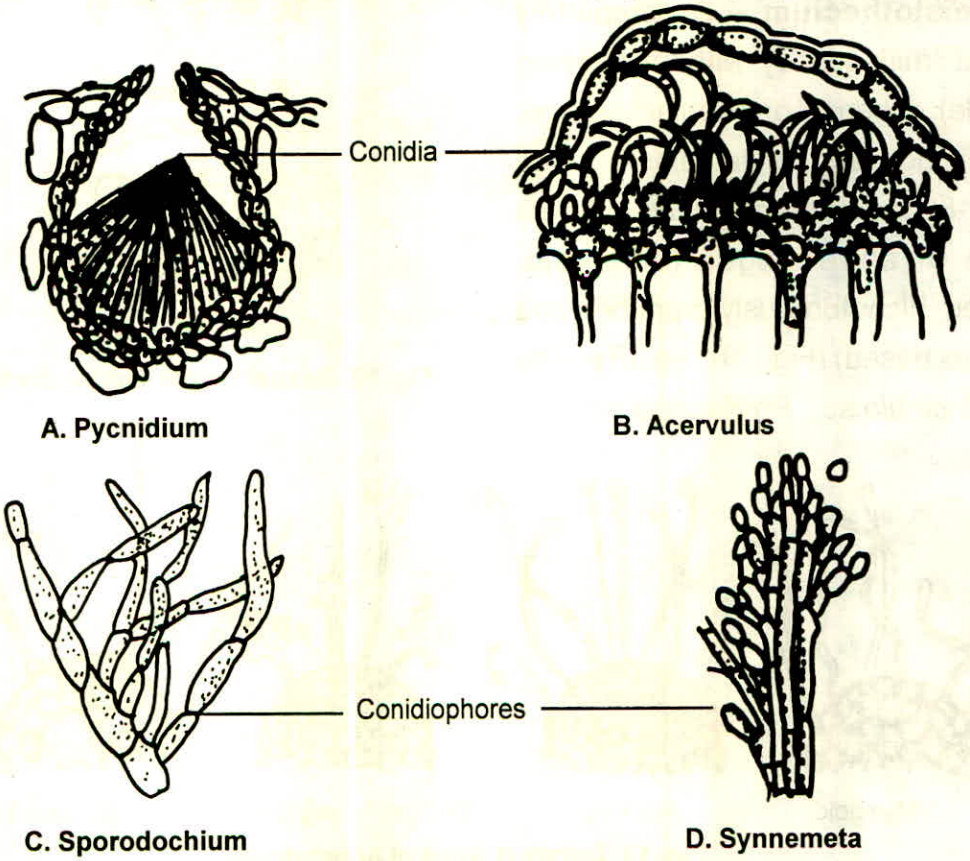
A. Asexual

1. Pycnidium – A globose or flask shaped hollow fruiting body, lined inside with conidiophores on which conidia are produced, have an opening called ostiole (Fig. 17 A). eg. *Phomopsis* sp.

2. Acervulus – A mat of hyphae giving rise to short conidiophores closely packed together and forming a bed like mass, setae (sterile hair like structure) also present (Fig. 17 B). eg. *Colletotrichum* sp.

3. Sporodochium – A cushion shaped stroma on which conidiophores are formed (Fig. 17 C). eg. *Fusarium* sp.

4. Synnemata – A group of conidiophores are grouped together at the base and free towards the tip on which conidia are formed (Fig. 17 D). eg. *Ceratocystis* sp.



A. Pycnidium **B. Acervulus**

C. Sporodochium **D. Synnemeta**

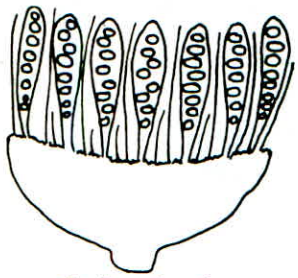
Fig. 17: Asexual fungal fruiting bodies.

B. Sexual

1. Apothecium – An open fruiting body, disc or cup or saucer shaped, produce asci on open upper surface, paraphyses may be present (Fig. 18 A). eg. *Sclerotinia* sp.

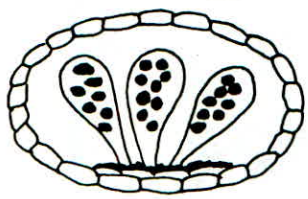
2.Perithecium - A flask shaped, hollow, closed fruiting body with a ostiole at the top, asci are lined along the inner surface of the fruiting body, sterile hair like structure paraphyres also present between asci (Fig. 18 B). eg. *Chaetomium* sp., *Nectria* sp., *Cleviceps* sp.

3.Cleistothecium – A completely closed fruiting body without opening (ostiole), spherical or globular in shape, containing asci inside, black, tough (Fig. 18C), ornamented with different types of appendages (myceloid, hooked, dichotomously branched and bulbous based) (Fig. 19). eg. *Erysiphe* sp., *Uncinula* sp., *Phyllactinia* sp.



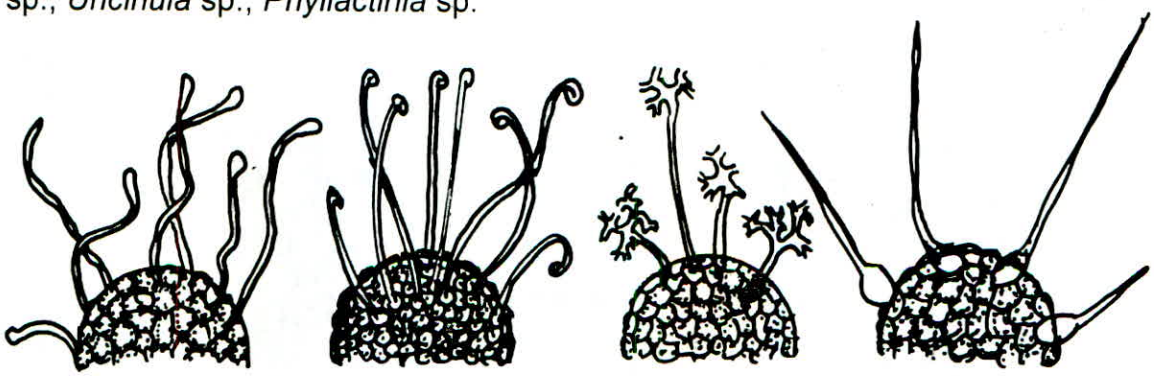
A. Apothecium

B. Perithecium



C. Cleistothecium

Fig. 18: Sexual fungal fruiting bodies



Myceloid Hooked Dichotomously branched Bulbous based

Fig. 19: Different types of appendages

Exercise No. 8 : Draw the diagram of asexual spores

Conidia

Chlamydospores

Oidia

Blastospores

Aplanospores

Zoospores

Stylospores

Exercise No. 9 : Draw the diagram of sexual spores

Oospores

Zygospores

Ascospores

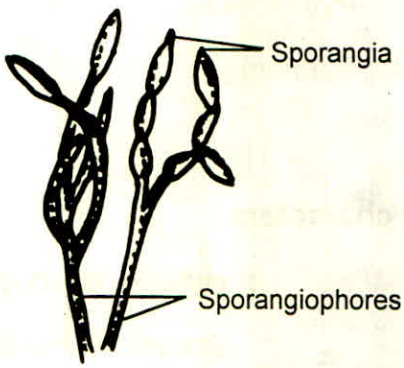
Basidiospores

CHAPTER - XII

SYSTEMATIC POSITION AND IDENTIFICATION OF IMPORTANT PLANT PATHOGENIC FUNGI

Phytophthora spp.

Kingdom - Stramenophila
Phylum - Oomycota
Class - Oomycetes
Order - Peronosporales
Family - Pythiaceae
Genus - *Phytophthora*



Identifying characters

Mycelium : Hyaline, coenocytic (nonseptate) and branched.

Sporangiophores : Hyaline, septate, branched, indeterminate, sympodial branching (zig-zag growth), nodulate (swellings or bulbous enlargement at the node), terminates in a sporangium.

Sporangia : Lemon or pear shaped, papillate at apex, hyaline, single celled.

Oospore : Spherical, thick and smooth walled

Importance :

1. *Phytophthora infestans* - Late blight of potato and tomato
2. *P. citrophthora* - fruit rot and gummosis of citrus
3. *P. palmivora* - Black pod and bud rot of palm
4. *P. cinnamomi* - Root rot of avacado

Pythium spp.

Kingdom - Stramenophila

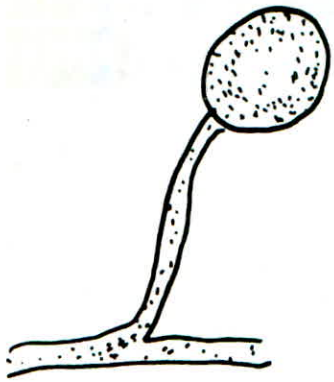
Phylum - Oomycota

Class - Oomycetes

Order - Peronosporales

Family - Pythiaceae

Genus - *Pythium*



Identifying characters

Mycelium : Hyaline, coenocytic (nonseptate) and branched.

Mycelium : Branched, hyaline, coenocytic, slender.

Sporangiophores : Simple like hypha, bears single sporangium terminally

Sporangia : Globose or lobed (some times club shaped).

Oospore : Smooth, double thick walled, spherical and aplerotic (does not fill the entire cavity of oogonium)

Importance :

1. *Pythium aphanidermatum*- Damping off of vegetable seedlings, fruit rot of cucurbits, soft rot of papaya.
2. *P. debaryanum* – Damping off of tobacco and chillies.
3. *P. graminicolum* – Rhizome and root rot of turmeric.

Albugo spp.

Kingdom - Stramenophila

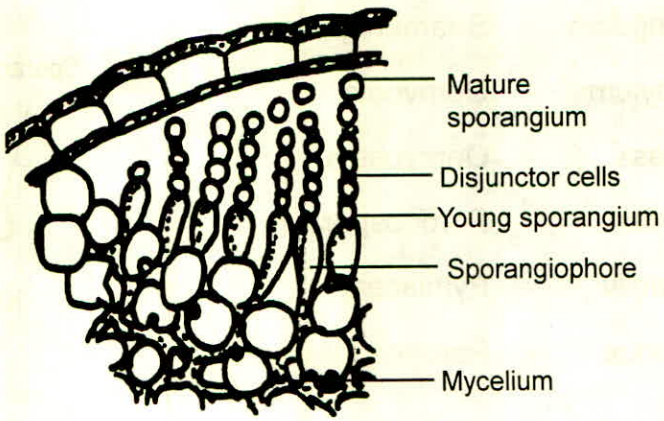
Phylum - Oomycota

Class - Oomycetes

Order - Peronosporales

Family - Albuginaceae

Genus - *Albugo*



Identifying characters

Mycelium : Hyaline, coenocytic, inter cellular, branched with short, knob-like haustoria.

Sporangiophores : Short, club-shaped (clavate), free from each other laterally, very thick, borne in close proximity to one another in palisade layers or beds just below the host epidermis.

Sporangia : Globose or nearly spherical, single celled, formed in chains in basipetal succession (oldest at top and the youngest at the base of sporangiophore) gelatinous pad between two successive sporangia function as 'disjunctor'.

Oospore : Double walled, outer wall is thick and rough (spiny or warty).

Importance :

1. *Albugo candida*- white rust (white blisters) of crucifers.
2. *A. bliti*– White rust of amaranthaceae.
3. *A. ipomoeae-panduranae*- White rust of sweet potato and morning glory.
4. *A. occidentalis* – White rust of spinach.

Peronospora spp.

Kingdom - Stramenophila

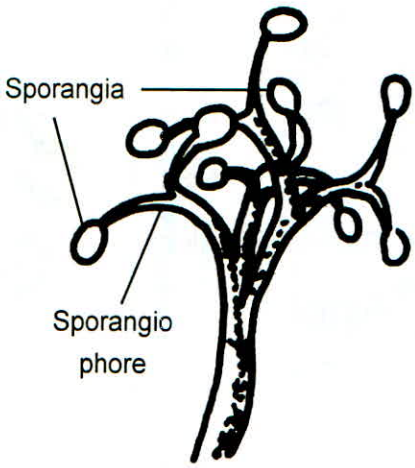
Phylum - Oomycota

Class - Oomycetes

Order - Peronosporales

Family - Pythiaceae

Genus - *Peronospora*



Identifying characters

Mycelium : Hyaline, coenocytic, profusely branched and inter-cellular.

Sporangiophores : Determinate, emerge in clusters from the stomata of under surface of the leaves, long, slender, unbranched for two third or more of their length, then dichotomously branched two to seven times. The ultimate branches called sterigmata, are at obtuse or acute angles with each other, bear conidia singly at the pointed tip of sterigmata.

Sporangia : Spherical, oval or elliptical, single celled, greenish yellow, borne singly at the pointed tip of sterigmata.

Oospore : Spherical, greenish yellow, thick outer wall marked by large raised reticulations

Importance :

1. *Peronospora parasitica*-Downy mildew of crucifers
2. *P. viciae*– Downy mildew of peas, beans and lucern
3. *P. destructor*-Downy mildew of onion
4. *P. farinosa* – Downy mildew of spinach and beet

Sclerospora spp.

Kingdom - ~~Stramenophila~~

Kingdom - Stramenophila

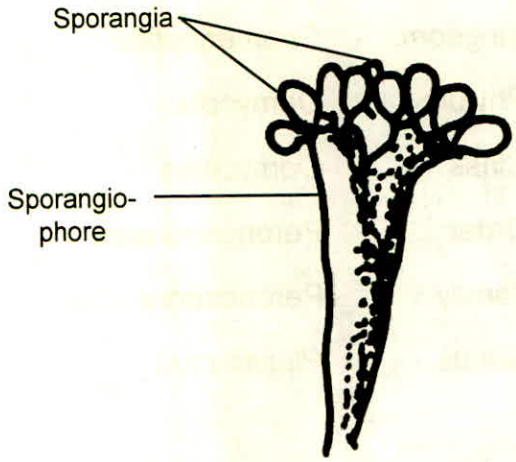
Phylum - Oomycota

Class - Oomycetes

Order - Peronosporales

Family - Peronosporaceae

Genus - *Sclerospora*



Identifying characters

Mycelium : Hyaline, coenocytic, branched and intercellular with small bulbous haustoria.

Sporangiophores : Determinate, broader at apex, unbranched in the lower part but at the apex repeatedly dichotomous or even trichotomous, bears single sporangium at tip of ultimate branch called sterigmata.

Sporangia : Hyaline, elliptical, single celled, borne singly, slightly pointed or papillate.

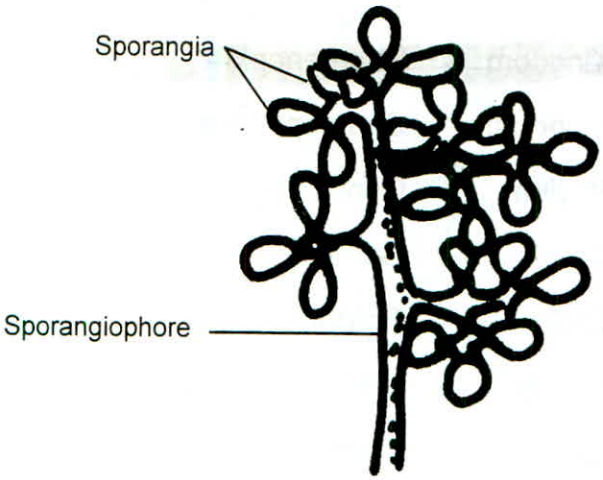
Oospore : Usually spherical but irregular or elliptical due to uneven thickenings of oogonial wall, shrinks to touch the new oosporial wall at many points, plerotic

Importance :

1. *Sclerospora graminicola* – Downy mildew and green ear of bajra
2. *S. sorghi* – Downy mildew of sorghum
3. *S. sacchari* & *S. spontanea* – Downy mildew of sugarcane

Plasmopara spp.

Kingdom - Stramenophila
Phylum - Oomycota
Class - Oomycetes
Order - Peronosporales
Family - Peronosporaceae
Genus - *Plasmopara*



Identifying characters

Mycelium : Hyaline, coenocytic, intercellular with knob shaped haustoria, branched.

Sporangiophores : Short, truncate branches nearly that are right angles to the axis.

Sporangia : Hyaline, single celled, lemon shaped and borne singly at tip of the branch.

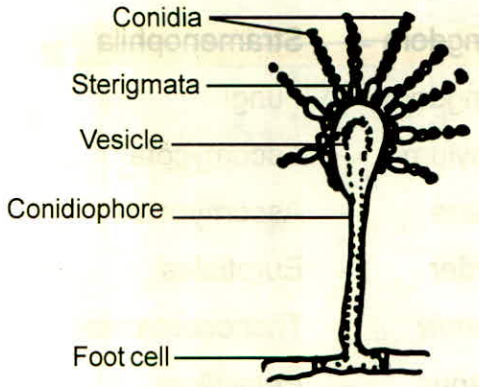
Oospore : Simple, double thick walled, spherical.

Importance :

1. *Plasmopara viticola* – Downy mildew of grapevine
2. *P. nivea* – Downy mildew of umbelliferae
3. *P. australis* – Downy mildew of cucurbits
4. *P. halstedii* – Downy mildew of sunflower and other compositae

Aspergillus spp.

Kingdom - Fungi
Phylum - Ascomycota
Class - Ascomycetes
Order - Eurotiales
Family - Trichocomaceae
Genus - *Aspergillus*



Identifying characters

Mycelium : Well developed, profusely branched, septate, multinucleate, hyaline which ramify on the substratum.

Conidiophores : Arise singly at right angle from thick walled, hyphal cell called 'foot cell', unseptate, long, erect, terminating in a bulbous shaped head 'vesicle'.

Vesicle : Spherical usually, occassionaly hemispherical or elliptical, with bottle shaped sterigmata or phialides or conidiogenous cells arise in one or two layers as primary and secondary sterigmata.

Conidia : Formed in chain (basipetal succession), single celled, typically globose with outer roughened walls.

Ascocarp : Small cleistothecia generally yellow coloured (colonies in petri-dishes are black, brown, yellow, green etc.).

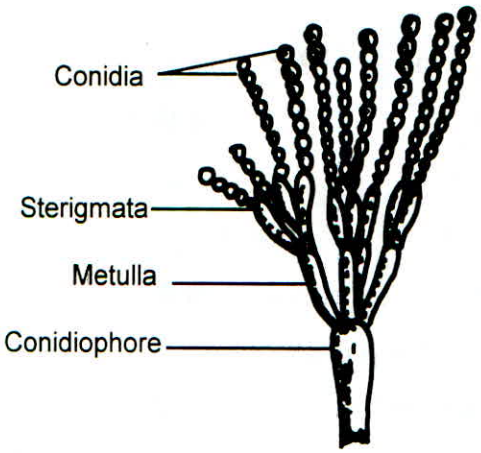
Importance :

1. *Aspergillus flavus*–Produce violent toxin 'Aflatoxin' in contaminated seeds.
2. Species of *Aspegillus*-Common contamination of cultures in plant pathological laboratories, food spoilage
3. *A. oryzae* – Used in alcoholic beverage 'Sake' from rice.
4. *A. wentii* - Processing of soybean.
5. *A. niger* - Used in bioassay of available amounts of copper, magnesium, potassium and molybdenum in the soil.

Penicillium spp.

Kingdom - Stramenophila

Kingdom - Fungi
Phylum - Ascomycota
Class - Ascomycetes
Order - Eurotiales
Family - Trichocomaceae
Genus - *Penicillium*



Identifying characters

Mycelium : Highly branched, septate, hyaline, uninucleate.

Conidiophores : Simple, long, erect, branched about 2/3 of the way to the tip, branches (metullae) more or less parallel, each metulla bears a tuft of sterigmata or phialides on which conidia are produced.

Conidia : Globose to ovoid, unicellular, hyaline, appear as glass beads, formed in basipetal succession on bottle shaped phialides. In abundance, they appear green, yellow or blue, responsible for colony colour in petridishes.

Ascocarp : Cleistothecia are yellow or light brown, thick walled. The conidiophore with is branches (metullae), sterigmata (phialides) and conidia give the appearance of a broom known as 'Penicillus'.

Importance :

1. *Penicillium expansum* – Blue mold of apple fruits.
2. *P. italicum* & *P. digitatum* – Blue and green mold of citrus fruits respectively.
3. *P. gladioli* – Storage rot of gladiolus.
4. *P. notatum* & *P. chrysogenum* – Produces wonder drug 'Penicillin'.
5. *P. griseofulvum* & *P. nigricans* – Produces antibiotic 'Griseofulvin'.
6. *P. rogueforti* & *P. camemberti* – Used in ripening of highly priced roguefort and camembert cheese.

Erysiphe spp.

Kingdom - Fungi

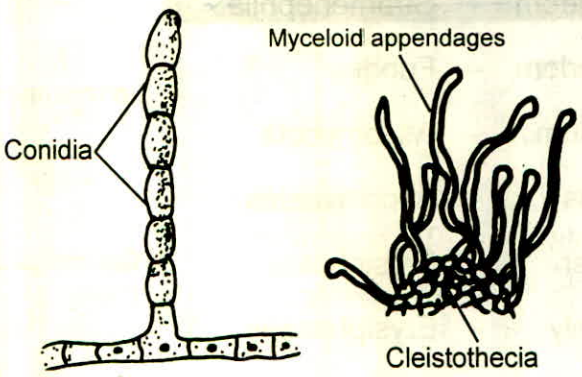
Phylum - Ascomycota

Class - Ascomycetes

Order - Erysiphales

Family - Erysiphaceae

Genus - *Erysiphe*



Identifying characters

Mycelium : External mycelium hyaline, rarely light brown septate without hyphopodia (one or two cells, more or less lobed serves as on organ of attachment), branched with intracellular haustoria either globular type or digitate (finger) type.

Conidiophores : Long, hyaline, erect, septate, arise singly, bears conidia in chain at the tip.

Conidia : Elliptical, barrel shaped (some times cylindrical) with rounded edges, hyaline, unicellular and formed in basipetal succession.

Ascocarp : Cleistothecia sharp, black, spherical, minute bodies with characteristic myceloid appendages.

Importance :

1. *Erysiphe polygoni* – Powdery mildew of legumes.
2. *E. pisi* – Powdery mildew of pea.
3. *E. graminis* – Powdery mildew of cereals and grasses.
4. *E. cichoracearum* – Powdery mildew of cucurbits.

Phyllactinia spp.

~~Kingdom - Stramenophila~~

Kingdom - Fungi

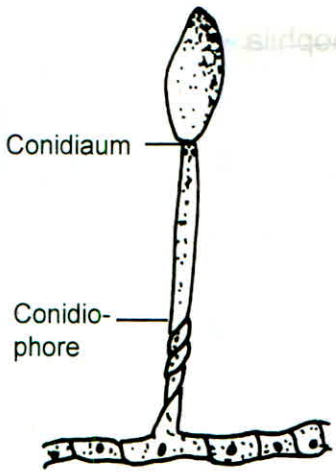
Phylum - Ascomycota

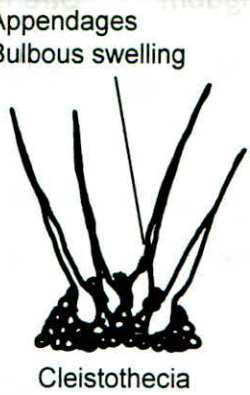
Class - Ascomycetes

Order - Erysiphales

Family - Erysiphaceae

Genus - *Phyllactinia*





Identifying characters

Mycelium : Septate, hyaline, without hyphopodia, branched with glubulor type haustoria.

Conidiophores : Unseptate, hyaline, spirally coiled, bears conidia singly.

Conidia : Pointed, reticulated surface, clavate and formed singly.

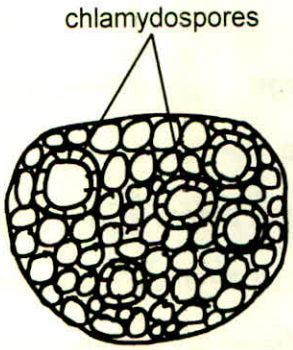
Ascocarp : Cleistothecia provided with straight, unbranched pointed appendages have bulbous swelling.

Importance :

1. *Phyllactinia dalbergiae* – Powdey mildew of sisam.
2. *P. corylea* – Powdery mildew of hazel (corylus) and *Morus alba.*

Protomyces spp.

Kingdom - Fungi
Phylum - Ascomycota
Class - Archiascomycetes
Order - Taphrinales
Family - Protomycetes
Genus - *Protomyces*



Identifying characters

Mycelium : Closely septate, broad, intercellular, endophytic, irregular branching, multinucleate.

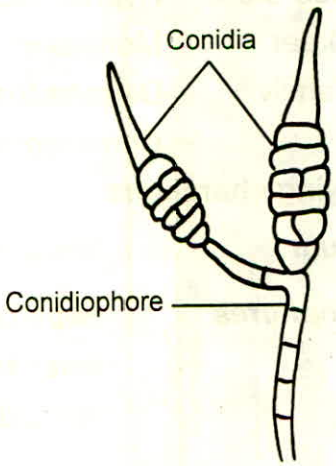
Chlamydospores : Multinucleate cells of hyphae become thick walled resting cells, ellipsoidal or globose.

Ascocarp : Synascus- an elongated, more or less cylindrical sac.

Importance : *Protomyces macrosporus* – Stem gall of coriander.

Alternaria spp.

Kingdom - Mycota (Fungi)
Subdivision - Deuteromycotina
Form Class - Deuteromycetes
Form Sub Class - Hyphomycetidae
Form Order - Moniliales
Form Family - Dematiaceae
Genus - *Alternaria*



Identifying characters

Mycelium : Frequently septate, intercellular, branched, light brown become darker with age.

Conidiophores : Short, dark coloured, simple, septate.

Conidia : Borne singly or in chains (acropetal succession), dark coloured, muriform (presence of 5-10 transverse septa and few longitudinal septa), obclavate.

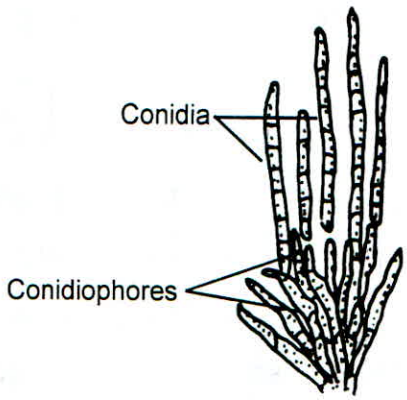
Perfect stage : *Pleospora.*

Importance :

1. *Alternaria solani*– Early blight of potato and tomato.
2. *A. brassicae* and *A. brassicicola* – Leaf spot crucifers.
3. *A. carthamii* – Leaf spot of safflower.
4. *A. triticina* – Leaf blight of wheat.
5. *A. tenuis* – Leaf spot of serpagandha.

***Cercospora* spp.**

Kingdom - Mycota (Fungi)
Subdivision - Deuteromycotina
Form Class - Deuteromycetes
Form Sub Class - Hyphomycetidae
Form Order - Moniliales
Form Family - Dematiaceae
Genus - *Cercospora*



Identifying characters

Mycelium : Septate, branched, intercellular, slightly grayish in colour.

Conidiophores : Septate, simple, dark in colour, arise in dense or very dense fascicles, olivaceous brown, unbranched and geniculate (shouldered, having knee joints).

Conidia : Long, cylindrical or obclavate, filiform, hyaline (sometimes coloured) and multiseptate, with rounded to distinctly truncate base and sub-acute tips, borne on knee shaped joints, formed in acropetal succession.

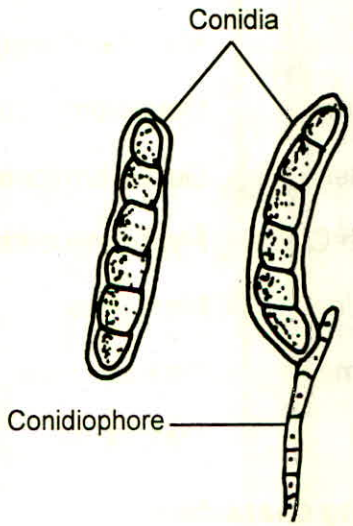
Perfect stage (Teleomorph): *Mycosphaerella.*

Importance :

1. *Cercospora arachidicola & C. personata* – Tikka disease of groundnut.
2. *C. musae* – Leaf spot (Sigatoka) of banana.
3. *C. beticola* – Leaf spot of beet and spinach.
4. *C. apii* – Leaf spot of celery.
5. *C. kikuchii* – Purple stain of soybean seeds.
6. *C. rodmanii* – Biological control of water hyacinth.

***Helminthosporium* spp.**

Kingdom - Mycota (Fungi)
Subdivision - Deuteromycotina
Form Class - Deuteromycetes
Form Sub Class - Hyphomycetidae
Form Order - Moniliales
Form Family - Dematiaceae
Genus - *Helminthosporium*



Identifying characters

Mycelium : Septate, prostrate, grayish brown to dark brown, inter cellular.

Conidiophores : Stout, erect, unbranched, dark obivaceous near the base and lighter towards the tip, arise in tufts, posses bent and knee joints.

Conidia : Cylindrical or obclavate, rounded ends, curved, multiseptate produced singly at tip (apically) and at knee

joints (laterally) of conidiophores.

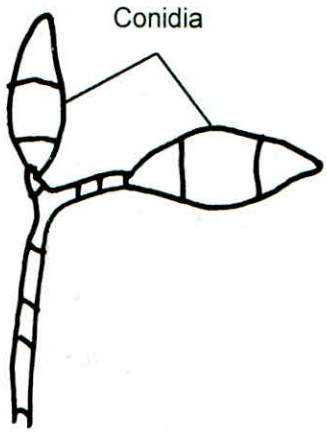
Perfect stage : *Cochliobolus, Trichometasphaeria* and *Pyrenophora.*

Importance :

1. *Helminthosporium oryzae* - Brown spot of paddy.
2. *H. maydis* - Southern leaf blight of maize.
3. *H. heveae* - Bird's eye spot of rubber.
4. *H. sacchari* - Eye spot and seedling blight of sugarcane.

***Pyricularia* spp.**

Kingdom - Mycota (Fungi)
Subdivision - Deuteromycotina
Form Class - Deuteromycetes
Form Sub Class - Hyphomycetidae
Form Order - Moniliales
Form Family - Dematiaceae
Genus - *Pyricularia*



Identifying characters

Mycelium : Septate, multinucleate, branched.

Conidiophores : Simple, rarely branched, grayish, septate, slender, thickened at the base.

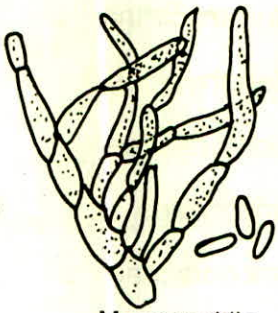
Conidia : Produced terminally, ovate or pear shaped (Pyriform), 2 septate (three-celled), pointed to blunt apex, multinucleate, sub hyaline.

Perfect stage : *Magnaporthe.*

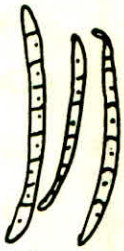
Importance : 1. *Pyricularia oryzae* - Blast disease of rice and grasses.

Fusarium spp.

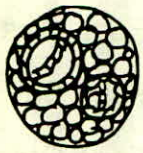
Kingdom - Mycota (Fungi)
Subdivision - Deuteromycotina
Form Class - Deuteromycetes
Form Sub Class - Hyphomycetidae
Form Order - Moniliales
Form Family - Tuberculariaceae
Genus - *Fusarium*



Macroconidia



Macro conidia



Mycelial strand inside the xylern vessel

Identifying characters

Mycelium : Extensive, hyaline, often with some tinge of pink-purple or yellow, septate, inter and intracellular.

Conidiophores : Simple, slender, short, aseptate or septate formed in the sporodochia.

Conidia : Two types- **Macro-conidia-** Boat shaped or crescent, hyaline, long, pointed at the tip, knotched at the base, septate (3-4 septa).

Micro-conidia- Small, elliptical or curved, unicellular or with one septa, gathering in short chains or in spore balls.

Chlamydospores : Spherical, thick walled, terminal or intercalary.

Perfect stage : *Gibberella, Nectria, Calonectria* and *Micronectriella.*

Importance :

1. *Fusarium oxysporum* fsp. *cubense* - Panama wilt of Banana.
2. *F. moniliforme* - Bakanae disease of rice and maize.
3. *F. o.* fsp. *vasinfectum*- cotton wilt.
4. *F. udum* - Pigeon pea wilt
5. *F. o.* fsp. *ciceris* - Gram wilt.

Colletotrichum spp.

Kingdom - Mycota (Fungi)

Subdivision - Deuteromycotina

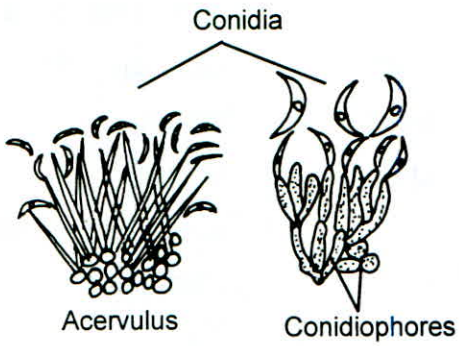
Form Class - Deuteromycetes

Form Sub Class - Coelomycetidae

Form Order - Melanconiales

Form Family - Melanconiaceae

Genus - *Colletotrichum*



Identifying characters

Mycelium : Septate, inter and intra cellular, branched.

Acervuli : Cushion shaped, provided with short conidiophore and sterile setae.

Setae : Straight, unbranched, tapered towards the apex, brown to black, smooth, thick walled and septate (3-4 septa).

Conidiophores : Hyaline to brown, septate, branched at the base, smooth, short, packed in acervuli.

Conidia : Falcate (sickle shaped), posses large oil globule in centre, hyaline, unicellular, borne singly on tip of short conidiophores.

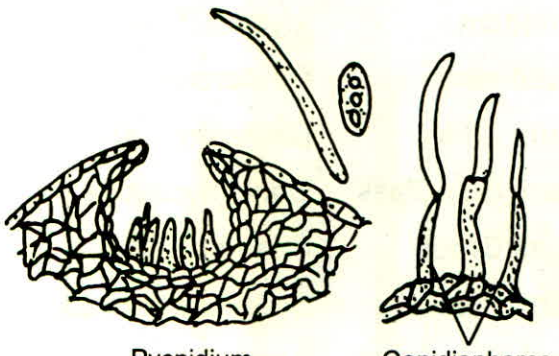
Perfect stage : *Glomerella.*

Importance :

1. *Collectotrichum falcatum* - Red rot of sugarcane.
2. *C. capsici* - Die back of chilli, Anthracnose of cotton.
3. *C. gloeosporioides* - Anthracnose of mango.
4. *C. dematium* - Anthracnose of spinach.

Phomopsis spp.

Kingdom - Mycota (Fungi)
Subdivision - Deuteromycotina
Form Class - Deuteromycetes
Form Sub Class - Coelomycetidae
Form Order - Shaeropsidales
Form Family - Shaeropsidaceae
Genus - *Phomopsis*



Identifying characters :

Mycelium : Septate, branched and well developed.

Pycnidium : Globose to irregular, wide ostiole, brown to black.

Conidiophores : Hyaline, simple /branched, septate, bears conidia singly.

Conidia : 1. Alpha - Hyaline, fusiform, straight, aseptate.

2. Beta (stylospores) - Hyaline, aseptate, filiform, curved or bent.

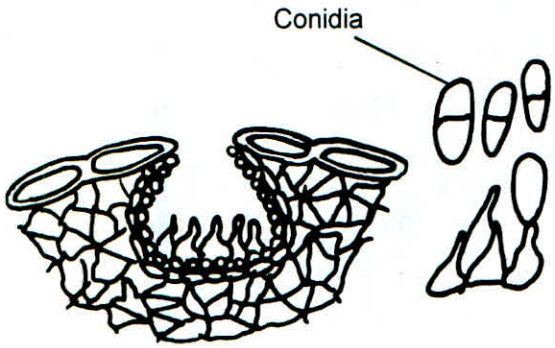
Perfect stage : *Diaporthe.*

Importance :

1. *Phomopsis vexans* – Stem, leaf blight and fruit rot of brinjal.
2. *P. cinerescens* - Fig canker.
3. *P. arnoldiae* - Canker and die back of *E. angustifolia.*

Ascochyta spp.

Kingdom - Mycota (Fungi)
Subdivision - Deuteromycotina
Form Class - Deuteromycetes
Form Sub Class - Coelomycetidae
Form Order - Shaeropsidales
Form Family - Shaeropsidaceae
Genus - *Ascochyta*



Identifying characters

Mycelium : Septate, branched, inter cellular.

Pycnidium : Globose, brown, with ostiole, produced below epidermis, later erumpent.

Phialides : Short, hyaline, unicellular, cylindrical or globose.

Conidia : Hyaline, smooth, thin walled, one septate, cylindrical to irregular, rounded to flattened apex and rounded to truncate base.

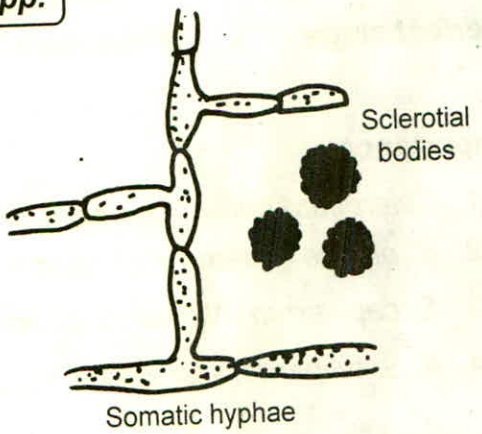
Perfect stage : *Mycosphaerella.*

Importance :

1. *Ascochyta pisi* - Leaf and pod spot of pea.
2. *A. gossypii* - Blight of cotton.
3. *A. rabiei* - Blight of gram.
4. *A. abelmoschi* - Spots of leaves, fruits and stem of okra

Rhizoctonia spp.

Kingdom - Mycota (Fungi)
Subdivision - Deuteromycotina
Form Class - Deuteromycetes
Form Order - Agonomycetales
Genus - *Rhizoctonia*



Identifying characters

Mycelium : Stout, septate, branching more or less at right angles, white when young and brown when old, constricted at the point of origin, multinucleate, cells are barrel shaped.

Sclerotia : Brown to black, uniform texture, more or less loosely packed, irregular in size, usually spherical.

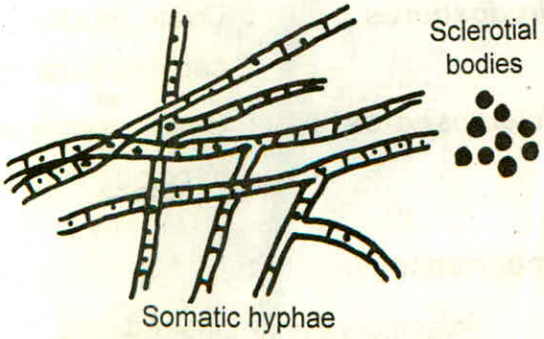
Perfect stage : *Thanatephorus, Ceratobasidium.*

Importance : .

1. *Rhizoctonia solani* - Sheath blight of paddy, web blight of beans, damping off of crucifers, beet root rot and blight.
2. *R. bataticola* - Charcoal rot of potato.

Sclerotium spp.

Kingdom - Mycota (Fungi)
Subdivision - Deuteromycotina
Form Class - Deuteromycetes
Form Order - Agonomycetales
Genus - *Sclerotium*



Identifying characters

Mycelium : Septate, white cottony, thin walled.

Sclerotia : Spherical, small as mustard seed, hard, dark brown.

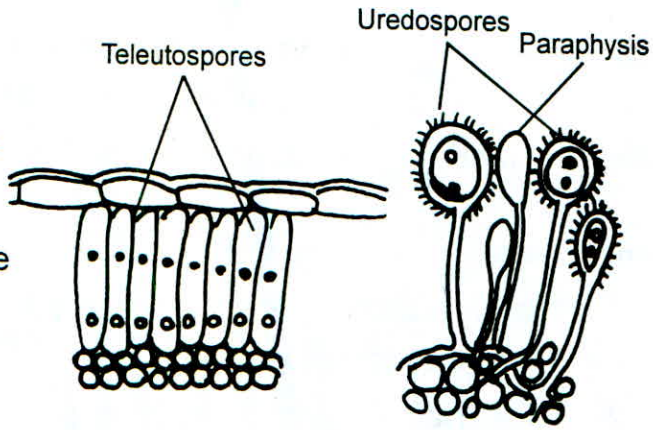
Perfect stage : *Pellicularia.*

Importance :

1. *Sclerotium rolfsii* - Damping off of seedlings, collar / stem rot of chickpea.
2. *S. oryzae* - Stem rot of paddy.
3. *S. cepivorum* - White rot of onion.
4. 4. *S. tuliparum* - Gray bulb rot of tulip.

***Melampsora* spp.**

Kingdom - Fungi
Phylum - Basidiomycota
Class - Uredinomycetes
Order - Uredinales
Family - Melampsoraceae
Genus - *Melampsora*



Identifying characters

Mycelium : Branched, septate (dolipore), uninucleate, produce haustoria.

Uredospores : Ovate, stalked, with fine spines (Echinulate), unicellular, capitate paraphyses intermingled.

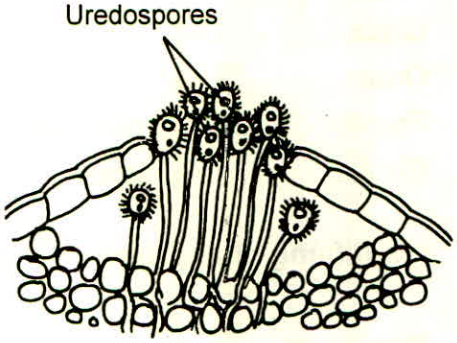
Teleutospores : Sessile, single celled, cylindrical, subepideral, reddish brown in colour, form a crust below the epidermis

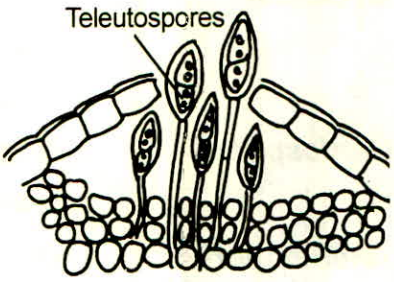
Importance :

1. *Melampsora lini* - Linseed rust.

Puccinia spp.

Kingdom - Mycota (Fungi)
Kingdom - Fungi
Phylum - Basidiomycota
Class - Uredinomycetes
Order - Uredinales
Family - Pucciniaceae
Genus - *Puccinia*





Identifying characters

Mycelium : Septate (dolipore) at long intervals, binucleate,inter cellular, sending small round or branched haustoria into the host cells.

Uredospores : Stalked, single celled, binucleate, oval, brown or brick red, with tiny spines (echinulate), thick walled.

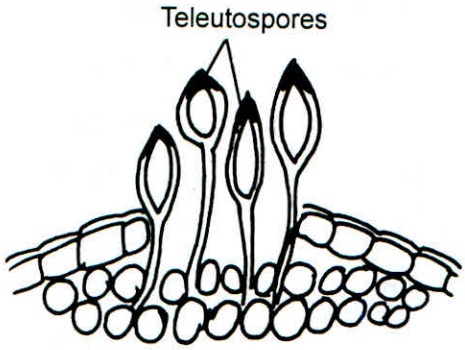
Teleutospores : Stalked, bicelled, binucleate, thick and smooth walled, brown, oblong-clavate in shape, slightly constricted at the septum, apex is pointed (P.g. tritici) or rouned (P. recondita) or flat (P. striiformis).

Importance :

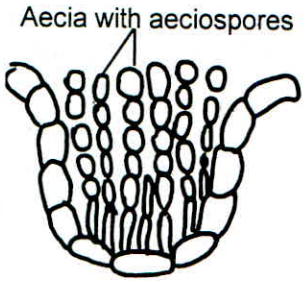
1. *Puccinia graminis tritici* - Black (stem) rust of wheat.
2. *P. recondita* - Brown or leaf rust of wheat.
3. *P. striformis* - Yellow (stripe) rust of wheat.
4. *P. menthi* - Rust of mint.
5. *P. helianthi* - Rust of sunflower.

Uromyces spp.

Kingdom - Fungi
Phylum - Basidiomycota
Class - Uredinomycetes
Order - Uredinales
Family - Pucciniaceae
Genus - *Uromyces*



Identifying characters



Mycelium : Septate at irregular intervals, intercellular and have knobed haustoria.

Uredospores : Globose, loosly echinulate, yellowish brown, stalked.

Teleutospores : Round or ovate or irregular with brown warty wall, roundish unthickened apex with papilla, stalked, unicellular.

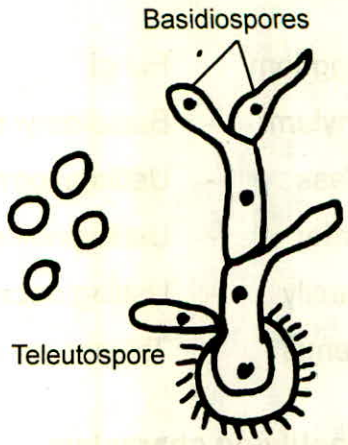
Aeciospores : Round to angular or elliptical, yellow in colour, with fine warts, formed in cup shaped aecia.

Importance :

1. *Uromyces fabae* & *U. pisi* - Pea rust.
2. *U. ciceris-arietini* - Gram rust.
3. *U. appendiculatus* - Bean rust.
4. *U. striatus* - Medicago rust.

Ustilago spp.

Kingdom - Fungi
Phylum - Basidiomycota
Class - Ustilaginomycetes
Order - Ustilaginales
Family - Ustilaginaceae
Genus - *Ustilago*



Identifying characters

Mycelium : Septate, binucleate, intercellular, with clamp connections.

Sori : Without peridium, black dusty teleutospores are covered by a delicate silvery membrane.

Teleutospores/ Chalamydospores/ Smut spores : Spherical or oval, thick walled, olivaceous brown, lighter on one side with fine spines produced singly.

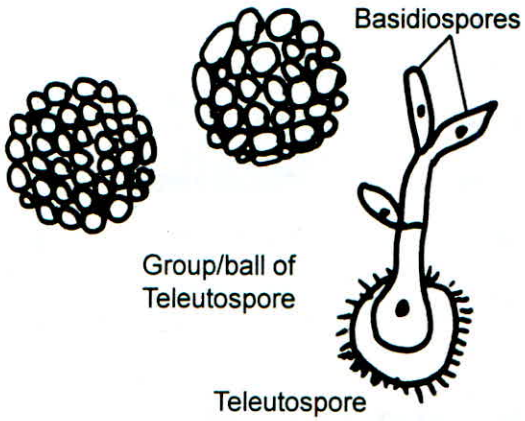
Sporidia : Uninucleate, ovate to oblong, formed secondary sporidia by budding.

Importance :

1. *Ustilago tritici* - Loose smut of wheat.
2. *U. hordei* - Covered smut of barley.
3. *U. avenae* - Loose smut of oats.
4. *U. scitaminea*- Whip smut of sugarcane.
5. *U. maydis* - Smut of maize.

Tolyposporium spp.

Kingdom - Fungi
Phylum - Basidiomycota
Class - Ustilaginomycetes
Order - Ustilaginales
Family - Ustilaginaceae
Genus - *Tolyposporium*



Identifying characters

Mycelium : Septate, binucleate, intercellular with clamp connections.

Sori : As spore balls, usually formed in the ovary, cylindrical, slightly curved, very prominent.

Teleutospores : Spores in compact balls or in groups, spore round to irregular or angular, light brown, slightly roughened wall.

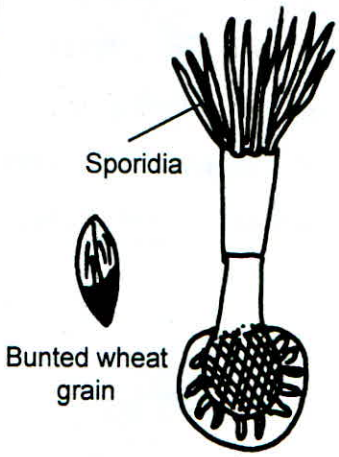
Sporidia : Oval, numerous, uninucleate, single or in chains.

Importance :

1. *Tolyposporium ehrenbergii* - Long smut of jowar.
2. *T. penicillariae* - Smut of bajra.

Neovossia spp.

Kingdom - Fungi
Phylum - Basidiomycota
Class - Ustilaginomycetes
Order - Ustilaginales
Family - Tilletiaceae
Genus - *Neovossia*



Identifying characters

Mycelium : Septate, binucleate, inter cellular and with clamp connections.

Sori : Black, in place of kernels, covered by a pericarp.

Teleutospores : Spherical to oval, reticulations on outer wall black, spines are hyaline or light coloured and curved, covered with thin hyaline membrane, long, pedicel like appendages.

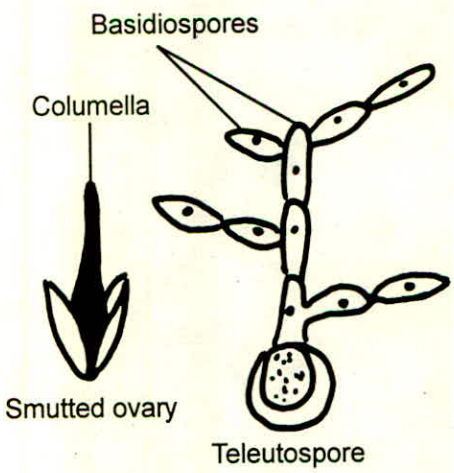
Sporidia : Filiform or needle shaped, curved in various ways, do not fuse in pair for H shaped.

Importance :

1. *Neovossia horrida* - Bunt of rice.
2. *N. indica* - Kernal bunt of rice.

***Sphacelotheca* spp.**

Kingdom - Fungi
Phylum - Basidiomycota
Class - Ustilaginomycetes
Order - Ustilaginales
Family - Ustilaginaceae
Genus - *Sphacelotheca*



Identifying characters

Mycelium : Septate, binucleate, intercellular with clamp connections.

Sori : Oval, dirty gray, conical at the tip, filled with mass of spores.

Columella : Slender, curved, central column of hard tissues, hollowed into depressions at the surface, depression filled with black spore mass.

Teleutospores : Round to slightly oval, dark brown, smooth walled, united into loose balls.

Importance :

1. *Sphacelotheca sorghi* - Grain smut of jowar.
2. *S. reiliana* - Head smut of jowar.
3. *S. cruenta* - Loose smut of jowar.

Exercise No. 10: Give the systemic position, figure and identifying characters of given specimen

Systemic position : **Kingdom -** **Phylum -** **Class -** **Order -** **Family -** **Genus -**	**Figure**
Identifying characters	
Systemic position : **Kingdom-** **Phylum-** **Class -** **Order-** **Family -** **Genus -**	**Figure**
Identifying characters	
Systemic position : **Kingdom -** **Phylum -** **Class -** **Order -** **Family -** **Genus -**	**Figure**
Identifying characters	

CHAPTER - XIII

PLANT DISEASE SYMPTOMS

The external or internal reactions or alterations of a plant as a result of infection.

or

A symptom of disease is expressed as a reaction of the host to a causal agent.

or

Sign or evidence of disease or disorder as shown by the plant or any objective evidence of disease or bodily disorder is called symptoms.

Syndrome

When a pathogen produce different types of symptoms collectively known as syndrome.

or

The totality of effects produced in a host by one disease, whether simultaneously or successively and whether detectable to the unaided eye or not.

A. PRESENCE OF VISIBLE PATHOGEN (ITS STRUCTURE OR ORGANS)

1. Powdery mildew : White to dirty white powdery mass of conidia, conidiophores and mycelium present on the host surfaces in patches or covering the entire surface.

eg. Powdery mildew of pea (*Erysiphe polygoni*), Powdery mildew of grape (*Uncinula necator*).

2. Downy mildew : Dirty white with slight pinkish tinged cottony fungal growth consists of sporangia, sporangiophores and mycelium present on lower surface of

leaves whereas light yellow or brown patches corresponding to downy fungal growth appear on upper surface of leaves.

eg. Downy mildew of Lathyrus (*Peronospora lathyri*), Downy mildew of Grape (*Plasmopara viticola*).

3. Rust : It gives rust like appearance of infected plant parts. The small pustule of spore of various colours, usually breaking through the host epidermis.

eg. Black rust of wheat (*Puccinia graminis tritici*), Groundnut rust (*Puccinia recondita*), Pea rust (*Uromyces fabae*).

4. Smut : Smut means charcoal like powder. The affected plant parts show black or black-purplish powdery mass of spores. Smut usually appear on floral parts but may also found on stem and leaves.

eg. Loose smut of wheat & grasses (*Ustilago nuda tritici* and *U. cynodontiae*), Whip smut of sugarcane (*U. scitaminiae*), Leaf smut of paddy (*Entyloma oryzae*).

5. Sooty mold : A sooty coating on foliage and fruits formed by the dark mycelium of fungi, live on the honey dew secreted by insects.

6. White rust (Blisters): Appearance of white powdery compact mass of fungal structure due to break open of host epidermis on lower surface of host leaves.

eg. White blisters of mustard (*Albugo candida*), White blisters of leafy vegetables (*Albugo bliti*).

7. Scab: Diseased organs showed roughened or crust like lesions.

eg. Potato scab (*Streptomyces scabies*).

B. CHANGE IN OR ON THE HOST PLANT

1. Mosaic: It is characterized by intermingled patches of normal and light green or yellowish colour.

eg. Mosaic of moong & urd, Yellow vein mosaic of Bhindi.

2. Mottle: It is a irregular pattern of indistinct light and dark areas.

eg. Mottle of papaya.

3. Overgrowths or hypertrophy: It is the abnormally increased the size of organ(s) due to either increase in cell size or increase in number (hyperplasia). They are of different forms :

***a. Galls*:** A gall is a localised swelling whose morphology (globose, elongated, irregular shape) is quite unlike that of any organ of a healthy plant. Smaller galls are called warts while larger galls are called knots.

eg. Wart of potato (*Synchytrium endobioticum*), Stem gall of coriander (*Protomyces macrosporus*), Root knot of vegetable (*Meloidogyne incognita*), Crown gall of apple (*Agrobacterium tumefaciens*).

***b. Curl*:** Folding or rolling of infected leaves either down ward or upward due to excessive growth of interveinal portion.

eg. Leaf curl of chilli (LCV).

***c. Fasciculation or witches broom* :** Number of small slender branches arise in clusters appearing like a broom.

eg. Green ear of Bajra (*Sclerospora graminicola*).

***d. Hairy root* :** Numerous fibrous roots are produced abnormally, replaces the main root system is called hairy root.

eg. Hairy root of apple (*Agrobacterium rhizogens*).

4. Atrophy or dwarfing (Hypoplasia): Reduction in normal cell size and/or cell number due to infection, sometimes results in dwarfing or stunting of whole plant or certain part or organs.

eg. Little leaf of brinjal (*Phytoplasma*), Grossy stunt of sugarcane (GSV).

5. Necrosis : This condition refers to death of cell or organ during host pathogen interaction. Necrosis is of different types depending upon the host pathogen combination.

***a. Blight*:** This means burnt appearance. The symptoms appear as continuous, rapidly advancing necrosis of leaves, blossom, twig or stem. The dead area turns brown to black and may soon disintegrate.

eg. Bacterial blight of Paddy (*Xanthomonas oryzae* pv *oryzae*), Stem and twig blight of Brinjal (*Phomopsis vexans*), Early blight of Potato & Tomato (*Alternaria solani*), Late blight of Potato (*Phytophthora infestans*).

***b. Streak*:** When elongated narrow brown lesions occurred in the intervenial portion of the leaf.

eg. Bacterial streak of paddy (*Xanthomonas oryzae* pv. *oryzicola*).

***c. Spots*:** Cells are killed in defined areas, circular or oval in shape, which a central necrotic area surrounded by a purple, red, yellow or brown margin. Sometimes fruiting bodies may also seen on the dead areas.

eg. Leaf spot (Tikka) of groundnut (*Cercospora arachidicola*), Leaf blast of rice (*Pyricularia grisea*).

***d. Blotch*:** When large, irregular necrotic lesions appear on leaves, twigs and stems.

eg. Purple blotch of onion (*Alternaria porri*).

***e. Canker*:** It is corky outgrowth formed on leaves, twigs, stem etc. They are mostly localized and confined to parenchymatous tissues of host plant and are usually some what sunken.

eg. Citrus canker (*Xanthomonas axonopodis* pv *citri*), Apple canker (*Nectria galligena*), Canker of cherries (*Pseudomonas mors-prunorum*).

***f. Damping off* :** Stems of seedlings are attacked near soil surface. The attacked portion becomes constricted and weak, incapable of bearing the load of the upper portion, ultimately seedlings topple down and die.

eg. Damping off of vegetables and ornamentals (*Pythium aphanidermatum*).

***g. Rot* :** It is a gangrene of plant tissues. The affected tissues die, decompose to greater extent and turn brown. According to plant organ attacked, the rot may be called root rot, leaf or stem rot, collar rot, bud rot and fruit rot.

eg. Black rot of cabbage (*Xanthomonas campestris*), Stem rot of paddy (*Sclerotium oryzae*), Collar rot of chickpea (*Sclerotium rolfsii*), Sheath rot of paddy (*Sarocladium oryzae*).

h. Anthracnose **:** It means coal like. This symptom is characterized by blackish lesion, the blackness being due to presence of dark spores, mycelium or both.

eg. Tomato anthracnose (*Colletotrichum gloeosporioides*).

6. Die back : Progressive death of twigs and branches from their tips towards the trunk.

eg. Die back of chilli (*Colletotrichum capsici*).

7. Wilts : The leaves and other green parts lose their turgidity, become flaccid and droop. These happen due to injuri to the root system, partial plugging of xylem vessels or phloem vessels.

eg. Bacterial wilt of solanaceous crop (*Ralstonia solanacearum*), Wilt of Linseed (*Fusarium oxysporum* fsp. *lini*).

CHAPTER - XIV

TYPICAL CHARACTERISTICS OF PLANT PATHOGENS

A. Bacteria

Bacteria are simple, unicellular micro organisms. They possess prokaryotic nucleus (nucleus not having nucleolus and nuclear membrane). Typical bacterial cell have cell wall, cell membrane, nuclear material, plasmids, pilli or fimbriae, ribosomes etc. The shape of the bacteria is variable i.e. Cocci-means spherical shaped {monococci, diplococci, streptococci, tetrads, staphylococci and sarcinae (Fig. 21 i, ii,iii, iv, v & vi)} ; Bacillus {rod shaped (Fig. 21 vii)} ; Spirillum {helical shaped (Fig. 21 viii)} and Vibrio {comma shaped (Fig. 21 ix)}. Generally bacterial cells measures 0.5-1.0 x 2–5 μ and 0.75- 1.25μ in diameter. Bacteria may be motile or non motile. The motility of bacteria performed by means of flexible, whip-like structure called flagella. The flagella may be monotrichous {with a single polar flagellum (Fig. 22 i)}, lophotrichous { with a tuft of flagella at one end or pole (fig. 22 ii)}, amphitrichous {

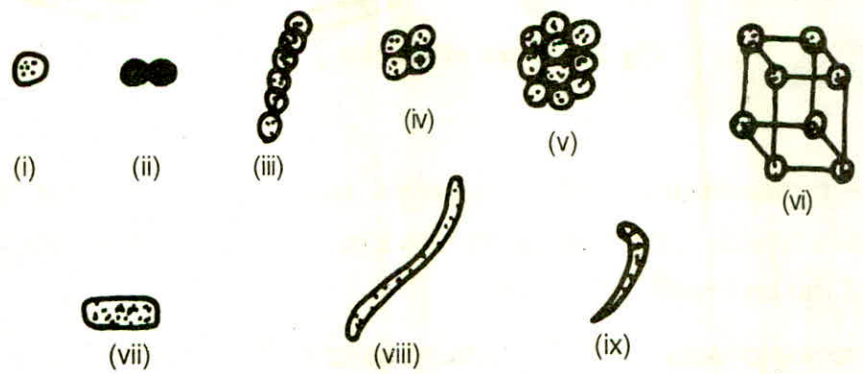


Fig. 21: Shape of Bacteria

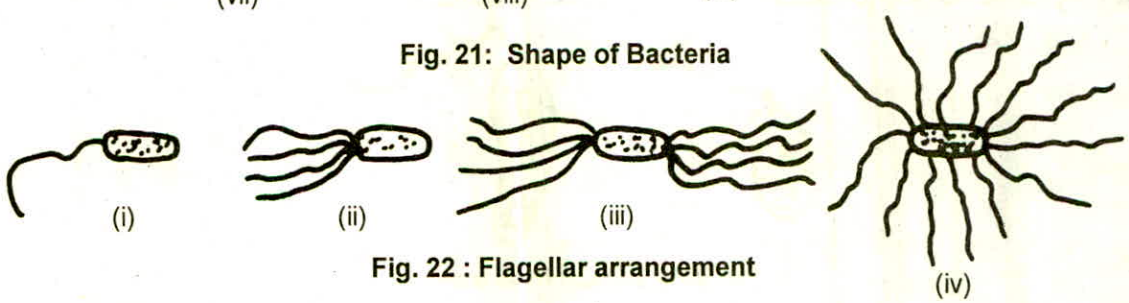


Fig. 22 : Flagellar arrangement

with a tuft of flagella at both ends or poles)fig. 22 iii)} and peritrichous {presence of flagella around the cell (Fig. 22 iv)}. Important plant pathogenic bacteria are *Xanthomonas* sp., *Ralstonia (Pseudomonas)* sp., *Erwinia* sp., *Agrobacterium* sp., *Corynebacterium* sp., *Xyllela* sp. Bacteria reproduce by binary fission shows differentiation in Gram's staining technique either as Gram positive or Gram negative

B. Virus

Viruses are ultramicroscopic, filter passing microorganism consist of nucleic acid (either RNA or DNA) and surrounded by protein coat. They can not be made to grow or multiply on artificial media. Viruses are basically anisometric (rigid or flexuous rods and bullet shaped) and isometric (polyhedral or spherical) in morphology. Majority of viruses are transmitted by insect vectors from diseased plant to healthy plant.

eg. TMV, TSWV, CMV.

Fig. 23 : Shape of viruses

C. Protozoa

The protozoa are mostly one celled, microscopic organism, generally motile and have typical nuclei. Protozoa move by flagella, by pseudopodia or by movements of the cell itself.

eg *Phytomonas leptovasorum* (Phloem necrosis of Coffee)

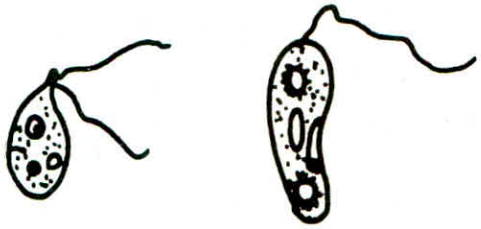


Fig. 24 : Typical Protozoa

D. Algae

The body of typical algae is undifferentiated thallus. The thallus is a pseudoparenchymatous tissue, one to several cells in thickness, in which the cells are arranged radially. The upper surface of the algal mass bears numerous unbranched filaments, some of these erect filaments are sterile hairs, others bear a cluster of sporangia or gametangia at their apex. The sporangia are borne in clusters and each lies at the end of short stalk- cell.

eg. *Cephaleuros virescens* (Syn. *C. parasiticus* and *C. mycoidea*) (Red rust of Mango)

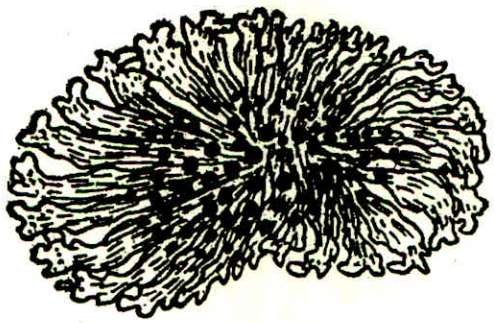


Fig. 25 : Thallus of Algae

ANNEXURE – I

LIST OF PHOTO PLATES

I	Powdery mildew of mung bean
II	Downy mildew of Lathyrus
III	Rust of pea
IV	Loose smut
V	White blisters of Amaranthus
VI	Yellow mosaic of mungbean
VII	Leaf curl of tomato
VIII	Little leaf of brinjal
IX	Bacterial blight of paddy
X	Purple blotch of onion
XI	Citrus canker
XII	Sheath rot of paddy
XIII	Fruit rot of bottle guard
XIV	Anthracnose of tomato
XV	Collar rot of brinjal
XVI	Fusarium wilt

STUDENT'S NOTES

STUDENT'S NOTES


I


II


III


IV


V


VI


VII


VIII

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