B.Sc. AGRICULTURE LAB MANUAL

2nd Semester

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Agriculture

MIDNAPORE CITY COLLEGE

FUNDAMENTALS OF GENETICS- AGS-201

PRACTICAL 1: STUDY OF MICROSCOPE.

Aim: To study parts and working of a typical compound microscope.

Apparatus and materials required:

A compound microscope

Principle:

It has a series of two lenses; (i) the objective lens close to the object to be observed and (ii) the ocular lens or eyepiece, through which the image is viewed by eye. Light from a light source (mirror or electric lamp) passes through a thin transparent object. The objective lens produces a magnified 'real image' first image) of the object. This image is again magnifiedby the ocular lens (eyepiece) to obtain a magnified 'virtual image' (final image), which canbe seen by eye through the eyepiece. As light passes directly from the source to the eye through the two lenses, the field of vision is brightly illuminated. That is why; it is a bright-field microscope.

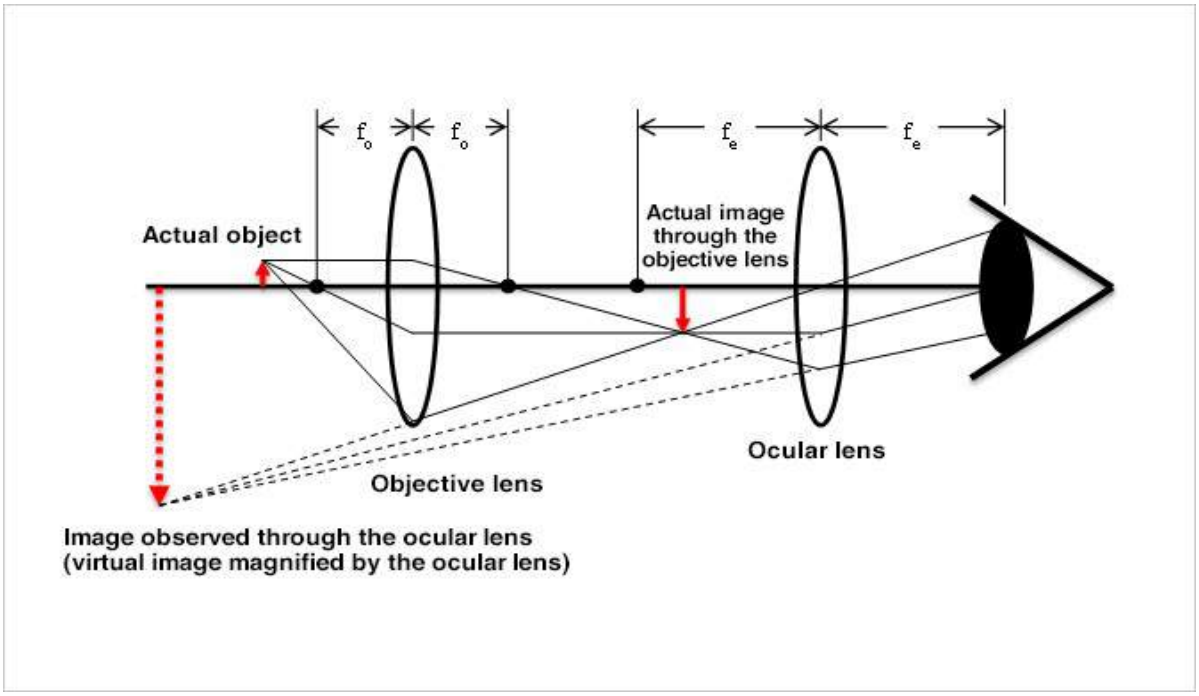


Figure: Ray diagram of a Compound Microscope

Parts of a Compound Microscope

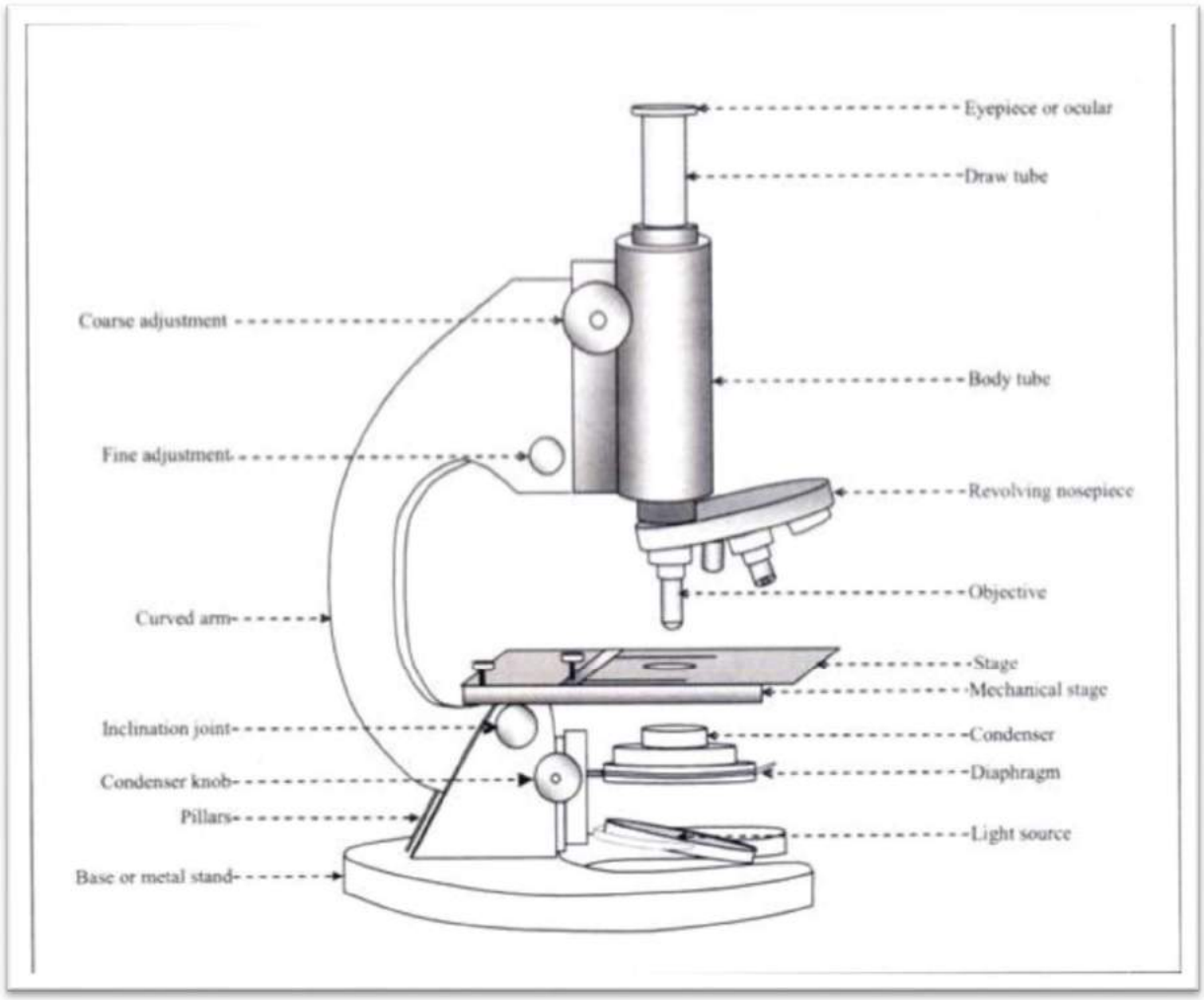


Figure: Parts of a compound microscope

Parts of compound microscope can be divided into two main categories:

A. Mechanical Parts

1. Base
2. Pillars
3. Inclination Joint
4. Curved arm
5. Body tube
6. Draw tube
7. Coarse adjustment
8. Fine adjustment
9. Stage
10. Mechanical stage

11. Revolving nosepiece

B. Optical Parts

1. Light Source
2. Diaphragm
3. Condenser or substage condenser
4. Objective
5. Eyepiece or ocular

A. Mechanical Parts

The components of mechanical parts are as follows:

1. Base or Metal Stand: The whole microscope rests on this base. Mirror, if present, is fittedto it.

2. Pillars: It is a pair of elevations on the base, by which the body of the microscope is held to the base

3. Inclination joint: It is a movable joint, through which the body of the microscope is held to the base by the pillars. The body can be bent at this joint into any inclined position, as desired by the observer, for easier observation. In new models, the body is permanently fixed to the base in an inclined position, thus needing no pillar or joint.

4. Curved Arm: It is a curved structure held by the pillars. It holds the stage, body tube, fine adjustment and coarse adjustment.

5. Body Tube: It is usually a vertical tube holding the eyepiece at the top and the revolving nosepiece with the objectives at the bottom. The length of the draw tube is called 'mechanical tube length' and is usually 140-180 mm (mostly 160 mm).

6. Draw Tube: It is the upper part of the body tube, slightly narrower, into which the eyepiece is slipped during observation.

7. Coarse Adjustment: It is a knob with rack and pinion mechanism to move the body tube up and down for focusing the object in the visible field. As rotation of the knob through a small angle moves the body tube through a long distance relative to the object, it can perform coarse adjustment. In modern microscopes, it moves the stage up and down and the body tube is fixed to the arm.

8. Fine Adjustment: It is a relatively smaller knob. Its rotation through a large angle can move the body tube only through a small vertical distance. It is used for fine adjustment toget the final clear image. In modern microscopes, fine adjustment is done by moving the stage up and down by the fine adjustment.

9. Stage: It is a horizontal platform projecting from the curved arm. It has a hole at the center, upon which the object to be viewed is placed on a slide. Light from the light source below the stage passes through the object into the objective.

10. Mechanical Stage (Slide Mover): Mechanical stage consists of two knobs with rack andpinion mechanism. The slide containing the object is clipped to it and moved on the stage in two dimensions by rotating the knobs, so as to focus the required portion of the object.

11. Revolving Nosepiece: It is a rotatable disc at the bottom of the body tube with three or four objectives screwed to it. The objectives have different magnifying powers. Based on the required magnification, the nosepiece is rotated, so that only the objective specified for the required magnification remains in line with the light path.

B. Optical Parts:

These parts are involved in passing the light through the object and magnifying its size.

The components of optical parts include the following:

1. Light Source:

Modern microscopes have in-built electric light source in the base. The source is connected to the mains through a regulator, which controls the brightness of the field. But in old models, a mirror is used as the light source. It is fixed to the base by a binnacle, through which it can be rotated, so as to converge light on the object. The mirror is plane on one side and concave on the other.

It should be used in the following manner:

(a) Condenser Present:

Only plane side of the mirror should be used, as the condenser converges the light rays.

(b) Condenser Absent:

(i) Daylight:

Plane or concave (plane is easier)

(ii) Small artificial light:

High power objective: Plane side Low power objective: Concave side

2. Diaphragm:

If light coming from the light source is brilliant and all the light is allowed to pass to the object through the condenser, the object gets brilliantly illuminated and cannot be visualized properly. Therefore, an iris diaphragm is fixed below the condenser to control the amount of light entering into the condenser.

3. Condenser:

The condenser or sub-stage condenser is located between the light source and the stage. It has a series of lenses to converge on the object, light rays coming from the light source. After passing through the object, the light rays enter into the objective.

The 'light condensing', 'light converging' or 'light gathering' capacity of a condenser is called 'numerical aperture of the condenser'. Similarly, the 'light gathering' capacity of an objective is called 'numerical aperture of the objective'. If the condenser converges light in a wide angle, its numerical aperture is greater and vice versa.

If the condenser has such numerical aperture that it sends light through the object with an angle sufficiently large to fill the aperture back lens of the objective, the objective shows its highest numerical aperture (Figure 4.7). Most common condensers have numerical aperture 1.25.

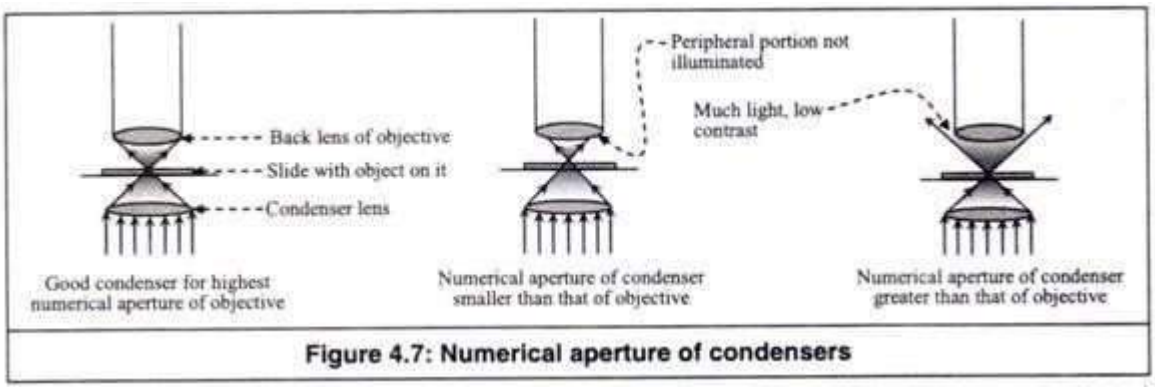


Figure 4.7: Numerical aperture of condensers

If the numerical aperture of the condenser is smaller than that of the objective, the peripheral portion of the back lens of the objective is not illuminated and the image has poor visibility. On the other hand, if the numerical aperture of condenser is greater than that of the objective, the back lens may receive too much light resulting in a decrease in contrast.

There are three types of condensers as follows:

(a) Abbe condenser (Numerical aperture=1.25): It is extensively used.

(b) Variable focus condenser (Numerical aperture =1.25)

(c) Achromatic condenser (Numerical aperture =1.40): It has been corrected for both spherical and chromatic aberration and is used in research microscopes and photomicrographs.

4. Objective:

It is the most important lens in a microscope. Usually three objectives with different magnifying powers are screwed to the revolving nosepiece.

The objectives are:

(a) Low power objective (X 10):

It produces ten times magnification of the object.

(b) High dry objective (X 40):

It gives a magnification of forty times.

(c) Oil-immersion objective (X100):

It gives a magnification of hundred times, when immersion oil fills the space between the object and the objective

The scanning objective (X4) is optional. The primary magnification (X4, X10, X40 or X100) provided by each objective is engraved on its barrel. The oil-immersion objective has a ring engraved on it towards the tip of the barrel.

Resolving Power of Objective:

It is the ability of the objective to resolve each point on the minute object into widely spaced points, so that the points in the image can be seen as distinct and separate from one another, so as to get a clear un-blurred image.

It may appear that very high magnification can be obtained by using more number of high power lenses. Though possible, the highly magnified image obtained in this way is a blurred, one. That means, each point in the object cannot be found as widely spaced distinct and separate point on the image.

Mere increase in size (greater magnification) without the ability to distinguish structural details (greater resolution) is of little value. Therefore, the basic limitation in light microscopes is one not of magnification, but of resolving power, the ability to distinguish two adjacent points as distinct and separate, i.e. to resolve small components in the object into finer details on the image.

Resolving power is a function of two factors as given below:

(a) Numerical aperture (n.a.)

(b) Wavelength of the light (λ)

(a) Numerical aperture:

Numerical aperture is a numerical value concerned with the diameter of the objective lens in relation to its focal length. Thus, it is related to the size of the lower aperture of the objective,

through which light enters into it. In a microscope, light is focused on the object as a narrow pencil of light, from where it enters into the objective as a diverging pencil (Figure 4.8).

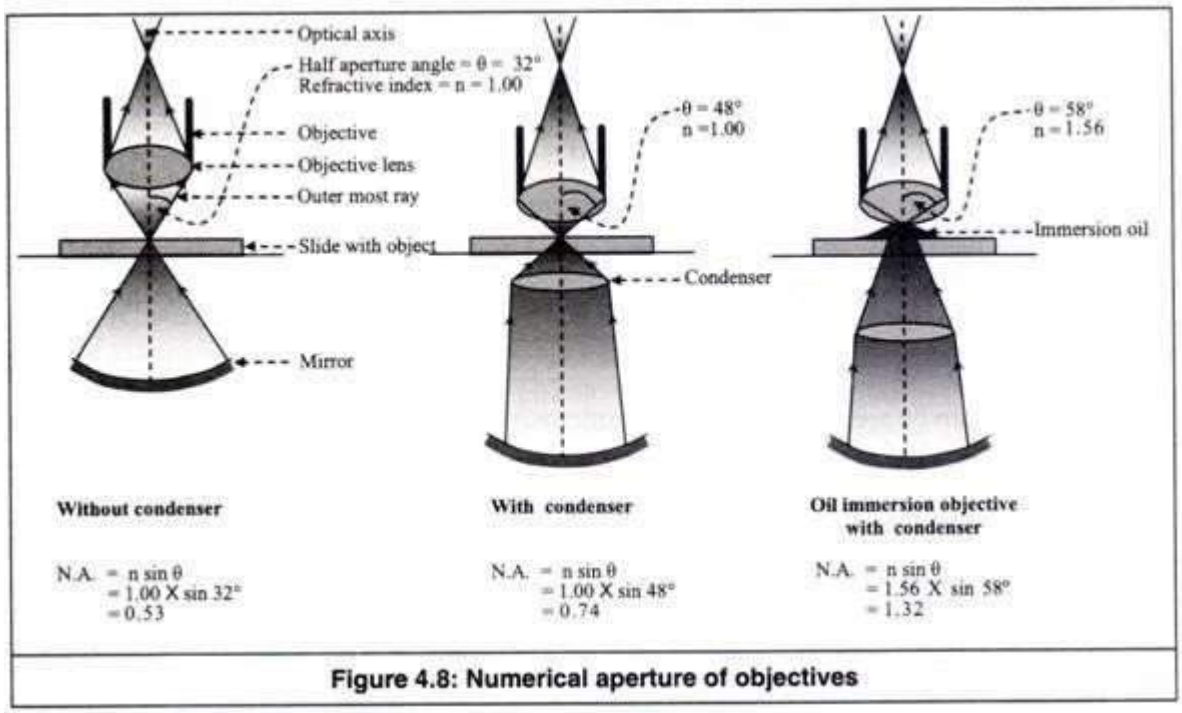


Figure 4.8: Numerical aperture of objectives

The angle 9 subtended by the optical axis (the line joining the centers of all the lenses) andthe outermost ray still covered by the objective is a measure of the aperture called ‘half aperture angle’.

A wide pencil of light passing through the object ‘resolves’ the points in the object into widely spaced points on the lens, so that the lens can produce these points as distinct and separate on the image. Here, the lens gathers more light.

On the other hand, a narrow pencil of light cannot ‘resolve’ the points in the object into widely spaced points on the lens, so that the lens produces a blurred image. Here, the lens gathers less light. Thus, the greater is the width of the pencil of light entering into the objective (29), the higher is its ‘resolving power’.

The numerical aperture of an objective is its light gathering capacity, which depends on the site of the angle 8 and the refractive index of the medium existing between the object and the objective.

Numerical aperture (n.a.) = n sin θWhere,

n = Refractive index of the medium between the object and the objective andθ = Half

aperture angle

For air, the value of 'n' is 1.00. When the space between the lower tip of the objective and the slide carrying the object is air, the rays emerging through the glass slide into this air are bent or refracted, so that some portion of it do not pass into the objective. Thus, loss of some light rays reduces numerical aperture and decreases the resolving power.

However, when this space is filled with an immersion oil, which has greater refractive index (n=1.56) than that of air (n=1.00), light rays are refracted or bent more towards the objective. Thus, more light rays enter into the objective and greater resolution is obtained. In oil immersion objective, which provides the highest magnification, the size of the aperture is very small.

Therefore, it needs bending of more rays into the aperture, so that the object can be distinctly resolved. That is why, immersion oils, such as cedar wood oil and liquid paraffin are used to fill the gap between the object and the objective, while using oil-immersion objective.

(b) Wavelength of light (λ):

The smaller is the wavelength of light (λ), the greater is its ability to resolve the points on the object into distinctly visible finer details in the image. Thus, the smaller is the wavelength of light, the greater is its resolving power.

Limit of resolution of objective (d):

The limit of resolution of an objective (d) is the distance between any two closest points on the microscopic object, which can be resolved into two separate and distinct points on the enlarged image.

Points with their in-between distance less than ‘d’ or objects smaller than ‘d’ cannot be resolved into separate points on the image. If the resolving power is high, points very close to each other can be seen as clear and distinct.

Thus, the limit of resolution (the distance between the two resolvable points) is smaller. Therefore, smaller objects or finer details can be seen, when’d’ is smaller. Smaller ‘d’ is obtained by increasing the resolving power, which in turn is obtained by using shorter wavelength of light (λ) and greater numerical aperture.

Limit of resolution = d = λ/2 n.a.Where,

λ = Wave length of light and

n.a. = Numerical aperture of the objective.

If λ_{green} = 0.55 p and n.a. = 1.30, then d = λ/2 n.a. = 0.55/2 X 1.30 = 0.21 μ. Therefore, the smallest details that can be seen by a typical light microscope is having the dimension of approximately 0.2 μ. Smaller objects or finer details than this cannot be resolved in a compound microscope.

5. Eyepiece:

The eyepiece is a drum, which fits loosely into the draw tube. It magnifies the magnified real image formed by the objective to a still greatly magnified virtual image to be seen by the eye (Figure 4.9).

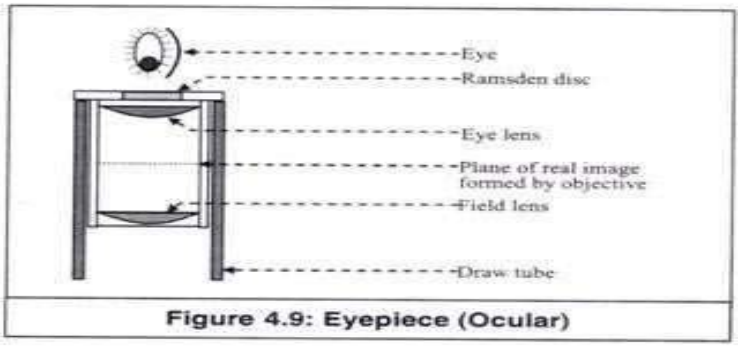


Figure 4.9: Eyepiece (Ocular)

Usually, each microscope is provided with two types of eyepieces with different magnifying powers (X10 and X25). Depending upon the required magnification, one of the two eyepieces is inserted into the draw tube before viewing. Three varieties of eyepieces are usually available.

They are the Huygenian, the hyper plane and the compensating. Among them, the Huygenian is very widely used and efficient for low magnification. In this eyepiece, two simple Plano-convex lenses are fixed, one above and the other below the image plane of the real image formed by the objective.

The convex surfaces of both the lenses face downward. The lens towards the objective is called 'field lens' and that towards eye, 'eye lens'. The rays after passing through the eye lens come out through a small circular area known as Rams-den disc or eye point, where the image is viewed by the eye.

Total magnification:

The total magnification obtained in a compound microscope is the product of objective magnification and ocular magnification.

$M_t = M_{ob} \times M_{oc}$ Where,

M_t = Total magnification,

M_{ob} = Objective magnification and M_{oc} = Ocular magnification

If the magnification obtained by the objective (M_{ob}) is 100 and that by the ocular (M_{oc}) is 10, then total magnification (M_t) = $M_{ob} \times M_{oc}$ =100 X 10 =1000. Thus, an object of lq will appear as 1000 μ.

PRACTICAL 2 : STUDY OF CELL STRUCTURE

Aim: To prepare a stained temporary mount of an onion peel and to recordobservations and draw labelled diagrams.

Apparatus and materials required:

An onion, glass slide, watch glass, coverslip, forceps, needles, brush, blade, filter paper, safranin, glycerine, dropper, water, and a compound microscope.

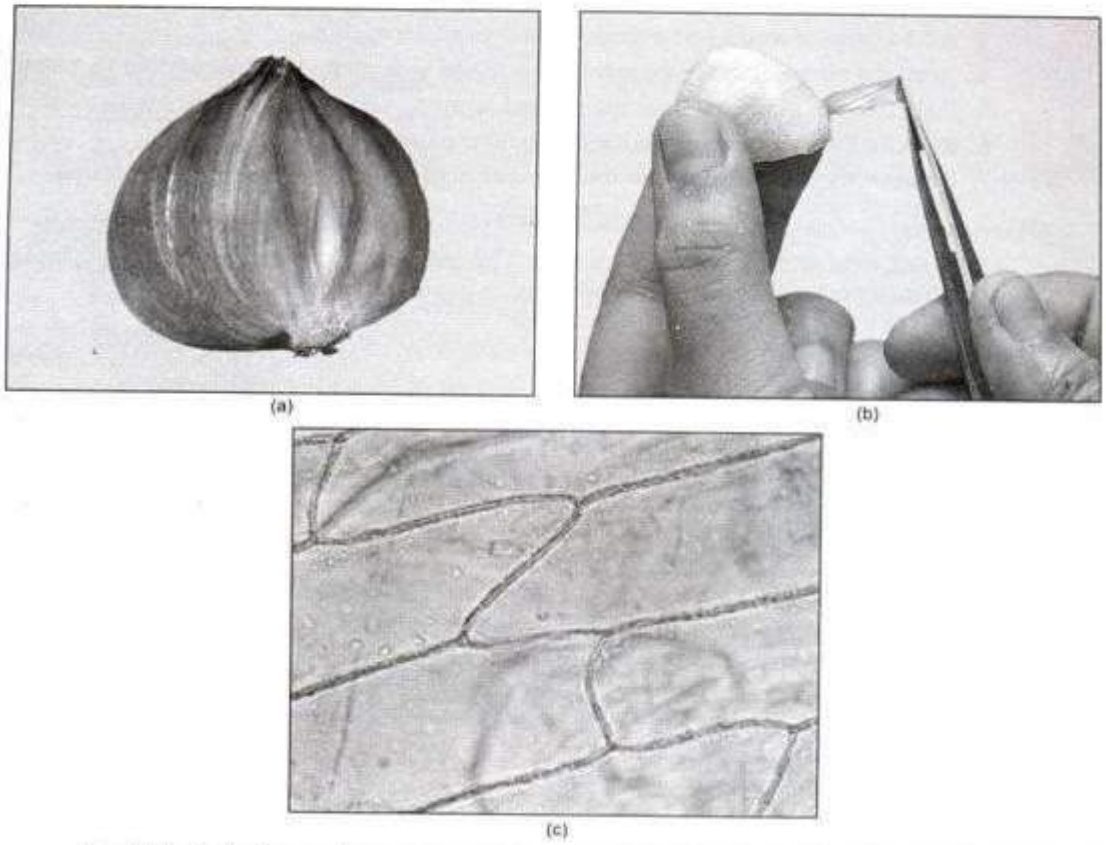
Principle: All living organisms are made up of cells. The shape, size and the number of these units vary in organisms. The three major components of a cellare the cell membrane, cytoplasm and nucleus. In a plant cell, a cell wall surrounds the cell membrane.

Procedure:

1. Take an onion and remove its outermost peel.
2. Now cut a small part from an inner scale leaf with the help of a blade.
3. Separate a thin, transparent peel from the convex surface of the scale leafwith the help of forceps.
4. Keep this peel in a watch glass/ glass slide containing water.
5. Add two drops of safranin stain in another watch glass/ glass slide to stain thepeel (around 30 seconds).
6. Take a clean slide and put a drop of glycerine in the centre of the slide.
7. With the help of a brush and needle transfer the peel on the slide. Glycerineprevents the peel from drying up.
8. Carefully cover it with a coverslip and avoid any air bubble from enteringinterring the coverslip.
9. Remove any excessive glycerine with a filter paper.
10. Observe the prepared mount of the peel under the low and highmagnification of a compound microscope.

Observations:

A large number of rectangular cells are visible. These cells lie close to each other with intercellular spaces between them. These cells are surrounded by distinct cell walls. These cells have a dark stained nucleus and a large vacuolein the centre.



(a)-(b) Methods of separating an onion peel (c) Structure of onion cells as seen under a microscope (450 ×)

Precautions:

1. Overstaining and under staining should be avoided.

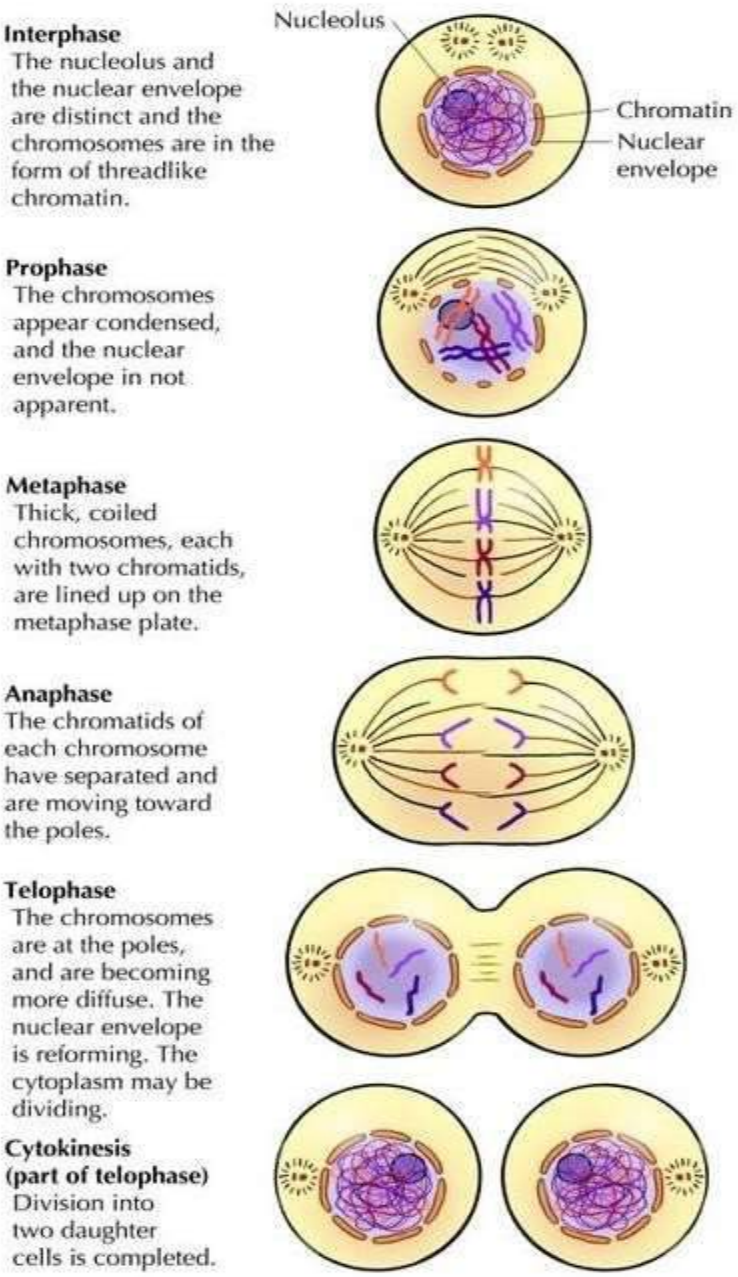
PRACTICAL 3: TO STUDY DIFFERENT TYPES OF CELL DIVISIONS (MITOSIS AND MEIOSIS).

Apparatus and materials required:

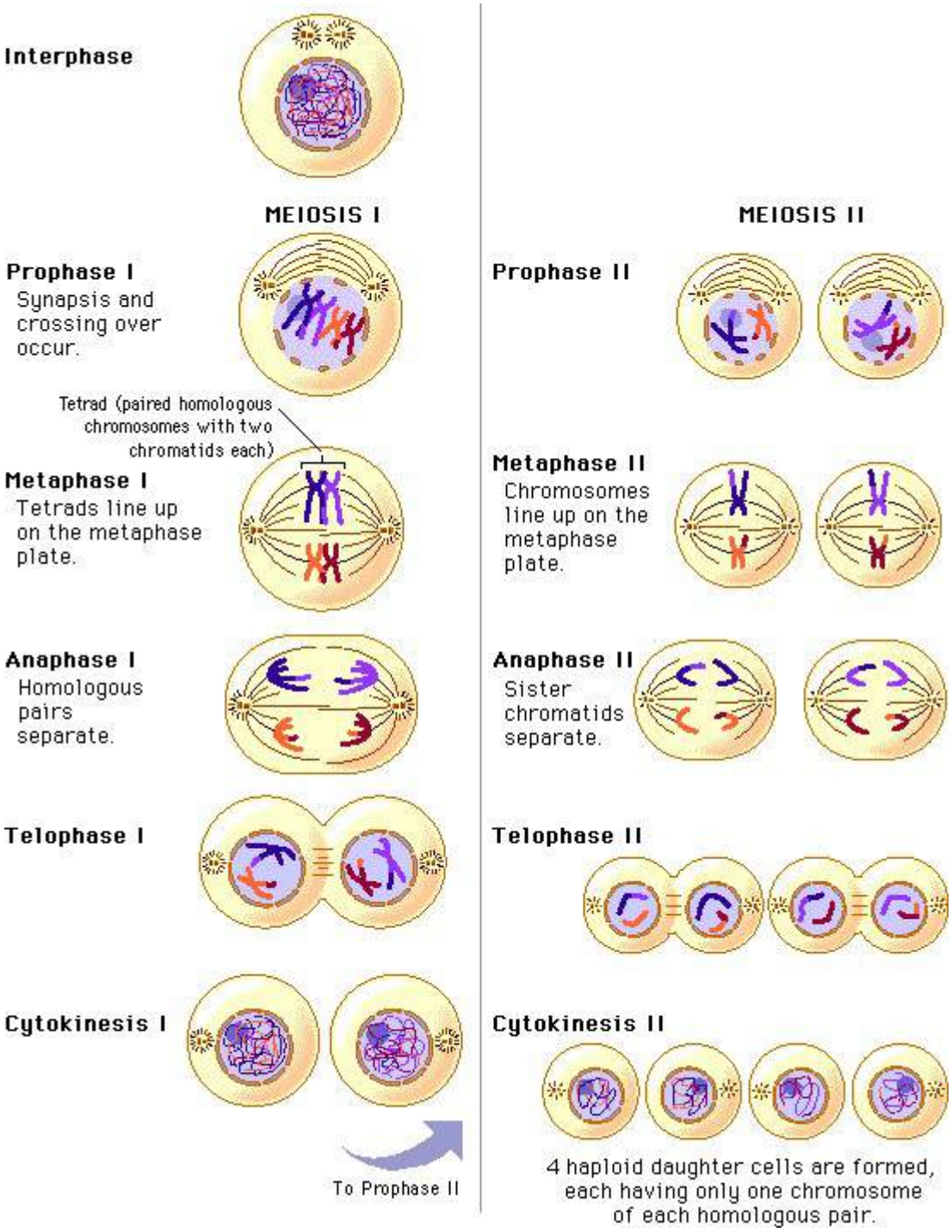
Permanent slides of Mitosis & meiosis and a compound microscope.

Cell Divisions

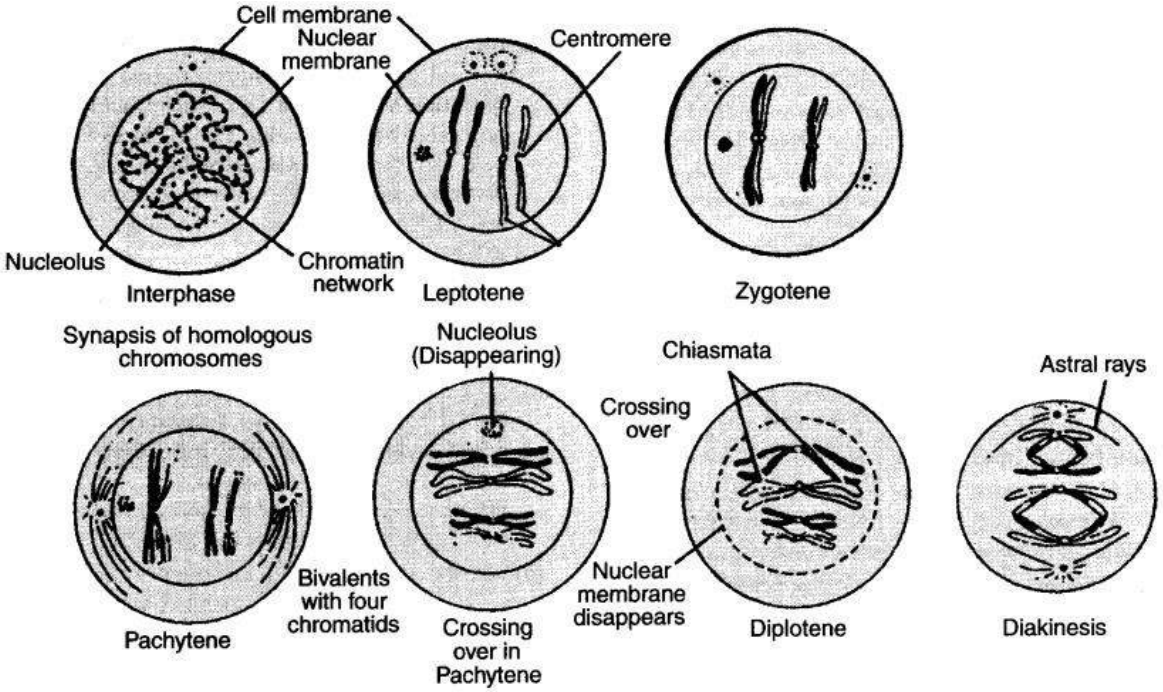
A. Mitosis



B. Meiosis


Interphase
MEIOSIS I
Prophase I
Synapsis and crossing over occur.
Tetrad (paired homologous chromosomes with two chromatids each)
Metaphase I
Tetrads line up on the metaphase plate.
Anaphase I
Homologous pairs separate.
Telophase I
Cytokinesis I
To Prophase II
MEIOSIS II
Prophase II
Metaphase II
Chromosomes line up on the metaphase plate.
Anaphase II
Sister chromatids separate.
Telophase II
Cytokinesis II
4 haploid daughter cells are formed, each having only one chromosome of each homologous pair.

Substages of Prophase I



PRACTICAL 4: EXPERIMENTS ON MONOHYBRID, DIHYBRID, TRIHYBRID, TEST CROSS AND BACKCROSS.

Q1. What will be the appearance of (a) F_1 and (b) F_2progenies when a pure (homozygous) tall pea plant is crossed with a pure (homozygous) dwarf pea plant?
Tallness (T) gene is dominant over dwarfness (t) gene.

Q2. When a plant homozygous for tall is crossed with a plant homozygous for dwarf, what will be the appearance of the offsprings of a cross of F_1 with its tall parent? What is the term given for such a cross?

Q3. When a plant homozygous for tall is crossed with a plant homozygous for dwarf, what will be the appearance of the off-springs of a cross of F_1 with its dwarf parent? What is the term given for such a cross?

Q4. Work out for the genotypes of the parents of the cross between a tall and a dwarf pea plant which result into about one half of the tall and one half of dwarf off-springs.

Q5. What will be the result of selfing the F_1 generation in a cross when round and yellow seeded pea plants (YYRR) are crossed with green and wrinkled (yyrr) seeded pea plant?

Q6. When round and yellow seeded pea plants (YYRR) are crossed with green wrinkled (y yr r) seeded pea plants the F_1 are yellow and round seeded plants (Yy Rr).
What will be the results when this F_1 is crossed with round and yellow seeded parents? What is the term given for such a cross?

PRACTICAL 5: EXPERIMENTS ON EPISTATIC INTERACTIONS INCLUDING TEST CROSS AND BACK CROSS.

1. In sweet pea, two allelic pairs CcPp are known to effect pigment formation in the plants flowers. The dominant C P are both necessary for coloured flower. Absence of either results in white flowering plant. A dihybrid plant is crossed to a white one (flowering plant) which is heterozygote at the 'C' loci.

a. What is the genotype of dihybrid plant?

b. What is the genotype of the white plant?

c. What kind of flower, coloured or white (include the ratio) are to be accepted from the crossabove.

d. What type of epistatic is being demonstrated?

2. Assumed that another allelic pair in sweet pea effect pigment formation in addition to the gene mentioned in question 1. The presence of dominant gene R is required for red flower while its recessive allele 'r' produces yellow flower. What would be the phenotype of the flowering plant in relation to flower colour.

3. In a certain breed of dog, the dominant 'B' is required for black fur, its recessive 'b' produces brown colour. However, the dominant 'I' is epistatic to the colour locus and inhibits pigment formation. What would be the phenotype of the following of the parents, show the ratio in F2 generation?

a. bbii × BbIi

b. bbii × Bbii

c. bbIi × BBIi

PRACTICAL 6: PRACTICE ON MITOTIC AND MEIOTIC CELL DIVISION.

Apparatus and materials required: Compound microscope, Onion root tip, glacial acetic acid, ethanol, acetocarmine, glass slide, cover slip, blade, needle.

Principle

All organisms are made of cells. For an organism to grow, mature and maintain tissue, new cells must be made. All cells are produced by division of pre-existing cells. Continuity of life depends on cell division. There are two main methods of cell division: mitosis and meiosis. Inthis tutorial we will learn about mitosis.

What is Mitosis?

Mitosis is very important to life because it provides new cells for growth and replaces dead cells. Mitosis is the process in which a eukaryotic cell nucleus splits in two, followed by division of the parent cell into two daughter cells. Each cell division consists of two events: cytokinesis and karyokinesis. Karyokinesis is the process of division of the nucleus and cytokinesis is the process of division of cytoplasm.

Events during Mitosis

1. Prophase:

1. Mitosis begins at prophase with the thickening and coiling of the chromosomes.
2. The nuclear membrane and nucleolus shrinks and disappears.
3. The end of prophase is marked by the beginning of the organization of a groupof fibres to form a spindle.

2. Metaphase

1. The chromosome become thick and two chromatids of each chromosomebecome clear.
2. Each chromosome attaches to spindle fibres at its centromere.
3. The chromosomes are arranged at the midline of the cell.

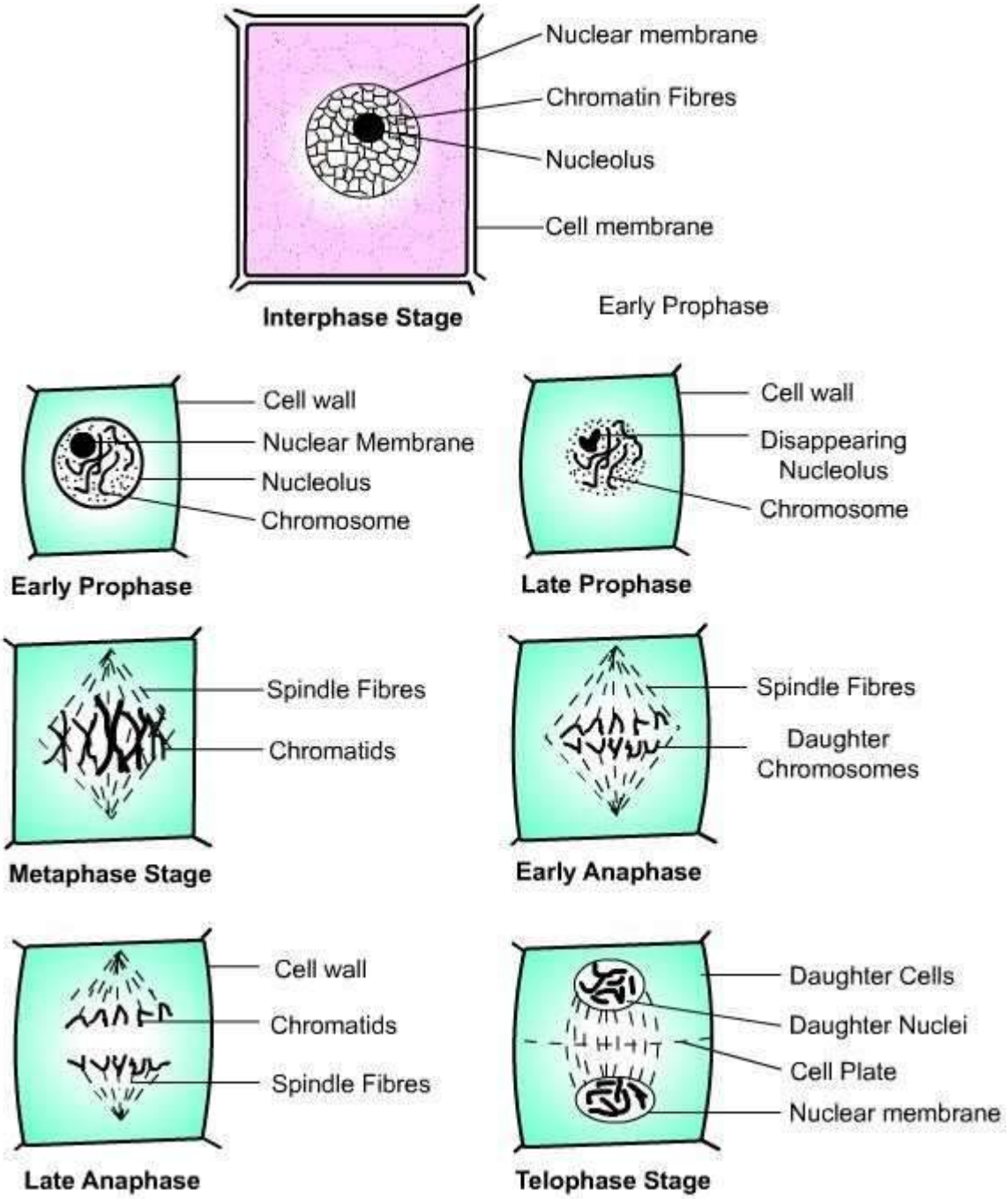
3. Anaphase

1. In anaphase each chromatid pair separates from the centromere and movetowards the opposite ends of the cell by the spindle fibres.
2. The cell membrane begins to pinch at the centre.

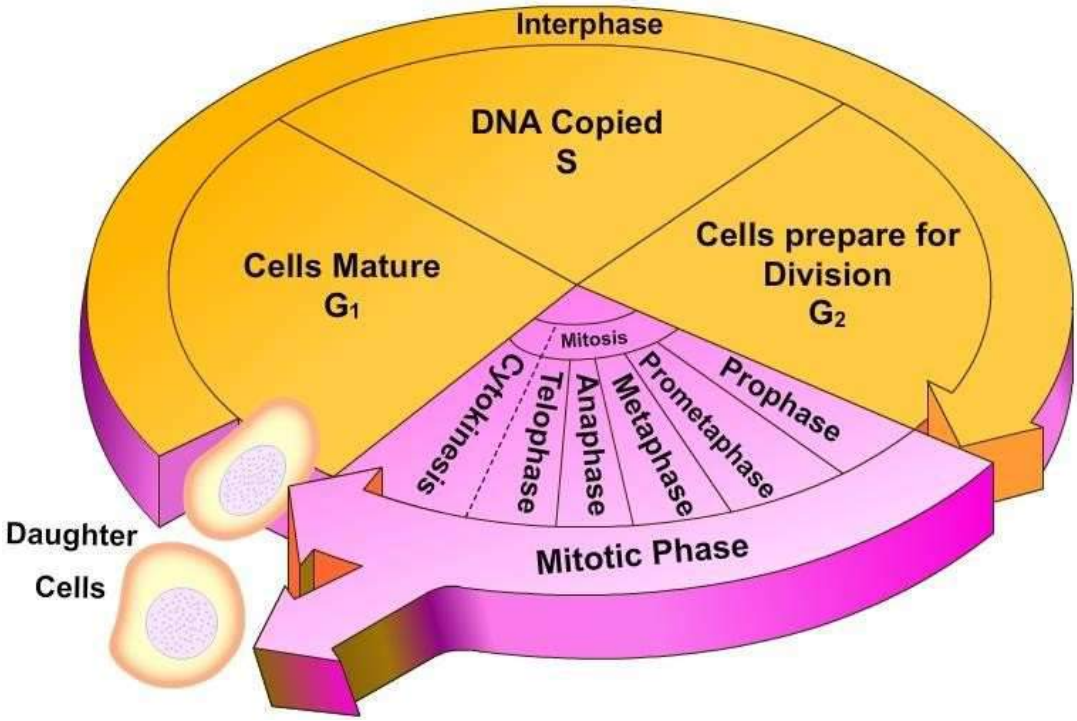
4. Telophase

1. Chromatids arrive at opposite poles of cell.
2. The spindle disappears and the daughter chromosome uncoils to form chromatinfibres.

3. The nuclear membranes and nucleolus re-form and two daughter nuclei appearat opposite poles.
4. Cytokinesis or the partitioning of the cell may also begin during this stage.

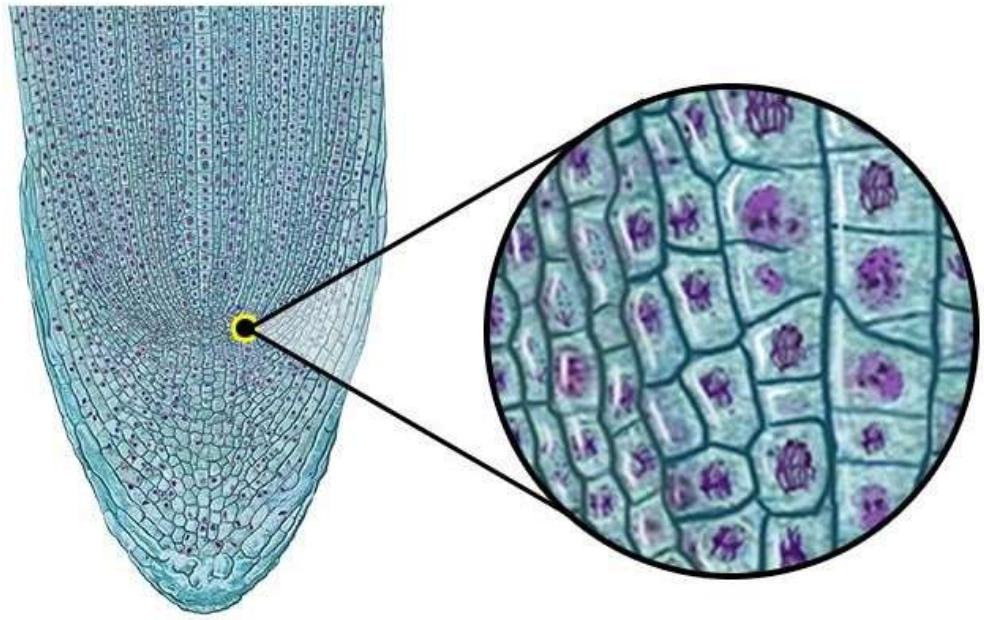


The stage, or phase, after the completion of mitosis is called interphase. It is the non dividing phase of the cell cycle between two successive cell divisions. Mitosis is only one part of the cell cycle. Most of the life of a cell is spent in interphase. Interphase consist of three stages call G1, S and G2.



Mitosis in Onion Root Tip

The meristamatic cells located in the root tips provide the most suitable material for the study of mitosis. The chromosome of monocotyledonous plants is large and more visible; therefore, onion root tips are used to study mitosis. Based on the kind of cells and species of organism, the time taken for mitosis may vary. Mitosis is influenced by factors like temperature and time.



Mitosis in Onion Root Tip

Procedure

- Take an onion and place it on the tile.
- Carefully remove the dry roots present using a sharp blade.
- Grow root tips by placing the bulbs in a beaker filled with water.
- New roots may take 3–6 days to grow.
- Cut off 2–3 cm of freshly grown roots and let them drop into a watch glass.
- Using a forceps, transfer them to the vial containing freshly prepared fixative of aceto-alcohol (1:3: glacial acetic acid: ethanol).
- Keep the root tips in the fixative for 24 hours.
- Using a forceps, take one root and place it on a clean glass slide.
- Using a dropper, place one drop of N/10 HCl on the root tip followed by 2–3 drops of acetocarmine stain.
- Warm it slightly on burner. Care should be taken that the stain is not dried up.
- Carefully blot the excess stain using filter paper.
- Using a blade, cut the comparatively more stained tip portion of the root, retain it on the slide and discard the remaining portion.
- After that, put one drop of water on the root tip.
- Mount a cover slip on it using a needle.
- Now, slowly tap the cover slip using the blunt end of a needle so that the meristematic tissue of the root tip below the cover slip is properly squashed and spread as a thin layer of cells.
- This preparation of onion root tip cells is now ready for the study of mitosis.
- Place the slide under the compound microscope and observe the different stages of mitosis.
- Various stages of mitosis are prophase, metaphase, anaphase and telophase.

PRACTICAL 7: EXPERIMENTS ON PROBABILITY AND CHI-SQUARE TEST.

Q1. A die is rolled, find the probability that an even number is obtained. Q2. Two coins are tossed, find the probability that two heads are obtained.

Q3 Two dice are rolled, find the probability that the sum is

a) Equal to 1 b) Equal to 4 c) Less than 13

Q4. In an experiment designed to study the inheritance of flower color in four-o'clocks, two plants with pink flowers were crossed. In the progeny from this cross, there were 42 plants with red flowers, 86 with pink flowers, and 39 with white flowers. Using a chi-square test, determine whether those numbers are consistent with the single-gene, incomplete dominanceinheritance pattern.

Q5. Trying to understand the inheritance of the dominant yellow gene in mice, researchers mated two yellow heterozygous mice. A typical result was 56 yellow progeny to 31 wild- type.

(a) Use a chi-square test to determine if the outcome of this cross is consistent with the usual 3:1 ratio predicted by Mendelian inheritance for a dominant gene.

(b) You will find that the chi-square test done in part (a) indicates the data are not consistent. Now try the hypothesis that the dominant allele is lethal in the homozygous condition. Repeatthe chi-square test.

Percentage Points of the Chi-Square Distribution

Degrees of Freedom	Probability of a larger value of x^2								
	0.99	0.95	0.90	0.75	0.50	0.25	0.10	0.05	0.01
1	0.000	0.004	0.016	0.102	0.455	1.32	2.71	3.84	6.63
2	0.020	0.103	0.211	0.575	1.386	2.77	4.61	5.99	9.21
3	0.115	0.352	0.584	1.212	2.366	4.11	6.25	7.81	11.34
4	0.297	0.711	1.064	1.923	3.357	5.39	7.78	9.49	13.28
5	0.554	1.145	1.610	2.675	4.351	6.63	9.24	11.07	15.09
6	0.872	1.635	2.204	3.455	5.348	7.84	10.64	12.59	16.81
7	1.239	2.167	2.833	4.255	6.346	9.04	12.02	14.07	18.48
8	1.647	2.733	3.490	5.071	7.344	10.22	13.36	15.51	20.09
9	2.088	3.325	4.168	5.899	8.343	11.39	14.68	16.92	21.67
10	2.558	3.940	4.865	6.737	9.342	12.55	15.99	18.31	23.21
11	3.053	4.575	5.578	7.584	10.341	13.70	17.28	19.68	24.72
12	3.571	5.226	6.304	8.438	11.340	14.85	18.55	21.03	26.22
13	4.107	5.892	7.042	9.299	12.340	15.98	19.81	22.36	27.69
14	4.660	6.571	7.790	10.165	13.339	17.12	21.06	23.68	29.14
15	5.229	7.261	8.547	11.037	14.339	18.25	22.31	25.00	30.58
16	5.812	7.962	9.312	11.912	15.338	19.37	23.54	26.30	32.00
17	6.408	8.672	10.085	12.792	16.338	20.49	24.77	27.59	33.41
18	7.015	9.390	10.865	13.675	17.338	21.60	25.99	28.87	34.80
19	7.633	10.117	11.651	14.562	18.338	22.72	27.20	30.14	36.19
20	8.260	10.851	12.443	15.452	19.337	23.83	28.41	31.41	37.57
22	9.542	12.338	14.041	17.240	21.337	26.04	30.81	33.92	40.29
24	10.856	13.848	15.659	19.037	23.337	28.24	33.20	36.42	42.98
26	12.198	15.379	17.292	20.843	25.336	30.43	35.56	38.89	45.64
28	13.565	16.928	18.939	22.657	27.336	32.62	37.92	41.34	48.28
30	14.953	18.493	20.599	24.478	29.336	34.80	40.26	43.77	50.89
40	22.164	26.509	29.051	33.660	39.335	45.62	51.80	55.76	63.69
50	27.707	34.764	37.689	42.942	49.335	56.33	63.17	67.50	76.15
60	37.485	43.188	46.459	52.294	59.335	66.98	74.40	79.08	88.38

PRACTICAL 8 : DETERMINATION OF LINKAGE AND CROSS-OVER ANALYSIS

Q1. An individual with cd genes was crossed with wild type + +. On test crossing F1, the progeny was + c 105, + d 115, cd 880, and + + 900. Distance between cd genes is:

a. 11 map units
b. 5.5 map units
c. 44 map units
d. 88 map units

Q2. A series of fruit fly mating shows that the recombination frequency between the gene for wing size and the gene for antenna length is 5% (i.e. the genetic distance between them is 5 centimorgans). List all possible recombination frequencies between the gene for colour and the gene for antenna length.

Q3. You are doing a genetics experiment with the fruit fly. In the "P" generation, you cross two true-breeding flies. The female parent is brown and wingless and the male parent is black with normal wings. All of the flies in the F1 generation (1600) are brown and have normal wings.

a. In case of complete linkage between the two genes, what would you the count of

i. brown, winged flies (of the genotype BbNn
ii. black, winged flies (of the genotype Bbnn)
iii. brown, wingless flies (of the genotype bbNn)
iv. black, wingless flies (of the genotype bbnn)

b. When you count the F2 generation, you really get: 85 brown winged flies 728 black winged flies 712 brown wingless flies 75 black wingless flies What is the genetic distance between thecolour and wing genes?

PRACTICAL 9: STUDY ON SEX LINKED INHERITANCE IN DROSOPHILA.

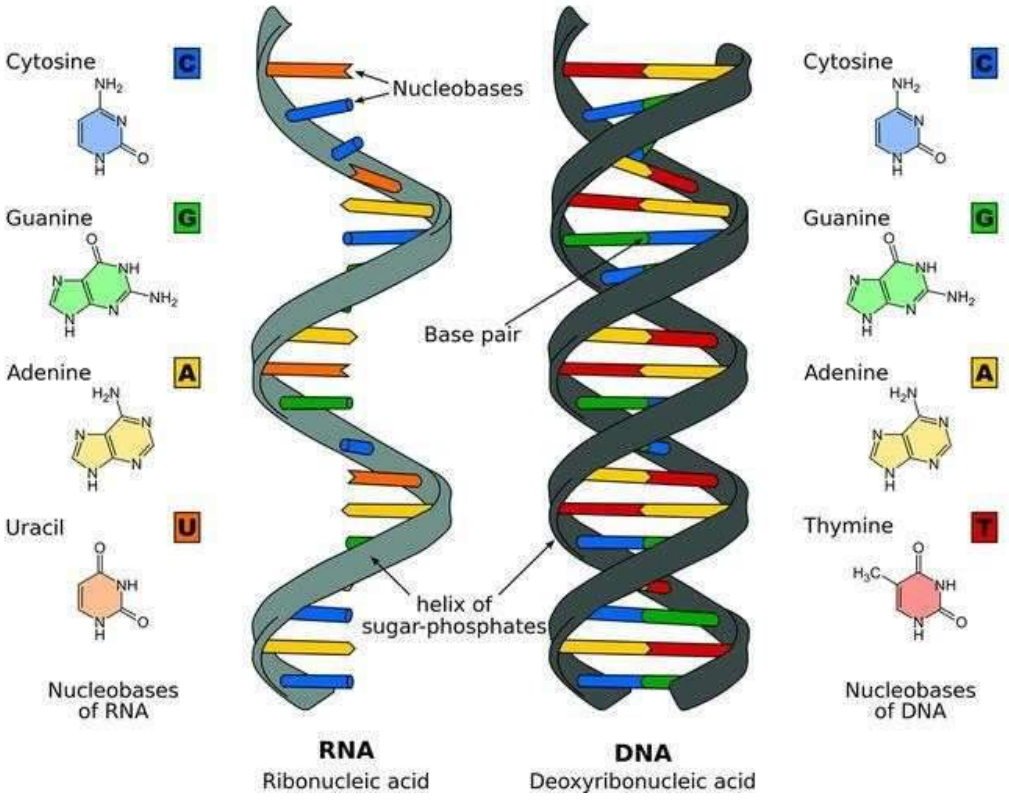
1. In a cross between a white eyed female fruit fly and red- eyed male, what percentage of thefemale offspring will have white eyed? (White eyes are X- linked recessive).

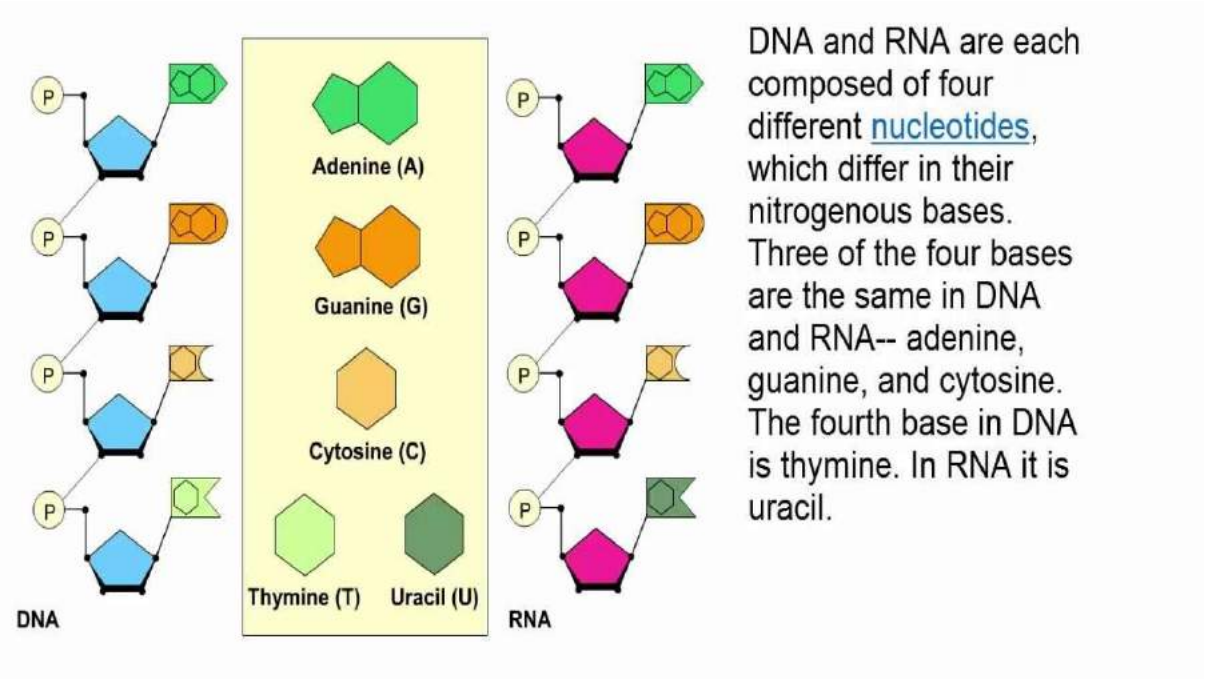
2. A female drosophila of unknown genotype was crossed with a white eyed male fly of genotype X^wY (X^wY is white eyed recessive and X^{w+} is red eyed dominant). Half of the male and half of the female offsprings were red- eyed. What is the genotype of female fly?

3. In a cross between a pure- bred red-eyed female fruit fly and white eyed male, what percent of the male offspring will have white eyes?

4. What is the genotype of red- eyed yellow – bodied female fruit fly, who is homozygous forthe eye colour allele? Red eyes (W^+) and tan- bodies (Y^+) are dominant allele.

PRACTICAL 10: STUDY OF MODELS ON DNA AND RNA STRUCTURES.





Basic chemical structure of DNA and RNA (heteropolymers of nucleotides)

• Monomer composition (nucleotide) – heterocyclic pentose sugar – phosphate – nitrogenousbase

• RNA: polar ribose phosphate backbone

• DNA: polar deoxyribose phosphate backbone (no 2′-hydroxyl)

• Nucleotides joined by 3′,5′- phosphodiester linkages

• Nitrogenous bases – side chains

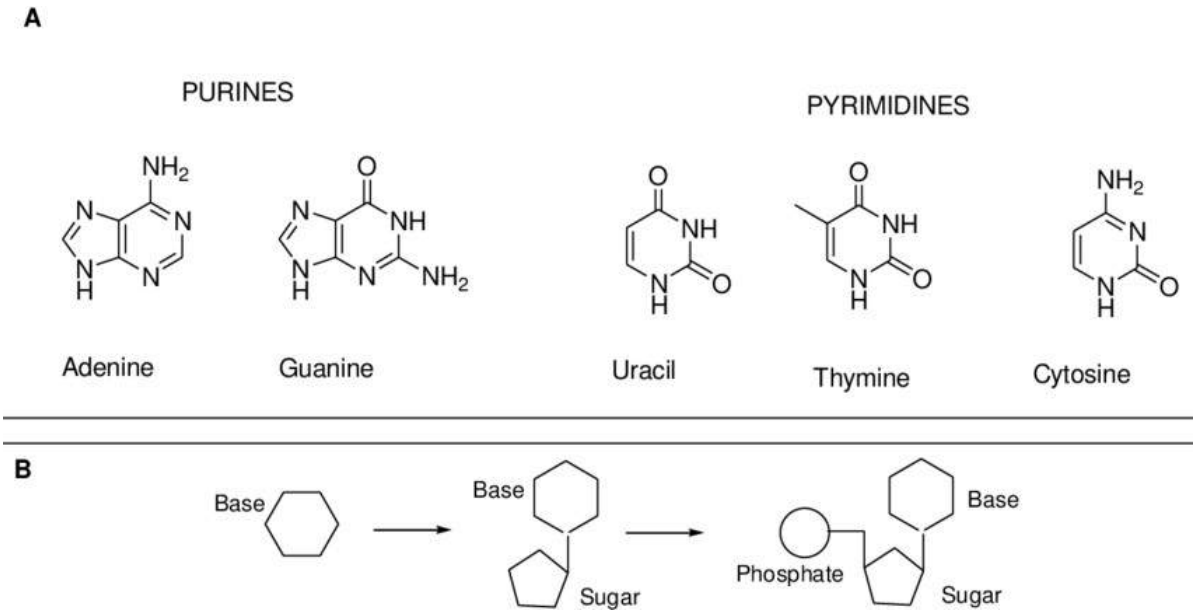
Major nitrogenous bases found in DNA and/or RNA (purines & pyrimidines)

• DNA: A, G, C, T

• RNA: A, G, C, U

• N - β-glycosyl bond: 1′ carbon of ribose and N9 of Pur base (A, G) or N1 of Pyr base (C, T,U)

• Pur or Pyr base + ribose = nucleoside



AGRICULTURAL MICROBIOLOGY- AGS-202

PRACTICAL 1 INTRODUCTION TO MICROBIOLOGY LABORATORY AND ITS EQUIPMENTS.

Following are the instrument:

1. Hot Air Oven for Sterilization:

It is used for sterilization of glassware's, such as test tubes, pipettes and petri dishes. Such dry sterilization is done only for glassware's. Liquid substances, such as prepared media and saline solutions cannot be sterilized in oven, as they lose water due to evaporation. The glassware's are sterilized at 180°C for 3 hours. An oven has a thermostat-control, using which the required constant temperature can be obtained by trial and error. The thermostat dial reading is approximate and the exact temperature is read by introducing a thermometer into the oven or on a built-in L-shaped thermometer. In a modern oven there is a digital temperature display and automatic temperature controller to set the desired temperature easily. Time is set by a digital timer. After loading the glassware's, the door is closed and oven switched on.

The required temperature is set. After the oven attains the set temperature, the required time of sterilization is set on the timer. The oven switches off automatically after the set time. The oven is opened, only after its temperature comes down near to room temperature. Otherwise, if the door is opened, while the inside of the oven is still very hot, cold air may rush in and crack the glassware's.

2. Drying Oven:

For preparation of certain reagents, the glassware's, after proper cleaning and rinsing with distilled water, are required to be dried. They are dried inside the drying oven at 100°C till the glassware's dry up completely.

3. Autoclave:

Autoclave is the nucleus of a microbiology laboratory. It is used not only to sterilize liquid substances such as prepared media and saline (diluents) solutions, but also to sterilize glassware's, when required.

It has the same working principle as a domestic pressure cooker. The maximum temperature that can be obtained by boiling water in an open container is 100°C (boiling point of water).

This temperature is sufficient to kill only the non-spore formers, but it is difficult to kill the spore-formingbacteria at this temperature, as they escape by forming heat resistant spores. It takes very long time to kill the spores at this temperature.

On the other hand, when water is boiled in a closed container, due to increased pressure inside it, the boiling point elevates and steam temperature much beyond 100°C can be obtained. This high temperature is required to kill all the bacteria including the heat resistant spore-formers. Steam temperature increases with increase in steam pressure (Table 3.1).

In operating a standard vertical autoclave, sufficient water is poured into it. If water is too less, the bottomof the autoclave gets dried during heating and further heating damages it.

If it has in-built water heating element, water level should be maintained above the element. On the other hand, if there is too much water, it takes long time to reach the required temperature.

The materials to be sterilized are covered with craft paper and arranged on an aluminium or wooden frame kept on the bottom of the autoclave, otherwise if the materials remain half-submerged or floating, they tumble during boiling and water may enter. The autoclave is closed perfectly airtight only keepingthe steam release valve open.

Then, it is heated over flame or by the in-built heating element. Air inside the autoclave should beallowed to escape completely through this valve. When water vapour is seen to escape through the valve, it is closed.

Temperature and pressure inside goes on increasing. The pressure increase is observed on the pressure dial. Usually sterilization is done at 121 °C (a pressure of 15 pounds per square inch i.e. 15 psi) for 15 minutes. The required time is considered from the point, when the required temperature-pressure is attained.

Once required temperature-pressure is attained, it is maintained by controlling the heating source. After the specified time (15 minutes), heating is discontinued and steam release valve slightly opened. If fully opened immediately, due to sudden fall in pressure, liquids may spill out from the containers.

Gradually, the steam release is opened more and more, so as to allow all steam to escape. The autoclave isopened only after the pressure drops back to normal atmospheric pressure (0 psi). The autoclave should never be opened, when there is still pressure inside. The hot sterilized materials are removed by holding them with a piece of clean cloth or asbestos- coated hand gloves.

In case of a steam-jacketed horizontal autoclave, a boiler produces the steam. It is released at a designated pressure, into the outer chamber (jacket). Air is allowed to escape and then its steam release valve is closed.

The hot jacket heats the inner chamber, thereby heating the materials to be sterilised. This prevents condensation of steam on the materials. Now, steam under pressure is released from the jacket into the inner chamber and air is allowed to escape from it.

Then, its steam release valve is closed. The steam under pressure in the inner chamber reaches temperatures in excess of 100°C, which can sterilise the materials kept inside it. The autoclave also has automatic shutting system i.e. unless temperature and pressure comes down near to room conditions, the door cannot be opened.

Besides the pressure dial, it also has separate temperature dial to indicate the temperature inside the inner chamber. Moreover, the autoclave maintains the temperature and pressure automatically and switches off after the set time of sterilization.

1. Microbiological Incubator:

Profuse growth of microbes is obtained in the laboratory by growing them at suitable temperatures. This is done by inoculating the desired microbe into a suitable culture medium and then incubating it at the temperature optimum for its growth.

Incubation is done in an incubator (Figure 3.7), which maintains a constant temperature specifically suitable for the growth of a specific microbe. As most of the microbes pathogenic to man grow profusely at body temperature of normal human being (i.e. 37°C), the usual temperature of incubation is 37°C.

The incubator has a thermostat, which maintains a constant temperature, set according to requirement. The temperature reading on the thermostat is approximate. Accurate temperature can be seen on the thermometer fixed on the incubator. Exact temperature, as per requirement, is set by rotating the thermostat knob by trial and error and noting the temperature on the thermometer.

Most of the modern incubators (Figure 3.8) are programmable, which do not need trial and error temperature setting. Here, the operator sets the desired temperature and the required period of time.

The incubator automatically maintains it accordingly. Moisture is supplied by placing a beaker of water in the incubator during the growth period. A moist environment retards the dehydration of the media and thereby, avoids spurious experimental results.

2. BOD Incubator (Low Temperature Incubator):

Some microbes are to be grown at lower temperatures for specific purposes. The BOD low temperature incubator (Figure 3.9), which can maintain temperatures from 50°C to as low as 2-3°C is used for incubation in such cases.

The constant desired temperature is set by rotating the knob of the thermostat. Rotation of the thermostat knob moves a needle on a dial showing approximate temperature. Exact required temperature is obtained, by rotating the knob finely by trial and error and noting the temperature on the thermometer fixed on the incubator.

Most of the modern BOD incubators (Figure 3.10) are programmable, which do not need trial and error temperature setting. Here, the operator sets the desired temperature and the required period of time. The incubator automatically maintains it accordingly.

1. Fridge (Refrigerator):

It serves as a repository for thermo labile chemicals, solutions, antibiotics, serums and biochemical reagents at cooler temperatures and even at sub-zero temperatures (at less than 0°C). Stock cultures of bacteria are also stored in it between sub-culturing periods. It is also used for the storage of sterilized media, so as to prevent their dehydration.

2. Deep-fridge:

It is used to store chemicals and preserve samples at very low sub-zero temperatures.

3. Electronic Top-pan Balance:

It is used for weighing large quantities of media and other chemicals, where precise weighing is not of much importance.

4. Electronic Analytical Balance:

It is used to weigh small quantities of chemicals and samples precisely and quickly.

5. Double-pan Analytical Balance:

It is used to weigh chemicals and samples precisely. Weighing takes more time, for which it is used in emergency only.

6. Distilled Water Plant:

Water is used in the preparation of media and reagents. If the media are prepared using tap water, the chemical impurities present in it may interfere with the growth of the microorganisms in the media. Moreover, the higher is the bacteria content of the media, the longer is the time required for their sterilization and greater is the chance of survival of some bacteria.

Distilled water, though not bacteria- free, contains less number of bacteria. That is why; it is preferred in the preparation of microbiological media. It is also used in the preparation of reagents, because the chemical impurities present in tap water may interfere with the proper functioning of the reagent chemicals.

As manufacture of distilled water by Liebig condenser is a time-taking process, in most laboratories, it is prepared by 'distilled water plants'. Usually a distilled water plant is made of steel or brass. It is also called distilled water still.

It has one inlet to be connected to the water tap and two outlets, one for distilled water to drop into a container and the other for the flow out of hot cooling water into the sink. The still is installed on the wall.It is heated by in-built electric heating elements (immersion heater).

The still works efficiently, when the water in-flow is so adjusted that, the temperature of the cooling water flowing out from the still into the sink is neither too high nor too low i.e., warm water should flow out. The distilled water may contain traces of metals corroded from the steel or brass container.

To get metal-free distilled water, glass distillation apparatus is used and still better is quartz distillation apparatus. However, for a microbiology laboratory, a steel or glass distillation apparatus is sufficient. For precision analyses, double- or triple- distilled water is used.

1. Ultrapure Water Purification System:

For precision analytical works, now-a-days, instead of using double- or triple-distilled water, micro- filtered water is used. In case of distilled water, there is chance that, few volatile substances present in the water get volatilized during heating of the water and subsequently get condensed into the distilled water collected.

Thus, there may be traces of such substances in the distilled water. To overcome this, ultrapure water is used. Here, water is allowed to pass through very fine microscopic pores, which retain the microscopic suspended particle including the microbes.

Then, the water passes through two columns of ion exchange resins. The anion exchange resin adsorbs the captions present in the water, while the caption exchange resin adsorbs the anions. The water that comes out is extremely pure.

2. Homogeniser:

For microbiological analysis, liquid samples are directly used, whereas solid samples have to be mixed thoroughly with a diluent (usually physiological saline), so as to get a homogenous suspension of bacteria. This suspension is assumed to contain bacteria homogenously.

The mixing of solid samples and diluents is done by a homogenizer, in which a motor rotates

an impeller with sharp blades at high speed inside the closed homogenizer cup containing the sample and the diluents. It has a speed regulator for controlling the speed of rotation of the impeller.

In some laboratories mixing is done manually by sterilized pestle and mortar. In modern laboratories, a disposable bag is used, inside which the solid sample and liquid diluents are put aseptically and mixed mechanically by peristaltic action of a machine on the bag. This machine is called stomacher.

3. pH Meter:

A pH meter is an instrument for determining the pH of liquid media, liquid samples and buffers. It has a glass pH electrode. When not in use, it should be kept half immersed in water contained in a small beaker and preferably be covered by a bell jar to avoid dust accumulation in the water and loss of water through evaporation.

Before use, the meter is calibrated using two standard buffers of known pH. Usually buffers of pH 4.0,

7.0 and 9.2 are available commercially. The instrument is switched on and left for 30 minutes to warm up. The temperature calibration knob is rotated to the temperature of the solutions whose pH is to the measured.

Then, the electrode is dipped into the buffer (pH 7.0). If the reading is not 7.00, the pH calibration knob is rotated till the reading is 7.00. Then, the electrode is dipped in another buffer (pH 4.0 or 9.2).

If the reading is same as the pH of the buffer used, the instrument is working properly. Otherwise, the electrode is activated by dipping in 0.1 N HC1 for 24 hours. After calibration, the pH of samples is determined by dipping the electrode into them and noting the reading.

Every time, before dipping into any solution, the electrode should be rinsed with distilled water. The samples should not contain any suspended sticky materials, which may form a coating on the tip of the electrode and reduce its sensitivity.

The old model pH meters have double electrodes (one of them acting as reference electrode), while new models have single combined electrode. Moreover, to overcome the problem of temperature correction, now pH meters with automatic temperature correction are available.

Here, another ‘temperature electrode’ is also put into the solution along with the pH electrode, which measures the temperature of the solution and automatically corrects the influence of temperature variations.Sophisticated pH meters have single gel electrode. Such electrodes have very little chance of breakage, as they are almost completely enclosed in a

hard plastic casing except at the tip. The tip has both pH and temperature sensors.

Moreover, they are easy to maintain, as they do not require constant dipping in distilled water, because the electrode tip is closed with a plastic cap containing saturated solution of potassium chloride, when not in use. However, in preparation of microbiological media, pH is determined by narrow-range pH papers and is adjusted to the required pH by adding acids or alkalis as required.

4. Hot Plate:

Hot plate is used to heat chemicals and reagents. The hot plate is made of an iron plate, which gets heated by an electric heating element from below. The required degree of heating is obtained by a regulator.

5. Shaking Water Bath:

Sometimes, heating at very precise temperatures is required. Such precise temperatures cannot be obtained in an incubator or oven, in which temperature fluctuates, though slightly. However, precise temperatures can be maintained in a water bath, which provides a stable temperature.

A water bath consists of a container containing water, which is heated by electric heating elements. The required water temperature is obtained by increasing or decreasing the rate of heating by rotating the thermostat by trial and error.

In a shaking water bath, the substance is heated at the required temperature and at the same time, it is shaken constantly. Shaking is done by a motor, which rotates and moves the containers to and fro in

rotation. The rate of shaking is again controlled by a regulator. Shaking agitates the substance and enhances the rate of the process.

Most modern water baths are programmable and do not need trial and error temperature setting. A desired water temperature can be maintained over a desired period of time by programming accordingly. It is used for cultivation of bacteria in broth medium at a specific temperature.

1. Quebec Colony Counter:

In enumeration of bacteria in samples, it is assumed that a single bacterium gives rise to a single visible colony, when grown on a plate of solidified nutrient medium. Thus, by counting the number of colonies, the number of bacteria in a sample can be estimated.

Sometimes, colonies are very small and too much crowded making it difficult to count. Counting becomes easy, when a mechanical hand counter, called Quebec colony counter (Figure 3.11), is used. It divides the plate into several square divisions and the colonies are magnified 1.5 times by a magnifying glass, which makes counting easy.

2. Electronic Colony Counter:

Electronic colony counter is of two types:

(1) Hand-held electronic colony counter and

(2) Table-top electronic colony counter.

The hand-held electronic colony counter is a pen-style colony counter with an inking felt-tip marker. For counting of colonies of bacteria grown in a petri dish, it is kept in an inverted position, so that the colonies are visible through the bottom surface of the petri dish.

The colonies are marked by touching the glass surface of the petri dish with the felt-tip of the colony counter. Thus, each colony is marked by a dot made by the ink of the felt-tip on the bottom surface of the petri dish. In a single motion, the electronic colony counter marks, counts and confirms with a beep sound.

The cumulative count of colonies is displayed on a four-digit LED display. In case of table-top electronic colony counter, the petri dish containing the colonies of bacteria is placed on an illuminated stage and the count bar is depressed. The precise number of colonies is instantly displayed on a digital read out.

3. Magnetic Stirrer:

In the preparation of solutions, certain chemicals require stirring for long time, to be dissolved in certain solvents. Magnetic stirrer is used to dissolve such substances easily and quickly. A small teflon- coated magnet, called ‘stirring bar’, is put into a container containing the solvent and the solute.

Then, the container is placed on the platform of the magnetic stirrer, below which a magnet rotates at high speed by a motor. Attracted by the rotating magnet, the teflon-coated magnet rotates inside the container and stirs the contents. Now, the solute dissolves quickly.

The teflon coating prevents the magnet from reacting with the solution, which comes in contact with it. After complete dissolution, the teflon-coated magnet is removed from the solution by mean of a long retriever, called ‘stirring bar retriever’.

4. Sonicator:

It is used to rupture cells using high frequency waves.

5. Vortex Mixer:

It is an instrument used for thorough mixing of liquids in test tubes. It has a rotor, whose speed can be controlled. On the tip of the rotor is a foam-rubber top. When the bottom of a test tube is pressed upon this foam-rubber top, the rotor starts rotating, thereby rotating the bottom of the test tube at high speed.

Due to centripetal force, the solution gets mixed thoroughly. It is particularly helpful during serial dilution in enumeration of bacteria, which needs homogenous suspension of bacteria cells.

6. Laminar Flow Chamber:

It is a chamber (Figure 3.12) used for aseptic transfer of sterilized materials, as well as for inoculation of microbes. Dust particles floating in the air harbour microbes. These microbe-laden dust particles may enter into the sterilized media and contaminate them, when they are opened for short periods of time during inoculation of microbe or transfer from one container to another.

To overcome this, when inoculation is done in open air, the air of the small inoculating area is sterilized by the flame of a bunsen burner. The heated air becomes light and moves upwards, thereby preventing the dust particles from falling on the media during the short opening process.

To further reduce the chance of contamination by the microbe-laden air, a laminar flow chamber is used. It is a glass-fitted cuboidal chamber. An air blower blows air from the surrounding and passes it through aHEPA filter (High Efficiency Particulate Air filter), so as to make it dust free (microbe-free).

This microbe-free air passes through the chamber in a laminar manner and comes out from the chamber through the open front door. This laminar flow of microbe-free air from the chamber to outside through the open door prevents the outside air from entering into the chamber.

Thus, the chamber does not get contaminated with the microbes present in the outside air, though the doors is kept opened during inoculation or transfer of media. An UV lamp fitted inside the chamber sterilizesthe chamber before operation.

It has a stainless steel platform with provision for gas pipe connection for a bunsen burner.

Before use, theplatform is cleaned and disinfected with lysol, the bunsen burner is connected and then the glass door is closed.

The UV light is switched on for 10 minutes to sterilise the environment inside the chamber and then switched off. The glass door should never be opened when the UV light is on, because UV light has detrimental effect on skin and vision. The blower is switched on and then the glass door is opened.

Now, the bunsen burner is lighted and media transfer or inoculation is carried out in the chamber aseptically. If extremely hazardous microbes are to be handled, a laminar flow chamber with gloves projecting into the chamber from the front glass door is used, as inoculation has to be done keeping the front door closed.

7. Electronic Cell Counter:

It is used to directly count the number of bacteria in a given liquid sample. An example of electronic cell counter is the 'Coulter counter'. In this equipment, a suspension of bacteria cells is allowed to pass through a minute orifice, across which an electric current flows.

The resistance at the orifice is electronically recorded. When a cell passes through the orifice, being non- conductor, it increases resistance momentarily. The number of times resistance increases momentarily is recorded electronically, which indicates the number of bacteria present in the liquid sample.

8. Membrane Filtration Apparatus:

Certain substances like urea disintegrate and lose their original properties, if sterilized by heat. Such substances are sterilized by membrane filtration apparatus. In this apparatus, the solution of the substance to be sterilized is filtered through a membrane filter, which does not allow bacteria cells to pass down. Filtration is done under suction pressure to increase the rate of filtration (Figure 2.19, page 30).

9. Microscopes:

Different types of microscopes are used for visual observation of morphology, motility, staining and fluorescent reactions of bacteria.

10. Computers:

Computers are generally used for analysis of results. They are also used for identification of bacteria easily within few hours. Otherwise, identification of bacteria is a tedious process and takes days together to identify one bacteria species.

The computers used for identification of bacteria are Apple II, IBM PC and TRS-80 and their modern variants. Each research personnel of the laboratory should be provided with a

computer, along with internet facility.

11. Spectrophotometer:

It is an instrument for measuring the differences in color intensities of solutions. A beam of light of a particular wavelength is passed through the test solution and the amount of light absorbed (or transmitted)is measured electronically.

A simple visible spectrophotometer can pass light with wavelengths within visible range, whereas a UV- cum-visible spectrophotometer can pass light with wavelengths in ultraviolet as well as in visible range. In microbiology lab, it is used for direct counting of bacteria in suspension as well as for other purposes.

12. Electrical Devices:

A fluctuation of electric voltage in the laboratory is one of the most important reasons, which reduces the longevity of the equipments and sometimes damage them. Therefore, all the voltage-sensitive equipments should be provided with voltage protection devices like stabilizers, servo stabilizers or constant voltage transformers (CVT) as per the recommendations of the manufacturers of the equipments.

Computers, balances and some sophisticated equipments should be connected through uninterrupted power supply (UPS), as any breakdown in the electric power supply during their operation may severely damage some of their sensitive components.

The laboratory should have a high capacity generator to supply electric current to the whole laboratory in case of power failure. This is because, power failure not only brings the activities of the laboratory to a standstill, it also brings about undesirable irreversible changes in the samples stored in the deep-fridges and refrigerators.

PRACTICAL 2: MICROSCOPE- PARTS, PRINCIPLES OF MICROSCOPY, RESOLVING POWER AND NUMERICAL APERTURE.

A high power or compound microscope achieves higher levels of magnification than a stereo or low power microscope. It is used to view smaller specimens such as cell structures which cannot be seen at lower levels of magnification. Essentially, a compound microscope consists of structural and optical components. However, within these two basic systems, there are some essential components that every microscopist should know and understand. These key microscope parts are illustrated and explained below.

STRUCTURAL COMPONENTS

The three basic, structural components of a compound microscope are the head, base and arm.

- Head/Body houses the optical parts in the upper part of the microscope
- Base of the microscope supports the microscope and houses the illuminator
- Arm connects to the base and supports the microscope head. It is also used to carry the microscope. When carrying a compound microscope always take care to lift it by both the arm and base,simultaneously.

OPTICAL COMPONENTS

There are two optical systems in a compound microscope: Eyepiece Lenses and Objective Lenses:

Eyepiece or Ocular is what you look through at the top of the microscope. Typically, standard eyepieces have a magnifying power of 10x. Optional eyepieces of varying powers are available, typically from 5x- 30x.

Eyepiece Tube holds the eyepieces in place above the objective lens. Binocular microscope heads typically incorporate a diopter adjustment ring that allows for the possible inconsistencies of our eyesight in one or both eyes. The monocular (single eye usage) microscope does not need a diopter. Binocular microscopes also swivel (Interpupillary Adjustment) to allow for different distances between the eyes of different individuals.

Objective Lenses are the primary optical lenses on a microscope. They range from 4x-100x and typically, include, three, four or five on lens on most microscopes. Objectives can be forward or rear-facing.

Nosepiece houses the objectives. The objectives are exposed and are mounted on a rotating turret so that different objectives can be conveniently selected. Standard objectives include 4x, 10x, 40x and 100x although different power objectives are available.

Coarse and Fine Focus knobs are used to focus the microscope. Increasingly, they are coaxial knobs - that is to say they are built on the same axis with the fine focus knob on the outside. Coaxial focus knobs are more convenient since the viewer does not have to grope for a different knob.

Stage is where the specimen to be viewed is placed. A mechanical stage is used when working at higher magnifications where delicate movements of the specimen slide are required.

Stage Clips are used when there is no mechanical stage. The viewer is required to move the slide manually to view different sections of the specimen.

Aperture is the hole in the stage through which the base (transmitted) light reaches the stage.

Illuminator is the light source for a microscope, typically located in the base of the microscope. Most light microscopes use low voltage, halogen bulbs with continuous variable

lighting control located within the base.

Condenser is used to collect and focus the light from the illuminator on to the specimen. It is located under the stage often in conjunction with an iris diaphragm.

Iris Diaphragm controls the amount of light reaching the specimen. It is located above the condenser and below the stage. Most high quality microscopes include an Abbe condenser with an iris diaphragm. Combined, they control both the focus and quantity of light applied to the specimen.

Condenser Focus Knob moves the condenser up or down to control the lighting focus on the specimen.

PRACTICAL 3: METHODS OF STERILIZATION

Sterilization:-Itis defined as the process where all the living microorganisms, including bacterial spores are killed.Sterilization can be achieved by physical, chemical and physiochemical means. Chemicals used as sterilizing agents are called chemisterilants.

Physical Methods of Sterilization:

Sunlight: The microbicidal activity of sunlight is mainly due to the presence of ultra violet rays in it. It is responsible for spontaneous sterilization in natural conditions. In tropical countries, the sunlight is more effective in killing germs due to combination of ultraviolet rays and heat. By killing bacteria suspended in water, sunlight provides natural method of disinfection of water bodies such as tanks and lakes. Sunlightis not sporicidal, hence it does not sterilize.

Heat: Heat is considered to be most reliable method of sterilization of articles that can withstand heat. Heat acts by oxidative effects as well as denaturation and coagulation of proteins. Those articles that cannot withstand high temperatures can still be sterilized at lower temperature by prolonging the duration of exposure.

Red heat: Articles such as bacteriological loops, straight wires, tips of forceps and searing spatulas are sterilized by holding them in Bunsen flame till they become red hot. This is a simple method for effective sterilization of such articles, but is limited to those articles that can be heated to redness in flame

Flaming: This is a method of passing the article over a Bunsen flame, but not heating it

to redness. Articles such as scalpels, mouth of test tubes, flasks, glass slides and cover slips are passed through the flame a few times. Even though most vegetative cells are killed, there is no guarantee that spores too would die on such short exposure.This method too is limited to those articles that can be exposed toflame. Cracking of the glassware may occur.

Hot air oven: This method was introduced by Louis Pasteur. Articles to be sterilized are exposed to high temperature (160o C) for duration of one hour in an electrically heated oven. Since air is poor conductorof heat, even distribution of heat throughout the chamber is achieved by a fan. The heat is transferred to the article by radiation, conduction and convection. The oven should be fitted with a thermostat control, temperature indicator, meshed shelves and must have adequate insulation.

Articles sterilized:Metallic instruments (like forceps, scalpels, scissors), glassware (such as Petri-dishes, pipettes, flasks, all-glass syringes), swabs, oils, grease, petroleum jelly and some pharmaceutical products.

Autoclave: Sterilization can be effectively achieved at a temperature above 100oC using an autoclave. Water boils at 100oC at atmospheric pressure, but if pressure is raised, the temperature at which the waterboils also increases. In an autoclave the water is boiled in a closed chamber. As the pressure rises, the boiling point of water also raises. At a pressure of 15 lbs inside the autoclave, the temperature is said to be121oC. Exposure of articles to this temperature for 15 minutes sterilizes them. To destroy the infective agents associated with spongiform encephalopathy (prions), higher temperatures or longer times are used;135oC or 121oC for at least one hour are recommended.

FILTRATION:

Filtration does not kill microbes, it separates them out. Membrane filters with pore sizes between

0.2-0.45 μm are commonly used to remove particles from solutions that can't be autoclaved. It is used to remove microbes from heat labile liquids such as serum, antibiotic solutions, sugar solutions, urea solution. Various applications of filtration include removing bacteria from ingredients of culture media, preparing suspensions of viruses and phages free of bacteria, measuring sizes of viruses, separating toxins from culture filtrates, counting bacteria, clarifying fluids andpurifying hydrated fluid. Filtration is aided by using either positive or negative pressure using vacuum pumps. The older filters made of earthenware or

asbestos are called depth filters.

PRACTICAL 4: NUTRIENT AGAR MEDIA AND THEIR PREPARATION

Nutrient Agar: Composition, Preparation and Uses

Nutrient Agar is a general purpose, nutrient medium used for the cultivation of microbes supporting growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for the bacterial growth.

Composition of Nutrient Agar

- 0.5% Peptone

It is an enzymatic digest of animal protein. Peptone is the principal source of organic nitrogen for the growing bacteria.

- 0.3% beef extract/yeast extract

It is the water-soluble substances which aid in bacterial growth, such as vitamins, carbohydrates, organic nitrogen compounds and salts.

- 1.5% agar

It is the solidifying agent.

- 0.5% NaCl

The presence of sodium chloride in nutrient agar maintains a salt concentration in the medium that is similar to the cytoplasm of the microorganisms.

- Distilled water

Water is essential for the growth of and reproduction of micro-organisms and also provides the medium through which various nutrients can be transported.

- pH is adjusted to neutral (7.4) at 25 °C.

Preparation of Nutrient Agar

1. Suspend 28 g of nutrient agar powder in 1 litre of distilled water.
2. Heat this mixture while stirring to fully dissolve all components.
3. Autoclave the dissolved mixture at 121 degrees Celsius for 15 minutes.
4. Once the nutrient agar has been autoclaved, allow it to cool but not solidify.
5. Pour nutrient agar into each plate and leave plates on the sterile surface until the agar has solidified.
6. Replace the lid of each Petri dish and store the plates in a refrigerator.

Uses of Nutrients Agar

1. It is frequently used for isolation and purification of cultures.
2. It can also be used as a means for producing the bacterial lawns needed for antibiotic sensitivity tests. In actuality, antibiotic sensitivity testing is typically performed on media specially formulated for that purpose.

PRACTICAL 5: ENUMERATION OF MICROBIAL POPULATION IN SOIL.

Soil microorganisms live in thin films of water that surround soil particles. These tinyorganisms include microflora (bacteria, fungi and actinomycetes) and microfauna(protozoa and nematodes). In terms of numbers and biological activity the microflora aredominant. Bacteria are small (about 1 - 10 µm) and occur in three general shapes rod(bacillus), spherical (coccus) and spiral (spirilla). Bacilli and cocci are more common insoil. Fungi are filamentous and much larger. The branched hyphae exhibit cell divisionsand fungal mycelia (hyphal mass) are often macroscopic. Actinomycetes are alsofilamentous andbranched but smaller.

Agar Plate Method for Microbial Count

In this method, soil is dispersed in an agar medium so that individual microbial cells, sporesor mycelial fragments develop into macroscopic colonies. The procedure involvessuccessive dilutions of soil. Depending upon extent of dilution, plates may be filled with ahuge number of colonies or very few. Enumeration of colony-forming units initially

presentin the soil is from plates in between these extremes. This method requires sterile techniqueto avoid introduction of extraneous microbes.

Procedure

A homogenized, field-moist sample of topsoil and bottles containing 90 mL of sterilized water was taken to perform the experiment.

1. Add a 10 g sub-sample of topsoil to the bottle of sterilized water. Tightly cap andshake vigorously for10 minutes to disperse the soil. This is the 10^{-1} dilution.

2. Transfer 10 mL of the 10^{-1} dilution to another bottle of sterilized water. Use a sterilepipette. Takesample from the middle. Tightly cap and shake to uniformly mix. Thisis the 10^{-2} dilution.

3. Repeat step 2 using the 10^{-2} dilution to make a 10^{-3} dilution and proceed similarly,making 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions.

4. From the 10^{-7} dilution, transfer 1 mL to each of 2 sterile petri dishes using a sterile 1mL pipette. Make similar transfers from the 10^{-6}, 10^{-5} and 10^{-4} dilutions.

5. Into each seeded petri dish, pour enough sterile, melted agar to fill dish about 2full.Immediately swirlit around to ensure good mixing of soil inoculant and agar.

6. After the agar has solidified, invert plates and incubate at 35ºC for 1 week.

7. Next week, count the number of colonies on plates from the dilution that containsfrom 30 to 300 colonies. Don't count from those plates that contain colonies largerthan 2 cm diameter. Multiply by dilution, take the average andcorrect to oven-dry moisture content of the soil. This gives the number of colonyforming units (CFUs) per gram of soil.

PRACTICAL 6: METHODS OF ISOLATION AND PURIFICATION OF MICROBIAL CULTURES

There are special techniques employed to obtain pure cultures of microorganisms. In few cases it is possible to secure pure culture by direct isolation or direct transfer. This can be done only in those situations in which pure culture occurs naturally. Kinds of specimens taken for culturing will depend on the nature and habitat of microbes.

Different pathogens can be isolated from body tissues and fluids such as blood, urine, sputum, pus, faces, spinal fluid, bile, pleural fluids, stomach fluids etc. In the blood stream of a patient suffering with typhoid fever, the bacteria Salmonella typhosa may be present.

A pure culture of this bacterium may be obtained by drawing blood sample using a sterilized hypodermic syringe and treating the blood with anticoagulant such as heparin and potassium oxalate. The presence of the anticoagulant prevents the pathogenic microbe from entrapping in fibrin clot. The sample of the bloodmay be inoculated into a suitable medium.

1. Streaking
2. Plating
3. Dilution
4. Enriched procedure, and
5. Single cell technique.

1. Streaking:

This is most widely used method of isolation. The technique consists of pouring a suitable sterile medium into sterile petriplate and allowing the medium to solidify. By means of a sterile loope or straight needleor a sterile bent glass-rod a small amount of growth preferably from a broth culture or bacterial suspension is streaked back and forth across the surface of agar until about one third of the diameter ofthe plate has been covered.

The needle is then flamed and streaking in done at right angles to and across the first streak. This servesto drag bacteria out in a long line from the initial streak. When this streaking is completed the needle is again flamed and streaking is done at right angles to the second

streak and parallel to the first.

2.Plating: It includes diluting of a mixture of microorganisms until only a few hundred bacteria are left in each millilitre of the suspension. A very small amount of the dilution is then placed in a sterile petriplateby means of a sterile loop or pipette. The melted agar medium is cooled to about 45°C and is poured into plate. The microorganism and agar are well mixed. When the agar is solidified the individual bacterium will be held in place and will grow to a visible colony.

3. Dilution: This method is used for the microorganisms which cannot be easily isolated by streaking or plating method. Sometimes when several organisms are present in a mixture, with one organism predominating, the predominating form may be isolated by this method. For example, when raw milk is allowed to sour at room temperature it will, at the time of curding, have a mixture of microorganisms withhigh percentage of Streptococcus lactis.

4. Single Technique:

This is one of the most ideal and difficult method of securing pure culture. In this method a suspension of the pure culture is placed on the under-side of a sterile cover-glass mounted over a moist chamber on the stage of the microscope.

While looking through the microscope, a single cell is removed with the help of sterile micropipette and transferred to a small drop of sterile medium on a sterile cover-glass and is mounted on a sterile hanging drop slide, which is then incubated at suitable temperature. If the single cell germinates in this drop, few cells are transferred into a tube containing sterile culture medium which is placed in the incubator to obtain pure culture originated from single cell.

Other methods:

The isolation of anaerobic microorganisms is very difficult. There are certain special techniques by whichthese organisms are isolated.

Cultivation of Microorganisms:

For cultivating microbes in laboratory, we require culture media. The various mixtures of

nutritive substances used for the laboratory cultivation of microorganisms are collectively known as culture media. The culture media serve as soil in which bacteria are planted for the purpose of study.

Culture Media:

Culture media must contain all the essential nutrients required by the organism for its growth and reproduction. A suitable source of energy, building materials and growth factors must be supplied in adequate amounts.

Carbohydrate (Sugar)	-	source of hydrogen, carbon & chemical-bond energy.
Nitrite ions or Ammonium ions	-	Source of nitrogen
Sodium / Magnesium sulphate	-	Minerals
Micronutrients		
Growth factors, Vitamins & certain amino acids.		

So a culture medium must contain:

Since microorganisms show a considerable variation in their nutritional requirements, no single mediumis suitable for growth of all of them.

The different types of culture media employed fall into three groups:

1. Defined or synthetic media:

These are the media prepared from chemical compounds. They are highly purified and specific, aninvestigator working in another laboratory can duplicate them.

2. Complex or non-synthetic media:

Media that are prepared from ingredients that have not been precisely defined. It contains hydrolysed proteins and vitamin extracts. This type of medium cannot be duplicated by another worker in another laboratory. Peptone is usually produced by boiling beef, by the hydrolysis of its protein. Casein peptone and milk peptone are also used in complex media as the source of amino acids and nitrogen.

All liquid media, whether complex or synthetic may be converted to solid media by adding either gelat in (a protein) or agar-agar, (a complex polysaccharide) extracted from red marine algae. The use of agar has an advantage. The most bacteria are unable to hydrolyze this molecule into more simple components. Since gelatin is a liquid at room temperature, the use agar allows the medium to remain in a solid form while microbes are growing on its surface.

3. Living cells:

These are used for the cultivation of viruses. For example, fertilized eggs incubated for 8 to 12 days are able to support the growth of many viruses.

In another classification culture media are grouped into following four types:

1. Natural media:

Includes substances occurring in nature, as 1) Milk 2) Eggs 3) Blood 4) Extract of plant and animaltissues.

2. Derived media:

Includes known substances but the chemical composition of each is unknown. Examples are 1. Nutrient broth (prepared by boiling beef to extract nutrients and adding an amino acid-nitrogen source.) 2. Nutrientagar 3. Nutrient gelatin.

3. Chemically defined media:

Exact chemical composition known.

4. Special media:

Include combinations of the other three types of media.

There are four categories of media used laboratory:They are:

1. Enrichment
2. Selective
3. Differential and
4. Propagation.

1. Enrichment media:

They are prepared with ingredients that will enhance the growth of certain microbes. Enrichment media encourage the growth of the suspected pathogen so that it will become the most pre-dominant type of microbe in the culture. Two types of enrichment media are blood agar and chocolate agar.

2. Selective media:

They are prepared with ingredients that inhibit the growth of unwanted microbes which might be in the specimen. The inhibitor may be an antibiotic, salt or other chemical. Mixed culture of microbes originally grown in enrichment media may be inoculated into selective media to isolate the desired microbe.

3. Differential media:

They are designed to differentiate among microbes. Different bacterial species may produce dissimilar colony colours when grown on differential agar. While in differential broth cultures, the media change colour. Differential media are used to confirm the identity of a microbe that has already been isolated by culturing in enrichment and selective media.

4. Propagation media:

They are used to propagate, or keep microbes growing for a long lime. Samples grown on these media may be taken for analysis. The most common propagation media are nutrient broth and agar.

Preparation of Media:

There are three main steps in the preparation of media:

(a) Preparation as solutions of chemicals and adjusting the pH.

(b) Dispensing the media, and

(c) Sterilization.

A broth is prepared by dissolving the appropriate amount of the components in distilled water and pH is adjusted by the addition of either dilute NaOH or Hcl. The broth is dispensed into clean rimless 'Pyrex' test tubes which are plugged with non-absorbant cotton wool plugs. The test tubes are placed in wire baskets which are covered with grease proof paper.

The media are sterilized by autoclaving at a temperature of 121 °C and a pressure of 151 b/in^2 for 15 minutes. But medium containing heat- sensitive substances are sterilized either by filtering the solution at room temperature, using bacteria-proof filter or by a process called Tyndallization.

In this method, the liquids are steamed for one hour a day on three consecutive days and the liquids are incubated at 25-30°C. During the first steaming, all the heat sensitive vegetative cells are killed, leaving only the spores. During the first incubation period, most of the spores germinate in to vegetative cells. These vegetative cells are killed by the second steam period.

In the second incubation period, the rest of the spores germinate into vegetative cells which are killed by the third steaming period. In this way, the liquids are sterilized without temperature rising above 100°C.

Maintenance of Pure Culture:

After obtaining the pure culture of a particular microbe, it may be grown and maintained as a pureculture in different ways:

1. The most common practice is to grow the culture on suitable medium until it reaches the stationary phase of growth, and then store in a refrigerator. If they are to be kept alive for a long period all culture must be transferred to a fresh sterile medium. Thus by successive transfer, a culture may be kept for an indefinite period.

2. A second method involves freezing of young culture and desiccating it under vaccum. The cells of a pure culture will remain viable for a long period of time if they are mixed with sterile blood serum, sterileskimmed milk, before freezing and drying. They dried cultures are kept in the sealed, evacuated tubes andare stored in cool places.

3. This method of maintaining pure culture is most suitable for spore forming species.

The microorganisms are grown in pure culture in suitable media. A suspension of microorganisms is then transferred to cotton stoppered tubes of sterilized dry soil. The spores remain viable, though dormant, for long periods of time, in dry soil. The organism can be grown after a desired period, by transferring thesoil into a suitable medium and incubating it under suitable temperature.

PRACTICAL 7: ISOLATION OF RHIZOBIUM FROM LEGUME ROOT NODULES

Rhizobium Medium is used for cultivation and isolation of Rhizobium species.

Composition**

Ingredients Gms / Litre

Mannitol 10.000

Dipotassium phosphate 0.500

Magnesium sulphate 0.200

Yeast extract 1.000

Sodium chloride 0.100

Agar 20.000

Final pH (at 25°C) 6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.8 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle and Interpretation

Rhizobium Medium is used in the large scale production of legumes and in their isolation from root nodules.

Rhizobium Medium is recommended for isolation and cultivation of mannitol-positive Rhizobium species. It is also useful for the maintenance of Rhizobium species by adding extra 1% mannitol to the medium as specified by the American Type Culture Collection (1).

The medium is well buffered for pH changes and osmotic changes by presence of phosphate

and sodium chloride salts. Yeast extract provides nitrogenous nutrients. Mannitol is the energy source while magnesium sulphate provides essential ions. The inocula are transferred from agar slants into starter flasks containing Rhizobium Medium. After 4 days of growth, the culture from starter flasks is transferred into a small seed tank fermentor. At this stage, Rhizobium Medium is used for large scale production. Rhizobium may be isolated from the root system of the leguminous plant. The healthy, pinkish nodule on the tap root is carefully cut out. The nodule is surface sterilized for 5 minutes and then washed with solvents like ethanol etc. The nodule is then crushed with a sterile glass rod in a small aliquot of sterile water. Serial dilutions are subsequently made to get sparse and distinct colonies. The dilutions are plated on Rhizobium Medium and incubated for upto 4 days at 25-30°C (2).

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.18% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

M408: Cultural characteristics observed after an incubation at 25-30°C for upto 4 days.

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period onthe label.

PRACTICAL 8: ISOLATION OF AZOTOBACTER FROM SOIL

Broth (Mannitol) M1722

Azotobacter Broth (Mannitol) is recommended for cultivation of mannitol positive *Azotobacter* speciesfrom soil.

Composition Ingredients Gms / Litre**

Dipotassium phosphate 1.000

Magnesium sulphate 0.200

Sodium chloride 0.200 Ferrous sulphate TRACESoil extract 5.000

Mannitol 20.000

Final pH (at 25°C) 8.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26.4 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Slight precipitate may occur after autoclaving, however it will not interfere with growth performance nor interfere with the interpretation of results.

Principle and Interpretation

Azotobacter is a free-living nitrogen-fixing bacterium (3), which is used as a biofertilizer in the cultivation of most crops. *Azotobacter* is found on neutral to alkaline soils, in aquatic environments, in the plant rhizosphere and phyllosphere. *Azotobacter* species are gram-negative aerobic soil-dwelling bacteria and are usually motile, oval, or spherical bacteria, form thick-walled cysts, and may produce large quantities of capsular slime. They are typically polymorphic, i.e. of different sizes and shapes. Their size of the cells ranges from 2-10 µm long and 1-2 µm wide. Plant needs nitrogen for its growth and *Azotobacter*fixes atmospheric nitrogen non-symbiotically. Therefore, all plants, trees, vegetables, get benefited. Beyond *Azotobacter*is used as a model it has biotechnological applications like use for alginate production and for nitrogen production in batch fermentations. This medium contains necessary nutrients for growth of *Azotobacter* species. For cultivation of mannitol positive *Azotobacter* species from soil, Azotobacter broth (Mannitol) can be used (1). It is used for cultivation of mannitol positive *Azotobacter* species from soil. It can also be

useful for maintenance of *Azotobacter* species by adding extra 1% Mannitol to the medium containing agar i.e solid media as specified by the American Type Culture Collection (2).

Quality ControlAppearance

White to Cream homogeneous free flowing powder

Colour and Clarity of Prepared medium

Colourless to off-white coloured clear to slightly opalescent solution with slight precipitate forms intubes

Reaction

Reaction of 2.64% w/v aqueous solution at 25°C. pH : 8.3±0.2

pH

8.10-8.50

Cultural Response

Cultural characteristics observed after an incubation at 25-30°C for 24-48 hours.

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry dateon the label.

PRACTICAL 9: ISOLATION OF AZOSPIRRILLUM FROM ROOTS.

Azospirillum Medium w/ 0.17% Agar (Twin Pack) M518Intended use

Azospirillum Medium with 0.17% Agar is used for the cultivation of *Azospirillum* species.

Composition Ingredients Gms / Litre**Part A -

Malic acid 5.000

Dipotassium hydrogen phosphate 0.500Ferrous sulphate 0.500

Manganese sulphate 0.010

Magnesium sulphate 0.200

Sodium chloride 0.100 Bromo thymol blue 0.002Sodium molybdate 0.002

Calcium chloride 0.020

Agar 1.750Part B -

Potassium hydroxide 4.000Final pH (at 25°C) 6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 8.08 grams of dehydrated Part A in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add required quantity of Potassium hydroxide (Part B) dissolved in 50 ml of sterile distilled water to obtain pH of 6.8±0.2. As per standard it is recommended to use 4.000 grams of Potassium hydroxide (Part B)

Principle And Interpretation

Azospirillum species occur as free-living in soil or in association with the roots of cereal crops, grassesand tuber plants

(1). *Azospirillum* species are plant-associated diazotrophs of the alpha subclass of *Proteobacteria*. Azospirillum Medium with 0.17% Agar is used for cultivation of *Azospirillum* species. Malic acid is used as the carbon source. *Azospirillum* species grow well in presence of Malic acid and are not overgrown by other nitrogen fixers. Dipotassium phosphate providesbuffering effect and other inorganic salt ingredients provide necessary growth nutrients. Agar at 0.17% concentrations provides microaerophillic conditions necessary for nitrogen fixation by *Azospirillum* species (1).

Type of specimen

Soil samples: For soil samples, follow appropriate techniques for sample collection, processing (1) After use, contaminated materials must be sterilized by autoclaving before discarding.

Specimen Collection and Handling:

Warning and Precautions:

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets

Limitations:

Further biochemical tests must be carried out for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within therecommended temperature.

Quality ControlAppearance

Part A: Cream to yellow homogeneous free flowing powder Part B :White to cream pellets

Gelling

Semisolid, comparable with 0.17 % Agar gel.

Colour and Clarity of prepared medium

Light yellow to pale green coloured clear to slightly opalescent solution.

Reaction

Reaction of 0.81% w/v aqueous solution (containing KOH) at 25°C pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

M518: Cultural characteristics observed after an incubation at 30°C for upto 8 days.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry.

PRACTICAL 10: ISOLATION OF BLUE GREEN Algae (BGA) Cyanophycean Agar M699

Cyanophycean Agar is used for the isolation and cultivation of Blue green Algae.

Composition Ingredients Gms / Litre**Potassium nitrate 5.000

Dipotassium phosphate 0.200

Magnesium sulphate 0.100

Agar 15.000

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 20.3 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 3 minutes. Cool to 45°C and aseptically add one drop of 1% separately autoclaved solution of ferrous ammonium citrate to 100 ml sterile medium. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Blue green algae are a type of photosynthetic bacteria, called *Cyanobacteria* that rely on sunlight for energy. They are present in almost all aquatic ecosystems, including creeks, rivers, lakes and wetlands. Algal blooms can cover large areas of a water supply. Like all photosynthetic organisms, blue-green algaerely on sunlight for energy, with their growth rate determined by the level of nutrients available in the water. Cyanophycean Agar is used for the isolation and cultivation of blue green algae. Potassium is required for maintenance of maximum growth rate of blue green algae (1). Nitrate serves as nitrogen source. Dipotassium phosphate buffers the media. Magnesium sulphate is a source of divalent cations. White to cream homogeneous free flowing powder

Quality Control appearance

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Colourless clear to slightly opalescent gel forms in Petri plates

Cultural Response

M699: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry dateon label.

PRACTICAL 11 : GRAM STAINING AND MICROSCOPIC EXAMINATION OF MICROBES.

Materials Required:

1. Clean glass slides
2. Inoculating loop
3. Bunsen burner
4. Bibulous paper
5. Microscope
6. Lens paper and lens cleaner
7. Immersion oil
8. Distilled water
9. 18 to 24 hour cultures of organisms

Reagents:

1. Primary Stain - Crystal Violet
2. Mordant - Grams Iodine
3. Decolourizer - Ethyl Alcohol
4. Secondary Stain - Safranin

Procedure:

Part 1: Preparation of the glass microscopic slide

Grease or oil free slides are essential for the preparation of microbial smears. Grease or oil from the fingers on the slides is removed by washing the slides with soap and water. Wipe the slides with spirit or alcohol. After cleaning, dry the slides and place them on laboratory towels until ready for use.

Part 2: Labeling of the slides

Drawing a circle on the underside of the slide using a glassware-marking pen may be helpful to clearly designate the area in which you will prepare the smear. You may also label the slide with the initials of the name of the organism on the edge of the slide. Care should be taken that the label should not be in contact with the staining reagents.

Part 3: Preparation of the smear

- **Bacterial suspensions in broth:** With a sterile cooled loop, place a loopful of the broth culture on the slide. Spread by means of circular motion of the inoculating loop to about one centimeter in diameter. Excessive spreading may result in disruption of cellular arrangement. A satisfactory smear will allow examination of the typical cellular arrangement and isolated cells.

- **Bacterial plate cultures:** With a sterile cooled loop, place a drop of sterile water or saline solution on theslide. Sterilize and cool the loop again and pick up a very small sample of a bacterial colony and gently stir into the drop of water/saline on the slide to create an emulsion.
- **Swab Samples:** Roll the swab over the cleaned surface of a glass slide.

Please note: It is very important to prevent preparing thick, dense smears which contain an excess of the bacterial sample. A very thick smear diminishes the amount of light that can pass through, thus making it difficult to visualize the morphology of single cells. Smears typically require only a small amount of bacterial culture. An effective smear appears as a thin whitish layer or film after heat-fixing.

Part 4: Heat Fixing

Heat fixing kills the bacteria in the smear, firmly adheres the smear to the slide, and allows the sample to more readily take up stains.

- Allow the smear to air dry.
- After the smear has air-dried, hold the slide at one end and pass the entire slide through the flame of a Bunsen burner two to three times with the smear-side up.

Now the smear is ready to be stained.

Please Note: Take care to prevent overheating the slide because proteins in the specimen can coagulate causing cellular morphology to appear distorted.

Part 5: Gram Stain Procedure

1. Place slide with heat fixed smear on staining tray.

2. Gently flood smear with crystal violet and let stand for 1 minute.

3. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
4. Gently flood the smear with Gram's iodine and let stand for 1 minute.
5. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle. The smear willappearas a purple circle on the slide.
6. Decolorize using 95% ethyl alcohol or acetone. Tilt the slide slightly and apply the alcohol drop by dropfor 5 to 10 seconds until the alcohol runs almost clear.
7. Immediately rinse with water.
8. Gently flood with safran in to counter-stain and let stand for 45 seconds.
9. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
10. Blot dry the slide with bibulous paper.

SOIL AND WATER CONSERVATION ENGINEERING, AGS-203

PRACTICAL – 1

GENERAL STATUS OF SOIL CONSERVATION IN INDIA

Soil degradation in India is estimated to be occurring on **147 million** hectares of land which includes;

- **94 Mha** from water erosion, **16 Mha** from acidification,
- **14 Mha** from flooding,
- **9 Mha** from wind erosion,
- **6 Mha** from salinity, and
- **7 Mha** from a combination of factors.

The causes of soil degradation are both natural and human-induced. Natural causes include earthquakes, tsunamis, droughts, avalanches, landslides, volcanic eruptions, floods, tornadoes, and wildfires. Human-induced soil degradation results from land clearing and deforestation, inappropriate agricultural practices, improper management of industrial effluents and wastes, over-grazing, careless management of forests, surface mining, urban

sprawl, and commercial/industrial development. Inappropriate agricultural practices include excessive tillage and use of heavy machinery, excessive and unbalanced use of inorganic fertilizers, poor irrigation and water management techniques, pesticide overuse, inadequate crop residue and/or organic carbon inputs, and poor crop cycle planning. Some underlying social causes of soil degradation in India are land shortage, decline in per capita land availability, economic pressure on land, land tenancy, poverty, and population increase.

Effects of Soil Erosion in India

- Soil erosion results in huge loss of nutrients in suspension or solution, which are washed away from one place to another, thus causing depletion or enrichment ofnutrients.
- Besides the loss of nutrients from the topsoil,
- There is also degradation through the creation of gullies and ravines.
- Water causes sheet-wash, surface gullies, tunnels and scours banks in rivers.
- In hot and dry climate of India, wind blowing is the main cause of soil erosion.

Indian government is adopting adequate measures to reduce the unpleasant effects of soil erosion in India particularly in the states like Punjab, Maharashtra.

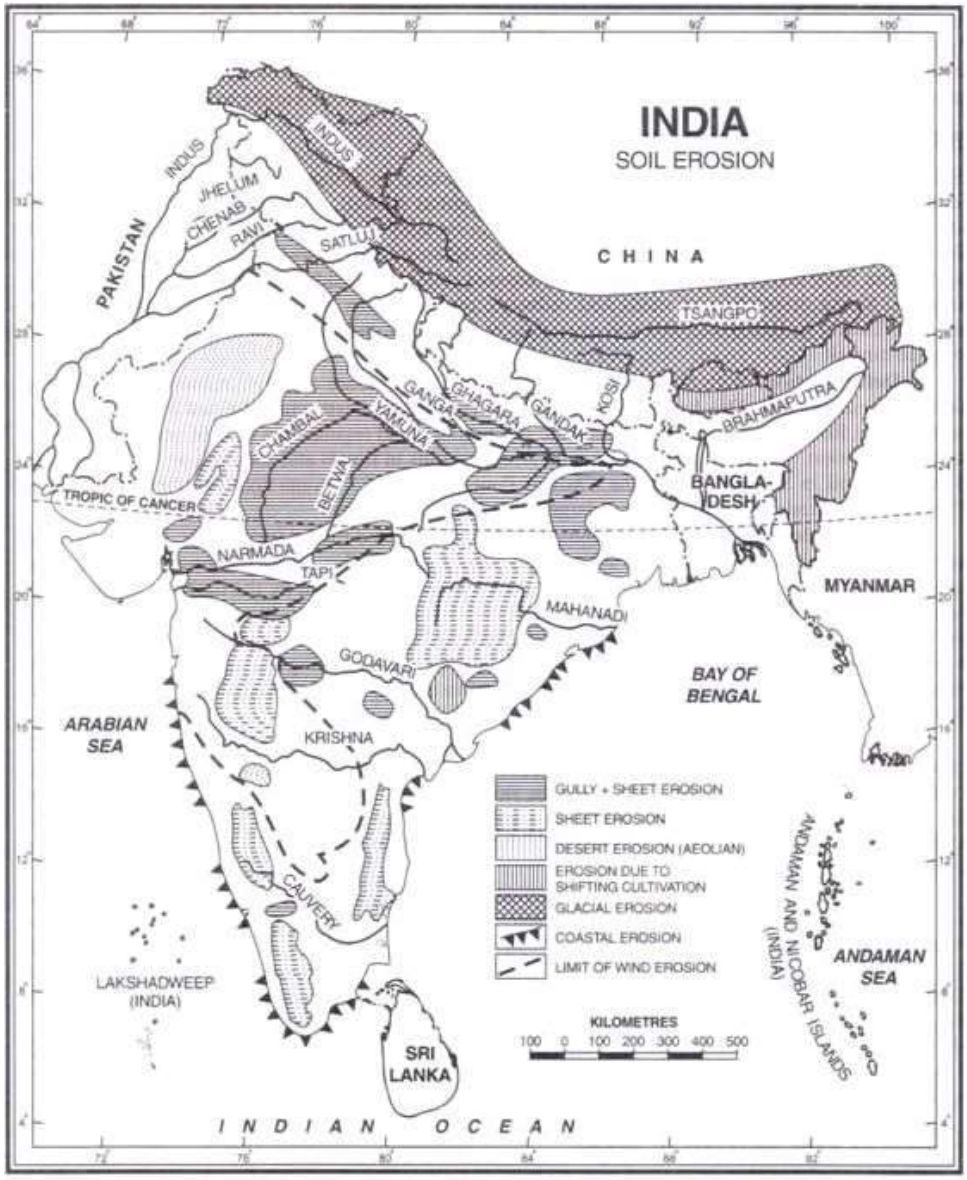


FIG. 7.2. India : Soil Erosion

Introduction to Soil Conservation

Soil and water conservation is essential to protect the productive lands of the world. In our country, where droughts, famines and floods cause crop damage almost every year, soil conservation will not only increase crop yields but also prevent floods and further deterioration of land.

Prior to the days of independence, while general problems of soil erosion were known, answers to them backed by scientific investigations were not known. Consequently, during the framing of the first year plan and early in the second five year plan, a chain of 9 Soil Conservation Research Demonstration and TrainingCentres were established

Table 12.1

Central Soil and Water Conservation Research Demonstration and Training Centres in India (Tejwani, 1980)

Location	Region/major problems	Date of start
(1)	(2)	(3)
Dehradun	North-western Himalayan region. Erosion control in Himalayas; training of torrents; stabilisation of landslides; development of techniques for crop production; establishment of pastures and forestry.	28.9.1954
Chandigarh	Sub-montane tracts in the north-western region of India with special reference to Shiwalik hills. Erosion control in Shiwaliks; training of chos.	28.9.1954
Ootacamund	Southern hill high rainfall region/soil and water conservation in the Nilgiris hills; development of techniques for crop production; establishment of pastures and forestry.	10.10.1954
(1)	(2)	(3)
Bellary	Semi-arid black soil region. Soil and water conservation in the black soil region.	20.10.1954
Kota	Along the Chambal river in Rajasthan. Ravine problem on the banks of Chambal river and its tributaries; survey, classification and reclamation of ravines for forage production and forestry.	19.10.1954
Vasad	Along the rivers of Gujarat State. Ravine problem specifically along the banks of Mahi river system; survey, classification and reclamation of ravines forage production and forestry.	11.5.1955
Agra	Along the Yamuna river and its tributaries. Ravine problems specifically on the banks of Yamuna river; survey, classification and reclamation of ravines for forage production and forestry.	22.7.1955
Hyderabad	Red soil, semi-arid region. Soil and water conservation in the red soils under low to medium rainfall regions.	10.1.1962
Chattra	North-eastern Himalayan region. Erosion control in the Kosi river catchment.	19.12.1956

Broad objectives of these Centre's are:

(i) To identify erosion problems and conservation of land and water resources under differentland use systems,

(ii) To evolve mechanical and biological methods of erosion control under different land usesystems,

(iii) To evolve methods of control of erosion and reclamation of ravines stabilization oflandslides and hill torrents,

(iv) To evaluate hydrological behavior and evolve techniques of watershed managementunder different systems.

(v) To set up demonstration projects for popularizing soil and water conservation measures,

(vi) To impart specialized training in soil and water conservation to gazetted and non-gazetted officers of State Governments.

Important Soil and Water Conservation Programmes implemented by Govt.

- Soil conservation in catchments of river valley project (RVP).
- Integrated Watershed Management in the catchments of flood Prone Rivers (FPR).
- Centrally Aided Drought Prone Area Development Program (DPAP), (as per 1995 guidelines) implemented by Government and NGO. Desert Development Programme(DDP)
- National Watershed Development Program for Rain fed Area (NWDPRA) implemented by Dept. of Soil Conservation & Watershed Management, Gov.M with financial support from Department of Agriculture, Gov.I
- Operational Research Projects on Integrated Watershed Management (ICAR)
- World Bank Project on Watershed Development in Rain fed Area.
- Council for Peoples Action & Rural Technology (CAPART) supported 38 WatershedDevelopment Programs in Maharashtra.
- DPAP & IWDP projects (of 2001 guidelines) in Satara, Sangali& Nasik Districts ofMaharashtra state.
- NABARD Holistic Watershed Development Programme
- Vasundhara Watershed Development Project
- Maharashtra government has launched a new programme named 'JalyuktaShivarAbhiyan' in a state on January 26, 2015. The programme aim to make 5000 villages free of water scarcity every year and to conserve and protect the soil from further degradation. This Abhiyan aims at initiating permanent measures to make the state drought free by 2019 and to harvest rain water within the village boundary thereby increasing ground water levels.

PRACTICAL – 2

CALCULATION OF EROSION INDEX BY EI_{30} METHOD

Topic: Calculation of erosion index by EI_{30} method

Objective: From this study students are able to know about EI_{30} index method and its calculation procedure.

EI_{30} Index Method

This method was introduced by Wischemier (1965). It is based on the fact that the product of kinetic energy of the storm and the 30-minute maximum rainfall intensity gives the best estimation of soil loss. The greatest average intensity experienced in any 30 minutes period during the storm is computed from recording rain gauge charts by locating the maximum amount of rain which falls in 30 minutes period and later converting the same to intensity in mm/hour. This measure of erosivity is referred to as the EI_{30} index and can be computed for individual storms, and the storm values can be added over periods of time to give weekly, monthly or yearly values of erosivity.

Wischemier (1965) gave following equation for calculation of kinetic energy (E)

$$E = 916 + 331 log_{10} I \quad (1)$$

Here, *E* is the kinetic energy in ft-tonnes/acre and *I* is the rainfall intensity in inch/ hour.

or

$$E = 210.3 + 89 log_{10} I \quad (2)$$

Here, *E* is the kinetic energy in m-tonnes/ha-cm and *I* is the rainfall intensity in cm/ hour.

or

Wischemier and Smith (1978) gave the following equations for the calculation of Kinetic energy (E)

$$E = 0.119 + 0.0873\ log_{10} I \quad \text{for } I \leq 76 \quad (3)$$

$$E = 0.283 \quad \text{for } I > 76 \quad (4)$$

Where, E= Kinetic energy in megajoules/hectare mm, and I is the rainfall intensity in mm/h.

Limitation of Method

The EI_{30} index method was developed under American condition and is not found suitable for tropical and sub-tropical zones for estimating the erosivity.

Calculation Procedure: Table 1 illustrates the determination of EI directly from rainfall data.

1. The rainfall hyetograph is divided into increments where intensity is assumed to be uniform (Column 1). Cumulative rainfall amounts are given in column (2). Duration, Amount, and Intensity for the increments are shown in column (3), (4) and (5).
2. Unit energy for each increment is shown in column (6). These values are obtained by substituting intensity for the increment (column 5) into Eq. 3 and 4 (in this example).
3. Rainfall energy for an increment (column 7) is the product of energy (column 6) and the volume of rainfall increment (column 4).
4. Total energy for the storm is the sum of the energies for each increment (column 7) or

8.64 MJ/ha in this case.

5. Maximum 30- minutes intensity, $I_{30} = 2 \times (6 + 18 + 3) = 54\, mm/h$.

6. Therefore, EI_{30} for this storm is $EI_{30} = 8.64 \times 54 = 466.56$ MJ-mm/ha h.

Table 1. Example computation of energy for a rainstorm

Chart Readings		**Storm Increments**			**Energy**	
Time	Depth (mm)	Duration (min)	Amount (mm)	Intensity (mm/h)	Per Unit Rainfall (MJ/ha-mm)	For Storm Increment (MJ/ha)
(1)	(2)	(3)	(4)	(5)	(6)	(7)
4 : 00	0					
: 20	1	20	1	3	0.161	0.16
: 27	3	7	2	17	0.226	0.45
: 36	9	9	6	40	0.259	1.55
: 50	27	14	18	77	0.283	5.09
: 57	30	7	3	26	0.243	0.73
5 : 05	32	8	2	15	0.222	0.44

: 15	32	10	0	0	0	0
: 30	33	15	1	4	0.219	0.22
Totals		90	33			8.64

Conclusion: EI_{30} for this storm is 8.64 × 54 = 466.56 MJ-mm/ha h.

Exercise: CalculateEI_{30} for different storm/ or changing values of depth, duration amount and intensity.

References:

1. Land and Water Management Engineering by V. V. N. Murthy and Madan K. Jha, KalyaniPublishers (6th edition). pp- 434.

2. Principles of Agricultural Engineering, Vol. II, by A. M. Michael and T. P. Ojha, JainBrothers Publication. pp- 524.

3. Fundamentals of soil and water conservation engineering by R. Suresh, Standard Publishers Distributors. pp: 334-336.

PRACTICAL – 3 ESTIMATION OF SOIL LOSS (USLE)

The universal soil loss equation (USLE) developed by Wischmeier & Meyer; & the same was published in the year 1973 by Wischmeier & Meyer.

This equation was designated as Universal Soil Loss Equation, and in brief it is now as USLE. Since, simple & powerful, tool for predicting the average annual soil loss in specific situations. The associated factors of equation can be predicted by easily available meteorological & soil data.

The term 'Universal' refers consideration of all possible factors affecting the soil erosion/soil loss; and also its general applicability.

The USLE is given as under:

A = R. K. LS. C. P.

Where,

A = computed soil loss, expressed in t/ha/y for a given storm event.

R = rainfall erosivity factor, which is the measurement of the kinetic energy of a specific rain event or an average year's rainfall.

K = soil erodibility factor. It is the soil loss rate per erosion index unit for a given soil as

measured on a unit plot. (22.1 m long with 9 % slope in continuous clean –tilled fallow)

L = slope length factor. It is the ratio of soil loss from the field plot under existing slope length to that from the 22.1 m slope length (Unit plot) under identical conditions.

S = slope gradient factor. It is the ratio of soil loss from the field slope gradient to that from the 9% slope (unit plot) under identical conditions.

C = cover or crop rotation (management) factor. It is the ratio of soil loss from the area under specified cover and management to that from an identical area is tilled continuous fallow (unit plot).

P = erosion control practices or soil conservation practices factor. It is the ratio of soil loss under a support practice like contouring, strip cropping or terracing to that under straight – row farming up and down the slope.

Rainfall Erosivity Factor (R):

It refers to the rainfall erosivity index, which expresses the ability of rainfall to erode the soil particles from an unprotected field. It is a numeral value. From long term fieldstudies, it has been observed that the extent of soil loss from a barren field is directly proportional to the product of two rainfall characteristics: 1) kinetic energy of the storm; and

2) its 30- minute maximum intensity.

Soil Erodibility Factor (K):

This factor is related to the various soil properties, by virtue of which a particular soil becomes susceptible to get erode, either by water or wind. Physical characteristics of the soil greatly influence the rate at which different soils are eroded. In general, the soil properties such as the soil permeability, infiltration rate, soil texture, size & stability of soil structure, organic content and soil depth, affect the soil loss in large extent.

Slope Length and Steepness Factor (LS):

The LS factor represents the erosive potential of a particular soil with specified slope length and slope steepness. This factor basically affects the transportation of the detached particles due to surface flow of rainwater, either that is the overland flow or surface runoff. And accordingly affects the value of soil erosion due to any given rainfall. The capability of runoff/overland flow to detach and transport the soil materials gets increased rapidly with increase in flow velocity. On steep ground surface the runoff gets increase because of increase in runoff rate. The factors- L and –S are described as under:

Slope Length Factor (L):

The slope length is the horizontal distance from the point of origin of overland flow to the point where either the slope gradient gets decrease enough to start deposition or overland flow gets concentrate in a defined channel.

Slope Steepness Factor (S):

Steepness of land slope influences the soil erosion in several ways. In general, as the steepness of slope increases the soil erosion also increases, because the velocity of runoff gets increase with increase in field slope, which allows more soil to detach and transport them along with surface flow.

Crop Management Practices Factor (C):

The crop management practices factor (C) may be defined as the ratio of soil lossfrom a land under specific crop to the soil loss from a continuous fallow land, provided that the soil type, slope & rainfall conditions are identical. The crop & cropping practices affect the soil erosion in several ways by the various features such as the kind of crop, quality of cover, root growth, water use by growing plants etc.

Soil Conservation Practices Factor (P):

It may be defined as the ratio of soil loss under a given conservation practice to the soil loss from up and down the slope. The conservation practice consists of mainly the contouring, terracing and strip cropping in which contouring appears to be most effective practice on medium slopes ranging from 2 to 7 per cent.

Example 1: Calculate the annual soil loss from a given field subject to soil erosion problem, for the following information:

- Rainfall erosivity index = 1000 m.tonnes/ha
- Soil erodibility index = 0.20
- Crop management factor = 0.50
- Conservation practices factor = 1.0
- Slope length factor = 0.10

Also explain, how the soil loss is affected by soil conservation practices.

Example 2: A field is cultivated on the contour for growing maize crop. The other details regarding USLE factors are as follows:

K = 0.40

R = 175 t/acreLS = 0.70

P = 0.55

C = 0.50

Compute the value of soil loss likely to take place from the field. Also, make a comment on soil loss when same field is kept under continuous pasture with 95 percent cover. Assume the value of factor- C for new crop is 0.003.

PRACTICAL – 4

MEASUREMENT OF SOIL LOSS (MULTI SLOT DIVISOR)

Object: Measurement of soil loss from the field by using multi slot divisor.

Multi slot divisor is useful for measuring runoff from small plots. It can measure quantity of runoff and can estimate soil loss from field. Its design and application is very simple. Mostly used for experiment purpose. It has mainly three parts:

- Collection tank
- Slot divisor (spout)
- Cistern tank

1. Collection tank:

The collection tank is used to collect the runoff water from the plot. The water is diverted from the plot and discharged into the collection tank. The tank has four compartments of different dimensions. The dimension of the collection tank varies according to the size of the plot and probable runoff to be collected. The probable runoff is calculated considering the plot size and maximum daily rainfall of the area. The collection tank is provided with roof cap to avoid rain water falling into the tank. The tank is provided with a provision to attach slot divisor.

2. Slot Divisor:

The slot divisor with number of slots is used for experimentation, in which one slot is connected to the cistern tank. The divisor is always provided with the odd number of slots. The number of slot are decided as per the volume of water is to collected from the experimental plot. Larger the quantity of runoff, more are the slots and vice versa. It is also covered with cap on its top. The middle slot connected to the cistern tank, to collect excess runoff.

3. Cistern tank:

It is the tank connected to the slot divisor to collect the runoff water for final measurement. The capacity of the cistern tank is decided as per the probable runoff and number of slots.

Procedure:

1. Select the particular field from where soil loss is to be measured.
2. Generally, the dimensions of the field are selected as 15 x 4 m.
3. Mark the plot boundary by erecting GI sheets along the boundary of plot such that norunoff water will enter into the experimental field.
4. The runoff collection channel is constructed to divert the runoff water towards collectiontank.
5. Pipe is used to convey the runoff water into the tank.
6. At the end of the plot pit is excavated to install the multi slot assembly to collect runoffwater and runoff samples.

Calculation of runoff volume and soil loss:

Runoff volume: The runoff water collected in the Cistern tank is measured by using following formula

Where, V = volume of runoff water, m^3r = radius of cistern tank, m

h = height of tank, m

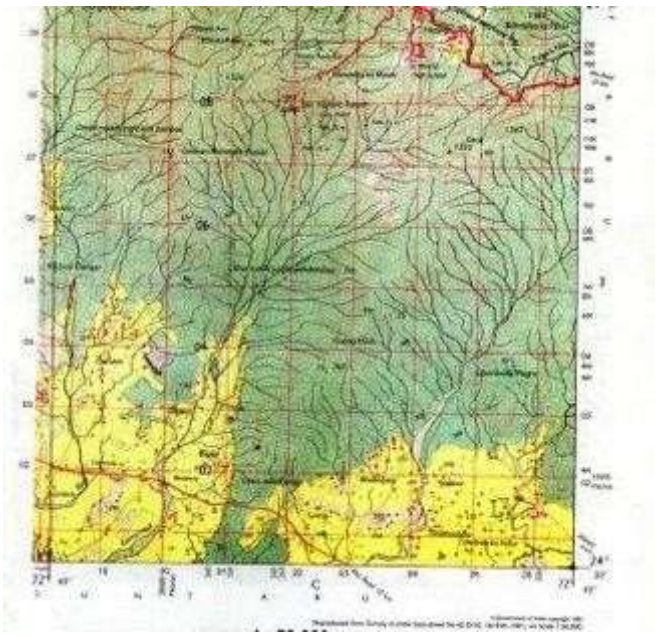
This is the volume of runoff water collected through one slot. Convert it into total volume of water collected from the plot considering the number of slots of the divisor. Then, calculate total volume of runoff water collected from one hector of land.

Soil loss:

1. The runoff samples are collected from the collection tank in the bottles with continuousstirring of water.
2. Add alum to the water samples to allow the settlement of sediment in the sample bottles.
3. Keep it for 24 hrs for settlement.
4. Remove water from bottles.
5. Keep the soil/sediment for 24 hrs at 105 ^{0}C in oven dryer.
6. Then take dry weight of soil.
7. Calculate the soil loss in kg per hector.

PRACTICAL – 5 PREPARATION OF COUNTER MAPS

What are contour lines? Contour lines connect a series of points of equal elevationand are used to illustrate relief on a map. They show the height of ground above mean sea level (MSL) either in metres or feet, and can be drawn at any desired interval. For example, numerous contour lines that are close to one another indicate hilly or mountainous terrain; when further apart they indicate a gentler slope; and when far apart they indicate flat terrain.



Purposes of Contouring Contour survey is carried out at the starting of any engineering project such as a road, a railway, a canal, a dam, a building etc.

1. For preparing contour maps in order to select the most economical or suitable site.

2. To locate the alignment of a canal so that it should follow a ridge line.

3. To mark the alignment of roads and railways so that the quantity of earthwork both incutting and filling should be minimum.

4. For getting information about the ground whether it is flat, undulating or mountainous.

5. To locate the physical features of the ground such as a pond depression, hill, steep or smallslopes.

Contour Interval & Horizontal Equivalent Contour Interval: The constant vertical distance

between two consecutive contours is called the contour interval. Horizontal Equivalent: The horizontal distance between any two adjacent contours is called as horizontal

equivalent. The contour interval is constant between the consecutive contours while the horizontal equivalent is variable and depends upon the slope of the ground.

Characteristics of Contours

1. All points in a contour line have the same elevation.

2. Flat ground is indicated where the contours are widely separated and steep-slope where they run close together.

3. A uniform slope is indicated when the contour lines are uniformly spaced and

4. A plane surface when they are straight, parallel and equally spaced.

5. A series of closed contour lines on the map represent a hill, if the higher values are inside

6. A series of closed contour lines on the map indicate a depression if the higher values are outside.

7. Contour line cross ridge or valley line at right angles. If the higher values are inside the bend or loop in the contour, it indicates a Ridge. If the higher values are outside the bend, it represents a Valley.

8. Contour lines cannot merge or cross one another on map except in the case of an overhanging cliff

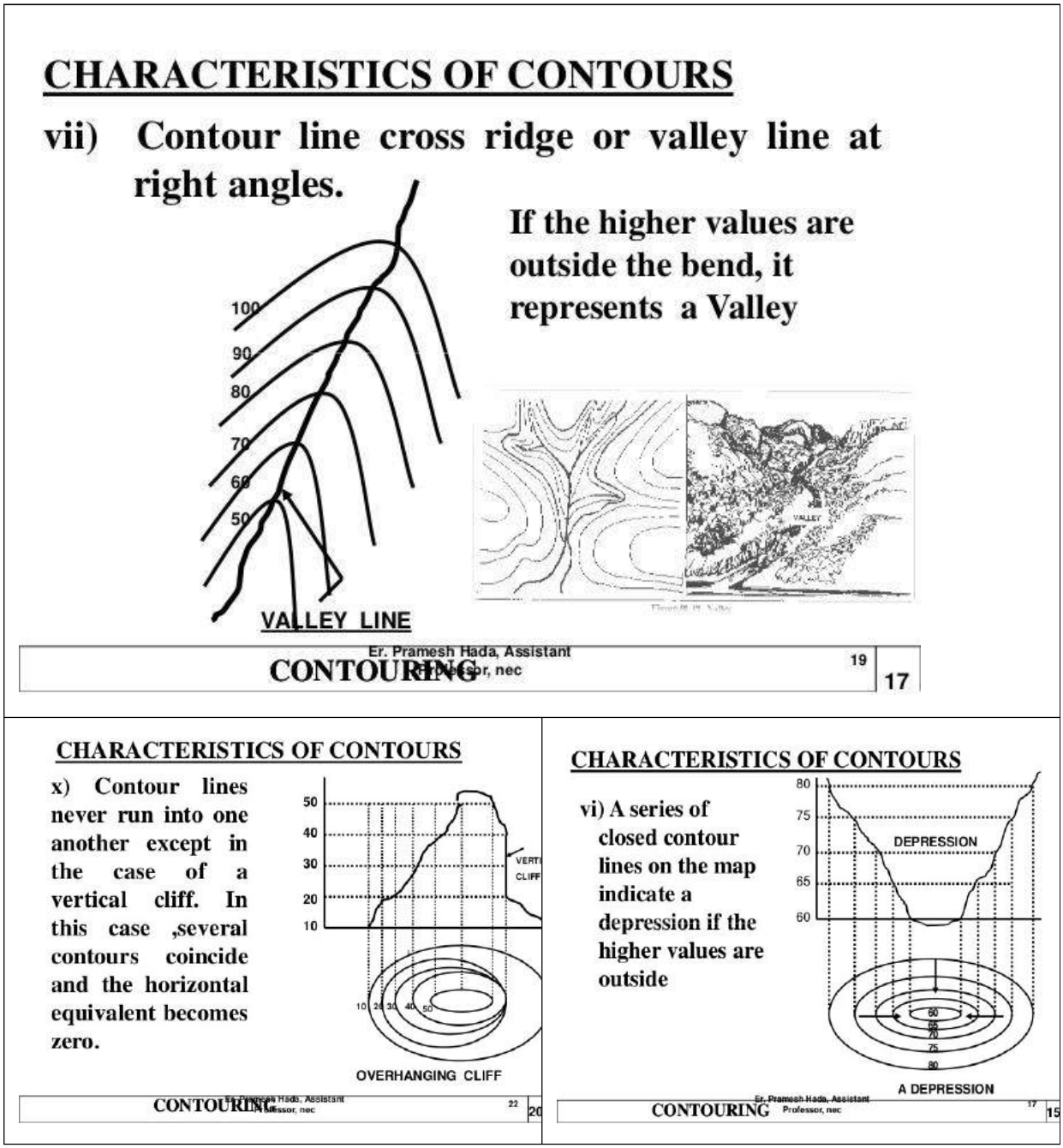
9. Contour lines never run into one another except in the case of a vertical cliff. In this case, several contours coincide and the horizontal equivalent becomes zero.

METHODS OF CONTOURING

There are mainly two methods of locating contours:-

(1) Direct Method: Direct Method: In this method, the contours to be located are directly traced out in the field by locating and marking a number of points on each contour. These points are then surveyed and plotted on plan and the contours drawn through them.

(2) Indirect Method: In this method the points located and surveyed are not necessarily on the contour lines but the spot levels are taken along the series of lines laid out over the area .The spot levels of the several representative points representing hills, depressions, ridge and valley lines and the changes in the slope all over the area to be contoured are also observed. Their positions are then plotted on the plan and the contours drawn by interpolation. This method of contouring is also known as contouring by spot levels.



PRACTICAL – 6

DESIGN OF GRASSED WATERWAYS

Vegetative waterways are natural or constructed waterways shaped to require dimensions and vegetated for safe disposal of runoff from a field, diversion, terrace or other structures. Satisfactory performance of vegetated waterways depends on its having the propershape, as well as the preparation of the area in a manner to provide conditions favourable to vegetation growth. The grass in the waterways should be established before any water turned into it. The velocity in the grassed waterways should be kept within the permissible limit for different types of soil and these limits are presented below table.

Permissible velocity in grassed waterways for different soil types

Type of soil	Maximum permissible velocity (cm/sec)
Sand and silt	45
Loam, sandy loam and silt loam	60
Clay loam	65
Clay	70
Gravelly soil	100

Design

Vegetative waterways are generally designed to carry the maximum runoff from a storm of 10-year recurrence interval. Runoff can be estimated by the Rational Method.

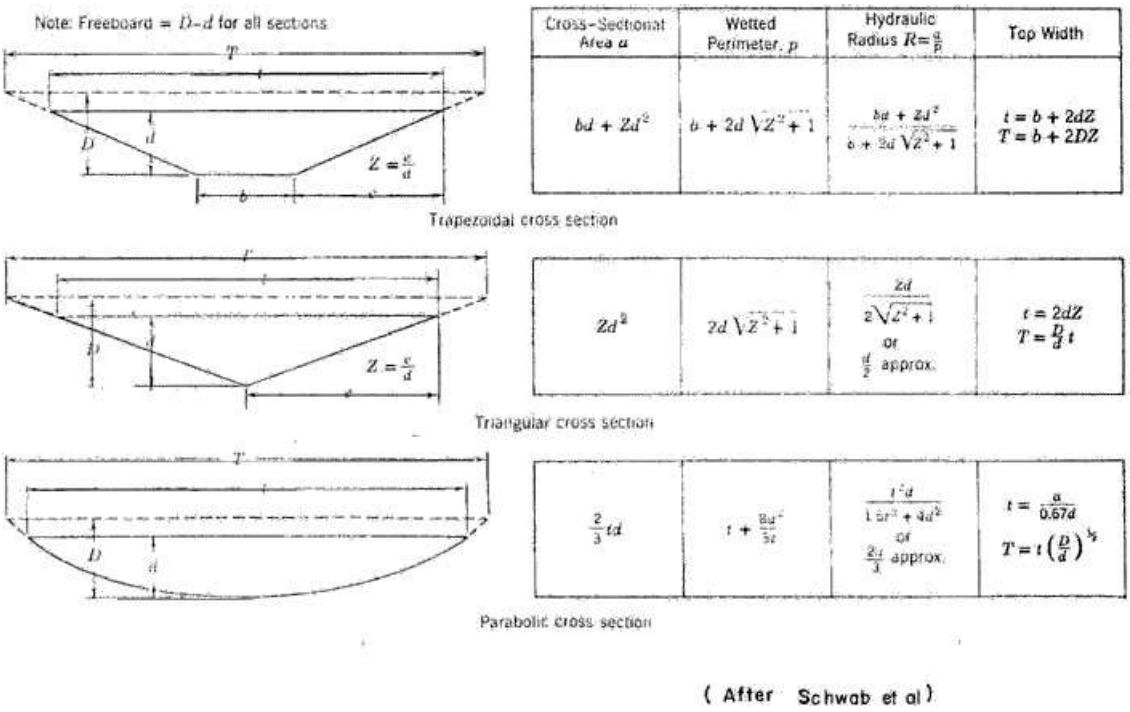
Shape

Vegetated waterways may be built to three general shapes or cross-sections, namely, parabolic, trapezoidal or V-shaped. Parabolic waterways are most common and generally are the most satisfactory. It is the shape ordinarily found in nature. V-shaped channels can be easily constructed with a V-ditcher and trapezoidal channels with a V-ditcher and a buck

scrapper, and hence these sections are preferred constructed channels. Broad-bottom trapezoidal channels require less depth of excavation than parabolic or V-shapes for the same capacity. Thus there are number of factors which govern the selection of shape.

Channel Grades

Grassed waterways generally run down the slope and the channel grade is usually governed by land slope. In any case, channel slope should not exceed 10 % while it is normally desirable to keep the grade within 5%.



Cross-Sectional Area a	Wetted Perimeter, p	Hydraulic Radius $R=\frac{a}{p}$	Top Width
$bd + Zd^2$	$b + 2d\sqrt{Z^2+1}$	$\frac{bd+Zd^2}{b+2d\sqrt{Z^2+1}}$	$t = b + 2dZ$ $T = b + 2DZ$
Zd^2	$2d\sqrt{Z^2+1}$	$\frac{Zd}{2\sqrt{Z^2+1}}$ or $\frac{d}{2}$ approx.	$t = 2dZ$ $T = \frac{D}{d}t$
$\frac{2}{3}td$	$t + \frac{8d^2}{3t}$	$\frac{t^2d}{1.5t^2+4d^2}$ or $\frac{2t}{3}$ approx.	$t = \frac{a}{0.67d}$ $T = t\left(\frac{D}{d}\right)^{\frac{1}{2}}$

(After Schwab et al)

Channel Dimensions

After the runoff, channel grade and design velocity have been determined, the next step is to decide on the channel dimensions. Design of vegetated waterways is based on the Manning's formula. The coefficients of roughness (n) usually assumed in grassed waterways design is 0.04. Side slopes of channel should be 4:1 or flatter to facilitate crossing of farm equipment. A freeboard of 10 - 15 cm should be provided to take care of the sediment deposition and variation in the value of 'n'.

Size of Waterway

The size of the waterway depends upon the expected runoff. A 10 year recurrence interval is used to calculate the maximum expected runoff to the waterway. As the catchment area of the waterway increases towards the outlet, the expected runoff is calculated for different reaches

of the waterway and used for design purposes. The waterway is to be given greater cross-sectional area towards the outlet as the amount of water gradually increases towards the outlet. The cross-sectional area is calculated using the following formula:

$$a = \frac{Q}{V}$$

where, a = cross-sectional area of the channel,Q = expected maximum runoff, and V = velocity of flow.

Shape of Water Way

The shape of the waterway depends upon the field conditions and type of the construction equipment used. The three common shapes adopted are trapezoidal, triangular, and parabolic shapes. In course of time due to flow of water and sediment depositions, the waterways assume an irregular shape nearing the parabolic shape. If the farm machinery has to cross the waterways, parabolic shape or trapezoidal shape with very flat side slopes are preferred.

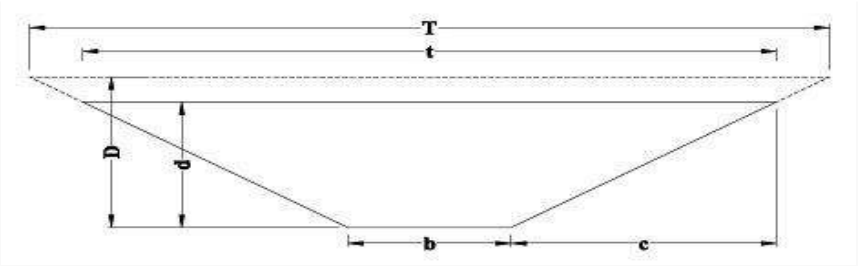


Table Design Dimensions for Trapezoidal Cross-

Cross-sectional Area, a	Wetted perimeter, P	Hydraulic Radius, $R = \frac{a}{p}$	Top width
$bd + zd^2$ Where, Z = c/d	$b + 2d\sqrt{Z^2 + 1}$	$\frac{bd + zd^2}{b + 2d\sqrt{z^2 + 1}}$	T = b + 2dz T = b + 2Dz

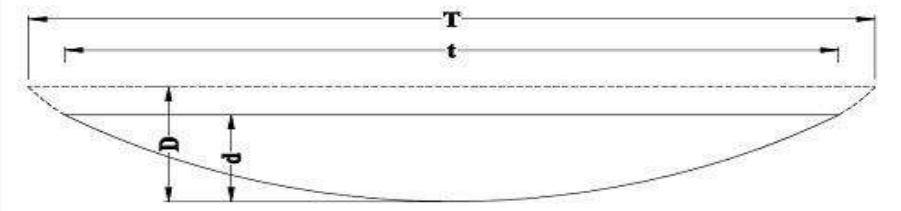


Fig. Parabolic Cross-section.

Cross-sectional Area, a	Wetted perimeter, P	Hydraulic Radius, $R = \frac{a}{p}$	Top width
$\frac{2}{3}td$	$t + \frac{8d^2}{3t}$	$\frac{t^2 \times d}{1.5t^2 + 4d^2}$ $\frac{2d}{3} approx$	$t = \frac{a}{0.67d}$ $T = t\left(\frac{D}{d}\right)^{\frac{1}{2}}$

Design of Cross-Section

The design of the cross-section is done using Equation 27.1 for finding the area required and Manning's formula is used for cross checking the velocity. A trial procedure is adopted. For required cross-sectional area, the dimensions of the channel section are assumed. Using hydraulic property of the assumed section, the average velocity of flow through the channel cross-section is calculated using the Manning's formula as below:

$$V = \frac{S^{1/2} R^{2/3}}{n} \qquad (27.2)$$

where, V = velocity of flow in m/s; S = energy slope in m/m; R = hydraulic mean radius of the section in m and n = Manning's roughness coefficient.

The Manning's roughness coefficient is to be selected depending on the existing and proposed vegetation to be established in the bed of the channel. Velocity is not an independent parameter. It will depend on n which is already fixed according to vegetation, R which is a function of the channel geometry and slope S for uniform flow. Slope S has to be adjusted. If the existing land slope gives high velocity, alignment of the channel has to be changed to get the desired velocity.

Problem : Design a grassed waterway of parabolic shape to carry a flow of 2.6 m^3/s down a slope of 3 percent. The waterway has a good stand of grass and a velocity of 1.75 m/s can be allowed. Assume the value of n in Manning's formula as 0.04.

Solution: Using, Q = AV for a velocity of 1.75 m/s, a cross-section of 2.6/1.75 = 1.485

m2 (~1.5 m2) is needed. Assuming, t = 4 m, d = 60 cm.

$$A = \frac{2}{3}t \times d = \frac{2}{3}4 \times 0.6 = 1.6\,m^2$$

$$P = t + 8\frac{d^2}{3t} = 4 + 8\frac{(0.6)^2}{3 \times 4} = 4.24\,m$$

$$R = \frac{A}{P} = \frac{1.6}{4.24} = 0.377\,m$$

$$V = \frac{S^{1/2}R^{2/3}}{n} = \frac{(0.03)^{1/2} \times (0.377)^{2/3}}{0.04} = 2.26\,m/s$$

$$A = \frac{2}{3}t \times d = \frac{2}{3}4 \times 0.6 = 1.6\,m^2$$

$$P = t + 8\frac{d^2}{3t} = 6 + 8\frac{(0.4)^2}{3 \times 6} = 6.45\,m$$

$$V = \frac{S^{1/2}R^{2/3}}{n} = \frac{(0.03)^{1/2} \times (0.248)^{2/3}}{0.04} = 1.70\,m/s$$

The velocity exceeds the permissible limit. Assuming a revised t = 6 m and d = 0.4 m

The velocity is within the permissible limit.Q = 1.6 × 1.7 = 2.72 m3/s

The carrying capacity (Q) of the waterway is more than the required. Hence, the design of waterway is satisfactory. A suitable freeboard to the depth is to be provided in the final dimensions.

PRACTICAL - 7 DESIGN OF CONTOUR BUNDS

Contour Bunding is the construction of small bund across the slope of the land on a contour so that the long slope is cut into a series of small ones and each contour bund acts asa barrier to the flow of water, thus making the water to walk rather than run, at the same time impounding water against it for increasing soil moisture. Contour bunds divide the length of the slope; reduce the volume of runoff water, and thus preventing or minimizing the soil erosion. Contour bunds are constructed in relatively low rainfall areas, having an annual rainfall or less than 700 mm, particularly in areas having light textured soils. For rolling and flater lands having slopes from 2 to 6% contour bunding is practiced, in red soils.

Location of contour bund:

Moderate slopes (up to 6%) with light or medium soil texture and less than 700 mm of rain per year.

Purpose of contour bund: -

To reduce soil erosion and to increase the amount of water the soil can hold.

Advantages of contour bund: - Simple to build. Bunds conserve topsoil and improve productivity. They keep water in the soil, allowing chemical fertilizers to be used effectively. They can be used both on cultivated and uncultivated land. Farmers can build contour bunds themselves without outside help.

Limitations of contour bund: - The bunds take some land away from cultivation (though some types of crops can be grown on the bunds to stabilize them). May create temporary waterlogging problems in heavy soil. May interfere with the farm operations if the bunds are too close together. A lot of labour is needed to maintain and repair the bunds.

General principles and design of contour bund:

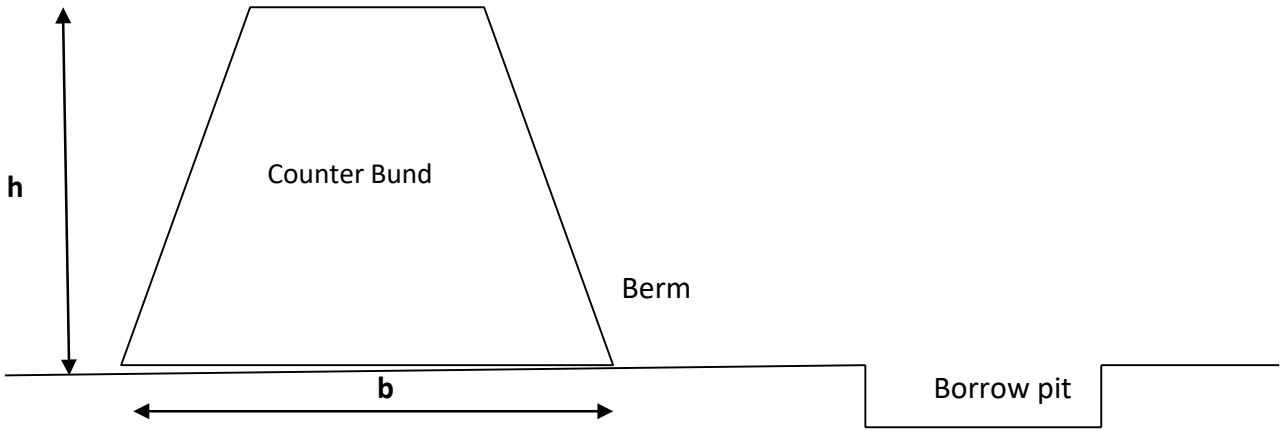
1. Spacing of Contour bund:

Bund spacing is expressed as the vertical or the horizontal distance between corresponding points on two adjacent bunds. Horizontal spacing is useful in determining the row arrangement. Vertical distance is commonly known as the vertical

interval or V. I. Bund spacing should not be so wide as to cause excessive soil erosion between adjacent bunds. Spacing may be increased or decreased 10 to 20% to suit local conditions.

Slope of land (%) vertical.	Interval (m) Approx	Horizontal distance (m)
0 to 1	1.05	105
1 to 1.5	1.2	98
1.5 to 2	1.35	75
2 to 3	1.5	60
3 to 4	1.65	52

T



2. Bund length:

In general, 400 to 500m is the maximum length of bund. The bund retains the runoff and carries it over the distance equal to bund length in one direction. The length of bund should be such that the velocity of water flowing between bunds should be non erosive.

3. Bund cross section: The height of bund should provide sufficient storage above the bund to handle the expected runoff. In normal practice sufficient practice is provided to take care of runoff from rains expected in 10 year recurrence interval. The cross section area of the storage space required can be calculated by the following formula

Cross section area of storage space = [Runoff, cm] X [Bund horizontal interval in m] / 100

Design steps:

Vertical Interval between bunds (V.I)

Where, S – land slope (%);

$VI = \{S/a + b\}\ 0.3$

a and b are constants a = 3 and b =2 for medium and heavy rainfall zonesa = 2 and b =2 for low rainfall zones

Horizontal Spacing in between bunds (H.I)

$$HI = (VI/S)\ X\ 100$$

Length of bund per hectare (L.B)

L. B. per ha = (100 S)/VI or 10000/H.I.

Depth of water impounding before the bund (h), $h = \{(D\ X\ R)/500\}^{1/2}$

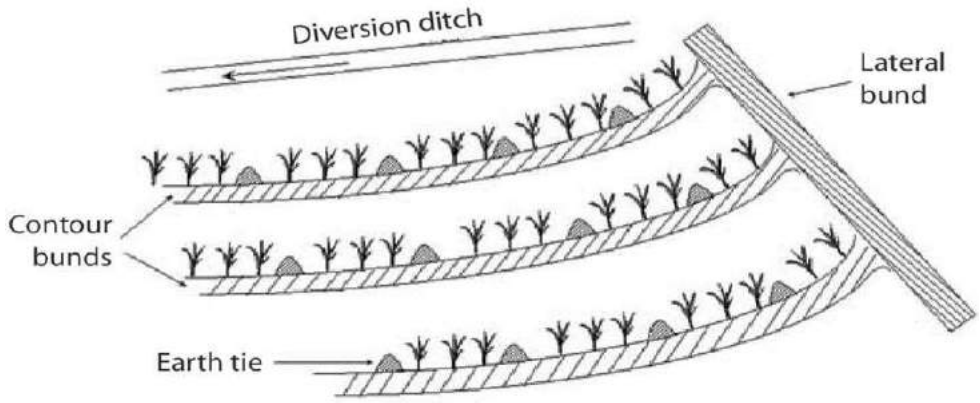
Where, D – vertical interval (m)

R – Maximum rainwater on area basis (mm)

Actual height of the bund = h + 20% of h as freeboard

Type of soil	Bottom width (m)	Top width (m)	Height (m)	Side slope
Gravel soil	1.2	0.3	0.6	0.75:1
Red soil	2.1	0.3	0.6	1.5: 1
Shallow to Medium Black Soil	2.4	0.45	0.75	1.3: 1
Deep Soil	3.3	0.6	0.675	2: 1

By knowing the cross section area of the bund, the volume of earthwork per hectare and thecost of earthwork per hectare can be determined.



Salient features:

- Bunding is an effective moisture conservation measure in dry land.
- It is suitable for lesser rainfall areas and the slope is< 1%.
- The lands are divided into small compartments with the dimension of 8 x 5 m.
- Store the rainfall for longer period.
- It increases water holding capacity of the soil.
- It can be formed while ploughing itself or before early sowing.
- Reduces the formation of cracks.
- It will overcome the disadvantages of contour bunding.

Exercise:

On a 3 per cent land slope calculate the horizontal spacing of bunds in medium rainfall zoneand the length of bunds per hectare.

VI= {S/a + b} 0.3, VI = *{3/3 + 2} 0.3*=0.9 m

Horizontal spacing = 0.9 x (100/3) = 30 meters Length of bund per ha = 10000/30 = 333 meters

PRACTICAL 8: DESIGN OF GRADED BUNDS

Graded bund

Graded bunds are constructed in medium to high rainfall areas having an annual rainfall of 600 mm and above and in soils having poor permeability (or) those having crust forming tendency (black soils), and in the lands having slopes between 2% and 6%.These bunds are provided with a channel if necessary. Uniformly graded bunds are suitable where the length of bund is less and the discharge behind the bund or in the channel is not much. Variable graded bunds are suitable when the length of bunds and discharge are more. Variable grades are provided in different sections of the bund. For uniform graded bund, a grade from 0.1% to a maximum of 0.4% is adopted and for variable graded bund the grade will vary with the length of the bund. The required capacity of the channel can be determined by using the Rational Method.

Function:

- These terraces act primarily as drainage channel to regulate and conduct runoff at nonerosive velocity.
- To make the runoff water to trickle rather than to rush out.

For graded bunds, the horizontal, length per hectare and cost estimation are similar as that of contour bunding

Design of Graded bund
Graded bund specifications

Type of soil	Bottom width (m)	Top width (m)	Height (m)	Side slope
Shallow soil	1.1	0.3	0.4	1:1
Red and alluvial soil	1.5	0.5	0.5	1:1
Heavy soil	2.1	0.5	0.5	1.5:1

For graded bunds, the horizontal, length per hectare and cost estimation are similar as that of contour bunding.

PRACTICAL 9: STUDY OF TERRACES

DESIGN OF BENCH TERRACING SYSTEM

A terrace is an embankment or ridge of earth constructed across a slope to control runoff and minimize soil erosion or Terracing is an agricultural technique for collecting surface runoff water thus increasing infiltration and controlling water erosion known from an ancient history and used to transform landscape to steeped agro-systems in many hilly or mountainous regions.

Features of terracing:

- These are constructed across the slope to intercept the surface runoff and convey it toa suitable outlet, at non erosive velocity.
- They reduce the length of slope by splitting the slope length in different parts.
- The terracing practice is adopted for soil and water conservation in that area where land slope is greater than 10%; soil is more erodible and prevails high rainfall intensity.
- Terraces not only control the soil loss by sheet flow, but also play an important role intrapping the splashed soil particles.
- This practice is not possible, particularly on those hill sloped areas, where soil depthis not sufficient.

Classification of terraces:

- **Diversion terraces** : used for intercepting the overland flow from hilly slope and channel it across the slope to a suitable outlet i.e. grassed water ways etc. built atslight down slope grade from contour. These are constructed in high rainfall area.
- **Magnum type**: It is constructed by taking the soil from both side of the embankment
- **Nichols type**: formed by taking the soil from side of up slope of the embankment,only.
- **Broad based type**: This terrace is constructed with embankment and channel

occupying a width of about 15 m.

- **Narrow based type**: These terraces are only 3 to 4 m wide; the banks have steeperslop which cannot be cultivated. For cultivation to make possible, the bank should not exceed 140 slop for use of small machines, otherwise it should be 8.50 for large size machines
- **Retention terraces**: these are levelled terrace, used particularly when water is required to conserve by making storage on hill sides. These are constructed in low rainfall areas.
- **Bench terrace**: Such types of terrace are constructed in form of alternate series of shelves and risers, used to cultivate the steep slopes. These are constructed in mediumrainfall areas.

Bench Terrace:

A bench terrace is shelf like embankment of earth with a level or nearly level top anda step or vertical downhill face constructed along the contour of sloping land.

Bench terracing consists of transforming relatively steep land into a series of levee or nearly level strips or steps running across the slope .the strips are separated by almost verticalrisers. The risers if sloping may be of earth construction .steep risers are supported by masonry [stones].bench terracing is adopted only on slopes steeper than 15%[for more than 8%]and where soil condition are favourable . The use of bench traces retards erosion losses and makes cropping operations on these slopes possible and safe.

Types of bench Terraces

A. Based on slope	B.Based on use /application
1] Level and table top	1] Hill type
2] Sloping inwards	2] Irrigated type
3] Sloping outwards	3] Orchard type
4] Puertorican or California type	

A. Classification based on slope

1. Table top bench terrace:

Table top bench terrace are suitable for areas receiving medium rainfall which is evenly distributed and which have highly permeable and deep soils .in paddy fields it may be used for slopes as mild as 1% and used where irrigation facilities are available.

2. Sloping inwards bench terrace:

In heavy rainfall areas, bench terraces of sloping inwards type are more effective. It prevents imponding of water and useful for crops susceptible to water logging.

3. Sloping outwards bench terrace:

Bench terraces sloping outwards are effective only in low rainfall areas whit a permeable soil of medium depth at lower ends graded channels are provided for safe disposal of runoff

4. Puertorican Type:

In this type of terrace, the soil is excavated little during every ploughing and gradually developing bench by pushing the soil downhill against a mechanical of vegetative barrier. Mechanical or vegetative barrier is established across the land at suitable interval and the terrace is developed gradually over the years, by pushing soil downhill and subsequent natural levelling.

B] Classification based on use:

Depending upon the purpose for which they are used, bench terraces are classified as follows:

1. Hill type Bench Terraces: It is used for hilly areas whit a reverse grad towards the hill.

2. Irrigated Bench Terraces: Level benches are adopted under irrigated conditions. The level table top terraces are referred to as irrigated bench Terries.

3. Orchard Bench Terraces: Narrow width terraces [about 1 mm] for individual trees are prepared in this type. These are also referred as intermittent terraces and step terraces. The conversion of land into bench terraces over a period of time is referred as gradual bench terracing.

PRACTICAL 10 :PROBLEMS OF WIND EROSION

Wind erosion is a serious environmental problem. It is in no way less severe than water erosion. High velocity winds strike the bare lands (having no cover), with increasing force. Fine, loose and light soil particles blown from the land surface are taken miles and miles away and thereby, causing a great damage to the crop productivity. It is a common phenomenon occurring mostly in flat, bare areas; dry, sandy soils; or anywhere the soil is loose, dry and finely granulated and where high velocity wind blows. Wind erosion, in India, is commonly observed in arid and semi-arid areas where the precipitation is inadequate, e.g. Rajasthan and some parts of Gujarat, Punjab and Haryana.

Factors Affecting Wind Erosion

Climate, soil and vegetation are the major factors affecting wind erosion at any particular location. The climatic factors that affect the wind erosion are the characteristics of wind itself (velocity and direction) in addition to the precipitation, humidity and temperature. Soil moisture conditions, texture, structure, density of particles, organic matter content are thesoil characteristics that influence erosion by wind. Soil movement is initiated as a result of wind forces exerted against the surface of the ground. For each specific soil type and surface condition there is a minimum velocity required to move soil particles. This is called the threshold velocity. Once this velocity is reached, the quantity of soil moved is dependent upon the particle size, the cloddiness of particles, and wind velocity itself. Surface features like vegetation or other artificial cover (mulching etc) have the protective effect on wind erosion problem as surface cover increases the roughness over the land surface and thus reduces the erosive wind force on the land surface.

Mechanics of Wind Erosion

The overall occurrence of wind erosion could be described in three distinct phases.

These are:

1. Initiation of Movement

2. Transportation

3. Deposition.

Movement of soil particles is caused by wind forces exerted against or parallel to the ground surface. The erosive wind is turbulent at all heights except very close to the surface. The lowest velocity occurs close to the ground and increases in proportion to the logarithm ofthe height above the surface. Soil particles or other projections on the surface absorb most of the force exerted by the wind on the surface. However, if the soil particles are lighter and loose, wind may lift them from the surface in the initiation process. A minimum threshold velocity (wind) is required to initiate the movement of soil particles. Thus, the threshold velocity is the minimum wind velocity needed to initiate the movement of soil particles. The magnitude of the threshold velocity is not fixed for all places and conditions but it varies withthe soil conditions and nature of the ground surface. For example, for the most erodible soils of particle size about 0.1 mm; the required threshold velocity is 16 km/h at a height of 30 cm above the ground.

Initiation of Movement: The soil particles are first detached from their place by the impact and cutting action of wind. These detached particles are then ready for movement by the wind forces. After this initiation of movement, soil particles are moved or transported by distinct mechanisms.

Transportation: The transportation of the soil particles are of three distinct types and occur depending upon size of the soil particles. Suspension, saltation, and surface creep are the three types of soil movement or transport which occur during wind erosion. While soil can be blown away at virtually any height, the majority (over 93%) of soil movement/transportation takes place at or within one meter height from land surface. These transportation mechanisms of soil particles due to wind are shown in Fig. 14.2.

Suspension: It occurs when very fine dirt and dust particles are lifted into the atmosphere. They can be thrown into the air through impact with other particles or by the wind itself. These particles can be carried very high and be transported over very long distances in the atmosphere by the winds. Soil moved by suspension is the most spectacular and easiest to recognize among the three forms of movement. The soil particles of less than 0.1 mm size are subjected to suspension and around 3 to 40 % of soil weights are carried by the suspension method of soil transport under the wind erosion.

Saltation: The major fraction of soil moved by the wind is through the process of saltation.

Saltation movement is caused by the pressure of the wind on soil particles as well as by the collision of a particle with other particles. Soil particles (0.1 to 0.5 mm) move in a series of bounces and/or jumps. Fine soil particles are lifted into the air by the wind and drift horizontally across the surface increasing in velocity as they move. Soil particles moved in the process of saltation can cause severe damage to the soil surface and vegetation. They travel approximately four times longer in distance than in height. When they strike the surface again they either bounce back into the air or knock other soil particles from the soil mass into the air. Depending on soil type, about 50 to 75% of the total weight of soil is carried in saltation. The height of the jump varies with the size and density of the soil particles, the roughness of the soil surface, and the velocity of the wind.

Surface Creep: The large particles which are too heavy to be lifted into the air are moved through a process called surface creep. In this process, the particles are rolled across the surface after coming into contact with the soil particles in saltation. In this process the largest of the erosive particles having diameters between 0.5 to 2 mm are transported and around 5 to 25% of the total soil weights are carried in this fashion. Overall, the mass of soil moved by wind is influenced primarily by particle size, gradation of particles, wind velocity and the distance along the eroding area. Winds being variable in velocity and direction produce eddies and cross-currents that lift and transport soil. The amount of soil moved/transported depends on the median particles (soil) diameter and the difference in threshold and actual wind velocity. The mass of soil moved can be related to the influencing parameters by the following equation:

Quantity of soil moved $(V - V^{th})^3 / D^{0.5}$

Where V = wind velocity, V^{th} = threshold velocity, and D = particle diameter.

Deposition: Deposition of soil particles occurs when the gravitational force is greater than the forces holding the particle in the air. This generally happens when there is a decrease in the wind velocity caused by vegetative or other physical barriers like ditches or benches. Raindrops may also take dust out of air.

PRACTICAL 11:IDENTIFICATION OF DIFFERENT TYPES OF EROSION AS PER FIELD VISIT

OBJECTIVE: Identification of different types of erosion as per field visit.

Sheet erosion

Sheet erosion is the uniform removal of soil in thin layers by the forces of raindrops and overland flow. It can be a very effective erosive process because it can cover large areas of sloping land and go unnoticed for quite some time.

Rill erosion

Rill erosion is the removal of soil by concentrated water running through little streamlets, or headcuts. Detachment in a rill occurs if the sediment in the flow isbelow the amount the load can transport and if the flow exceeds the soil's resistance todetachment.

Gully erosion

Gully erosion is the removal of soil along drainage lines by surface water runoff. Once started, gullies will continue to move by headward erosion or by slumping of the side walls unless steps are taken to stabilise the disturbance.

Stream bank erosion

Rivers and streams are products of their catchments. They are often referred to as dynamic systems which mean they are in a constant state of change. The factors controlling river and stream formation are complex and interrelated. These factors

include the amount and rate of supply of water and sediment into stream systems, catchment geology and the type and extent of vegetation in the catchment. As these factors change over time, river systems respond by altering their shape, form and/or location. In stable streams the rate of these changes is generally slow and imperceptible.

Exercise: Identification of different types of erosion observed during field visit

- Date of visit-
- Location of visit-
- Observations-

SN	Name of Erosion	Land slope	Soil type	Soil depth	Locationof affected area	Area affected (approx.)in ha	Suggested control measures	Remarks
1	Sheet erosion							
2	Rill erosion							
3	Gully Erosion							Width= Depth=

4	Stream Bank Erosion							
5	Any other							

FUNDAMENTALS OF PLANT BIOCHEMISTRY AND BIOTECHNOLOGY- AGS-204

Practical No 1: Preparation of solution, pH & buffers

Materials required:pH meter,Beakers, Wash bottle, tissue paper, Glacial acetic acid,Sodium acetate.

Theory: The pH meter measures the electrical potential developed by pair ofelectrode pins in a solution. For measurement of pH, an electrode system sensitiveto change in H+ ion concentration of solution is taken. The electrode systemconsists of sequence of electrode whose potential raise with pH (H+ concentrationof the solution).

$pH = pKa + \log [A^-] / [HA]$

Procedure

pH measurement:

1. Take some water in a clean beaker.
2. Take the electrode out, wash with distilled water and wipe clean with tissue paper.
3. Tare the meter and dip the electrode in the sample (here water).
4. Note the reading.

Result: pH of the given Sample

Preparation of buffer:

Acetic acid- sodium acetate buffer:

Reagents required:

Acetic Acid 0.2M: 1.5 ml of glacial acetic acid is made up to 100ml withdistilled water.

Sodium Acetate Solution: 0.64 gm. of sodium acetate or 2.72gm of sodiumacetate tri-hydrate is dissolved in 100ml Distilled water.

Procedure:

Pipette out exactly 36.2ml of sodium acetate solution into 100ml ofstandard flask and add 14.8ml of glacial acetic acid, make the volume 100ml usingdistilled water using distilled water. This gives 0.2 M of acetic acid and sodiumacetate buffer. The pH is measured with pH meter.

The pH meter is first standarized with pH buffer. Wash electrode with distilled water and introduced into 0.2M acetic acid-sodium acetate bufferprepared, the pH of solution is 4.6.

Result:

Precautions:

- Weigh the chemical properly
- Mix the contents in around 50mL of distilied water and later scale up to the final

Practical No 2: Qualitative test of carbohydrate

Materials required: test tubes, pipette, beaker, deionized water, benedict's reagent,Fehling's reagent A& B, boiling water,samples (glucose, sucrose & starch).

Principle: Sugar that is capable of acting as a reducing agent is known as reducing sugar because it has a free aldehyde group or a free ketone group. These free groups are oxidized to carboxylic acid. In order for oxidation to occur, the cyclic form must first ring-open to give the reactive aldehyde. Thus any sugar that contains a hemi acetal will be a reducing sugar.

Benedict's test: the reagent that is used in the Benedict 's test is known as Benedict's reagent. It is a clear blue solution containing copper (II) sulphate & sodium tartrate. Copper sulphate provides the cu^{2+} ions and sodium tartarate provides the required alkaline environment. In the test free aldehyde or keto group in the reducing sugar reduce the alkaline cupric oxide to red coloured cuprous oxide.

Fehling's test: It is a test that is done so as to differentiate between reducing and non-reducing sugar. The test was developed by German chemist Hermann van Fehling in 1849. Fehling's solution is a preparation of two separate solution, known as Fehling's solution A and Fehling's solution B. Fehling's solution A is an aqueous solution of copper sulphate (II), which is deep blue. Fehling's solution B is a colourless solution of aqueous potassium sodium tartarate (also known as rochere salt), made in strong alkali, commonly with sodium hydroxide.

The reducing sugar when reacts with Fehling's solution gives yellow or brownish red colour precipitation.

Procedure:

For Benedict's test:

(1) Take a sample in a test tube.

(2) Add few amount of benedict's solution. Then the mixture is shaken well and placed inboiling water for a while.

(3) If the sample is reducing sugar a brick red precipitate will be formed otherwise themixture would remain clear blue.

For Fehling's Test:

(1) Fehling's solution A and B should add in equal amount and then sample will be addedto it.

(2) Then the mixture is shaked well and placed in boiling water for a while.

(3) If a sample is reducing sugar a brick red precipitate will be formed, otherwise themixture would remain clear blue.

Result and observation

Sample	Reagent Added	Inference	Result

Precaution:

- Chemical should not be wasted.
- The reaction takes place in alkaline environment only.
- In case the mixture is acidic the cu^{2+} ion would be stabilized and will not be easilyoxidized thus the reaction will not be completed.

Practical No 3: Qualitative tests of Protein

Materials Required: Ninhydrin solution, Acetone, HNO_3, NaOH, Spirit lamp,Glasswares

Principle

Ninhydrin test

Ninhydrin is a chemical used to detect ammonia or primary or secondary amines. Amino acid react with Ninhydrin at pH 4, then reduction product is obtained from Ninhydrin to yield a blue coloured substance. Amino acid is extremely sensitive to this test.

Xanthoprotic test

Some amino acid contains aromatic group that are derivatives of Benzene. These aromatic group undergoes reaction that have characteristics of benzene and benzene derivatives. One such reaction is nitration of benzene ring with nitric acid . Activated benzene ring of amino acid undergoes nitration. The nitration reaction in the presence of activated benzene ring forms yellow product. This test is application to aromatic amino acid like Tyrosin , Tryptophan, Phenylalanine, and non- aromatic acid like glutamic acid.

Procedure:

Ninhydrin test

- 1ml of sample (ex: filtered wheat flour or crushed seeds solution) solution and 5 dropsof 0.2% Ninhydrin solution in acetone was added.
- It was incubated in water bath for 2 minutes.
- Allow it to cool for some time and blue color complex was observed.

Xanthoprotic test

- 2ml of sample was taken in test tube and equal volume of concentrated Nitric acid

was added and heated over flame for 2 minutes till there was a colour change.

- It was cooled under running tap water and 40% NaOH was added to make thesolution alkaline in nature.
- The colour change in nitro derivative of aromatic structure was observed.

Result:

Precautions:

- Wash the apparatus before and after the experiment.
- Make sure that amount you are taking of samples and liquid drops are the same as mentioned in the procedure.
- Also, make sure that the test tube you are using must be clean and wash very neatly. There must not remain any impurity other than the natural stuff present in the test tube.
- Observe colours at the end of the tests very carefully and note down results on a notepad to avoid future inconvenience.
- Prevents prolonged heating of the test tube otherwise, it will give a false result.
- Carefully handle all the chemicals.

Practical 4: Quantitative estimation of glucose

Materials required: Test tube and test tube rack, Measuring cylinder, Distilled water, H_2SO_4, Anthrone reagent, Colorimeter, Water bath,Carbohydrate sample (sucrose solution)

Principle: Anthrone dissolved in 2% concentrated sulphuric acid is used for the quantitative analysis of different carbohydrates .Quantitative determination is only possible where the identity of sugar component is known because colour development varies with different sugars. Nevertheless,the anthrone method is widely used for the determination of starch and soluble sugar in plant material.

Generally, sugar and carbohydrate are extracted from dried and ground plant material. First soluble sugar are extracted with aqueous ethanol, later starch is extracted with an acid. Estimation of total carbohydrates moiety in a sample can be done by anthrone method which is a simple colorimetric method. The preparation of anthrone assay does not require the addition of distilled water and anthrone is directly dissolved in sulphuric acid at 2% concentration. This acid is a powerful dehydrating agent involved in dehydrating sugars leading to formation of furfural which condenses with Anthroneto give a bluish-green complex, which can be measured colorimetrically at 620nm by using a spectrometer (10-keto-9,10-dihydroanthrocene).This is a rapid and continuous method for the determination of hexoses, aldopentoses and hexuronic acid , either in the free form or when present polysaccharide.

Procedure:

1. 200mg of anthrone powder was dissolved to 100ml of chilled 95% sulphuric acid.
2. The standard sucrose solution of concentration 500mg/ml was prepared. From this 0.5 ml of sucrose solution is diluted in distilled water. As a result, the solution curve was plotted for calibrating curve.
3. To all the test tubes 2ml of Anthrone reagent was added.
4. The test tubes were incubated in boiling water bath for 10 minutes. The test tubes wereallowed to cool down.
5. In all the test tubes green to dark green color variation was observed.
6. O.D of different aliquots was taken at 620 nm by using UV visible spectrometer.
7. The standard curve was plotted by taking O.D on Y-axis against sugar

concentrationon X-axis.

8. The amount of sugar present in the sample was obtained from standard plot.

Result: Precautions:

- Make all the measurements very carefully. All the solutions and samples should be ofexact amount as mentioned in the experiment.
- While heating the solution the boiling water bath, do it very carefully. Also bring downthe temperature rapidly to room temperature. Take care of the time that you are giving to heat the sample as well as to the accurate temperature.

Practical 5: Quantitative estimation of proteins (Bradford Method)

Materials Required: Bradford reagent (Coomassie Blue G250, 95% ethanol, 85% phosphoric acid). BSA standard, Colorimeter, Distilled water

Principle

Bradford protein assay is a simple and accurate procedure for determining the concentration of protein in solution using the coomassie G250. This method was first described by Marion Bradford in the year 1976. This method was based on the principle of binding of coomassie G250 dye to proteins. The dye exists in three different forms:

(1) Cationic

(2) Anionic

(3) Neutral

Under acidic conditions the dye is in the protonated cationic form. When the dye binds to the proteins in an acidic condition, it is converted to a stable unprotonated blue form, resulting in a spectral shift.

The presence of certain basic amino acids such as Arginine, Histidine, Lycine in the protein are responsible for the development of color in the bradford's assay.

Mechanism

The dye first donates its free electrons to the protein bring treated causing disruptions of its native states and exposing its hydrophobic pockets. These hydrophobic sites on the protein chain then bind to the non-polar region of dye by Vanderwall's force.

The positive amino groups' position with the negative charge of the dye ionic interaction, this ionic interaction gets further strong and stabilizes the blue color. The amount of the color complex present in the solution, estimates the protein concentration which can be measured at 595 nm.

Procedure

1. Reconstitute 1 vial of standard protein (BSA) (10 mg/ml concentration) with 10 ml of distilled water to get the concentration of 10mg/ml.

2. Diluted solution of 120 µl of 10 mg/ml BSA solution with 1880 µl of bradford's reagent was mixed. Bradford reagent (100 mg of Coomassie Blue G250 + 50 mL of 95% ethanol + 100 mL of 85% phosphoric acid and made up to 1 L with distilled water + filtered through whatman no. 1, storage- amber bottle).

3. Pipette out diluted BSA, test sample and distilled water at given in the table and adjustthe volume.

4. Now, incubate at room temperature for 10 mins but less than ½ an hour.

5. The absorbance was taken for the standard tubes and samples respectively at 595 nm. A standard curve was drawn by plotting absorbance on Y-axis and concentration (µg/ml) on X-axis.

6. Determination of the concentration of protein of unknown sample has been observed by using standard graph.

Result:

PRACTICAL 6: TITRATION METHODS FOR ESTIMATION OF AMINOACIDS

Materials Required: 0.1 N solution NaOH, Formaldehyde, Phenolphthalein indicator, Amino acid solution, glasswares.

Principle: The acid group present in the glycine can be titrated with NaOH. It is not easyin this case because the amino group present will interfere at the end point. Topreventit excess of formaldehyde is used by which the amino group is blocked by th eformation of methylene glycine. Then it is titrated with NaOH using phenolphthaleinindicator.

Procedure:

Estimation of Amino acid

1. Make up the given amino acid solution with distilled water to 100ml in a volumetricflask. Shake the solution well for uniform concentration.
2. Pipette out 20ml of amino acid solution into a clean conical flask.
3. Add5 ml of HCHO and keep it for 2 minutes.
4. Then titrate it against the NaOH solution takenin the burette.
5. Phenolphthalein is used as the indicator.
6. The end point is the appearanceof permanent pale pink colour.
7. Repeat the titration for concordant values.

Blank titration

1. Pipette out 20ml of distilled water in a clean conical flask.
2. Add 5ml of HCHOand keep it aside for a few minutes.
3. Add 1-2 drops of phenolphthalein indicator.
4. Titrateit against the 0.1N NaOH solution taken in the burette.
5. The end point is the appearance of permanent pale pink colour.
6. Repeat the titration for concordant values.

Result:

The amount of amino acid present in the whole of the given solution =_____g

Titration I (Blank titration)

S. No.	Content on conical flask (ml)	Burette Reading		Concordant Value (ml)
		Initial	Final	
1				
2				
3				

Titration II (Estimation of Amino acid)

S. No.	Content on conical flask (ml)	Burette Reading		Concordant Value (ml)
		Initial	Final	
1				
2				
3				

Calculation I

Volume of NaOH, (Test value) = ml Volume of NaOH, (Blank value) = ml

Volume of NaOH used to titrate amino acid = Test value – Blank value= ml

CalculationII

Volume of amino acid, V_1= ml Normality of amino acid, N_1= NVolume of NaOH, V_2= ml Normality of NaOH, N_2= N

Strength of amino acid = $V_{(NaOH)}$x $N_{(NaOH)}$= $V_{(amino\ acid)}$x $N_{(amino\ acid)}$ Amount of amino acid present in 100ml of the given solution= Equivalent weight x Normality x 100/1000

Precautions

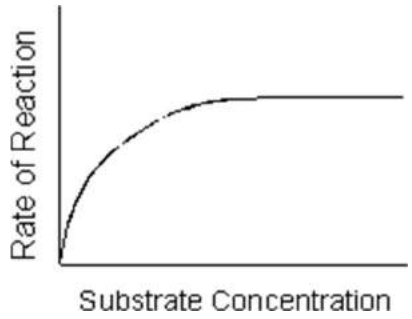
1. Use clean glass wares. Wash the glassware with distilled water before use.
2. Check that there is no air bubble in the burette or pipette.
3. Avoid inconsistent burette reading

PRACTICAL 7: EFFECT OF PH, TEMPERATURE AND SUBSTRATE CONCENTRATION ON ENZYME ACTION.

Effect of substrate concentration:

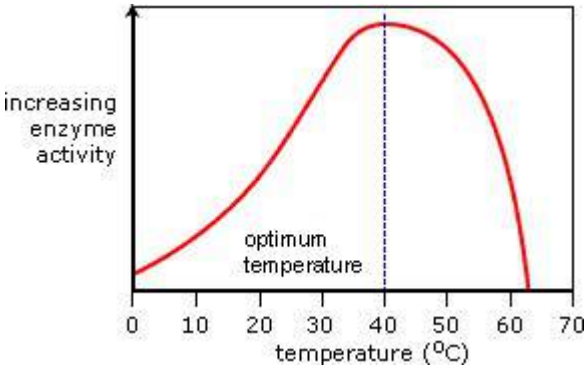
Reaction velocity of an enzymatic process increases with constant enzyme concentration and increase in substrate concentration. The velocity (V) is expressed in micromoles of substrate converted per minute. As the concentration of substrate increases, the velocity of the reaction increases. Continued increase in substrate concentration may lead to a reduction in rate of the reaction and leads to flattened curve. The maximum velocity obtained from an enzymatic reaction is called as V_{max}. V_{max} represents the maximum reaction rate possible in the presence of excess substrate. Though V_o approaches but never reaches V_{max}. At lower concentration of substrate, V_o increases almost linearly with an increase in [S]. The substrate concentration at which V_o is half maximum is K_m, the Michaels constant. The relationship between substrate concentration and reaction rate can be expressed quantitatively through

$V_o = V_{max}[S] / K_m + [S]$.



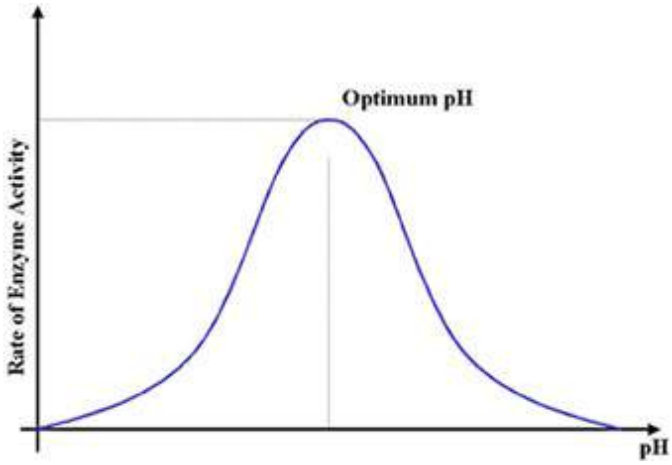
Effect of temperature:

Velocity of enzymatic reaction increases with temperature of the medium which they are most efficient and the same is termed as optimum temperature. As temperatures increases it leads to denaturation; a molecular arrangement which causes a loss of the active sites of the enzyme surfaces and results in a loss of efficiency.



Effect of pH:

Like temperature, all enzymes have a optimum pH, at which the enzymatic activity will be at maximum. Many enzymes are most efficient in the region of pH 6-7, which is the pH of the cell. Outside this range, enzyme activity drops off very rapidly. Reduction in efficiency caused by changes in the pH is due to changes in the degree of ionization of the substrate and enzyme. Highly acidic or alkaline conditions bring about a denaturation and subsequent loss of enzymatic activity. Some exceptions such as pepsin (with optimum pH 1-2), alkaline phosphatase (with optimum pH 9-10) and acid phosphatase (with optimum pH 4-5) are even noticed.



Practical 8: **TLC demonstration for separation of amino acids.**

Chromatography:

Chromatography is by far the most useful general group of techniques available for the separation of closely related compounds in a mixture. Here the separation is effected by differences in the equilibrium distribution of the components between two immiscible phases, viz., the stationary andthe mobile phases. These differences in the equilibrium distribution are a result of nature and degree of interaction of the components with these two phases. The stationary phase is a porous medium like silica or alumina, through which the sample mixture percolates under the influence of a moving solvent (the mobile phase). There are a number of interactions between the sample and the stationary phase and these have been well exploited to effect the separation of compounds.

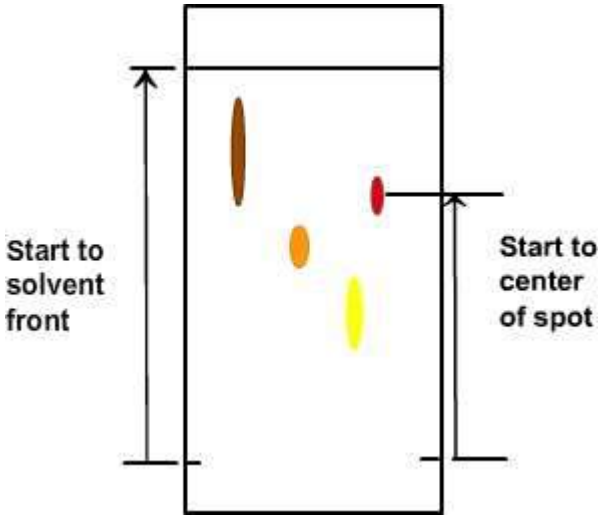
Thin layer chromatography [TLC]:

Thin layer chromatographic (TLC) technique readily provides qualitative information and with careful attention to details, it is possible to obtain quantitative data. Thin layer chromatography is a technique used to separate and identify compounds of interest. A TLC plate is made up of a thin layer of silica adhered to glass or aluminum for support. The silica gel acts as the stationary phase and the solvent mixture acts as the mobile phase. In the ideal solvent system the compounds of interest are soluble to different degrees. Separation results from the partition equilibrium of the components in the mixture.

In the simplest form of the technique, a narrow zone or spot of the sample mixture to beseparated is applied near one end of the TLC plate and allowed to dry. The strip or plate is then placed with this end dipping in to the solvent mixture, taking care that the sample spot/zone is notimmersed in the solvent. As the solvent moves towards the other end of the strip, the test mixtureseparates into various components. This is called as the development of TLC plates. The separation depends on several factors; (a) solubility: the more soluble a compound is in a solvent, the faster it will move up the plate. (b) attractions between the compound and the silica, the more the compound interacts with silica, the lesser it moves.The plate is removed after an optimal development time and dried and the spots/zones are detected using a suitable location reagent. An important characteristic used in thin layer

chromatography is Rf value.The plate is removed after an optimal development time and dried and the spots/zones are detected using a suitable location reagent. An important characteristic used in thin layer chromatography is Rf value.

$$Rf = \frac{distance\ moved\ by\ the\ substance\ from\ origin}{distance\ moved\ by\ solvent\ from\ origin}$$



Chromatographic Separation of Amino acids:

The present experiment employs the technique of thin layer chromatography to separate the amino acids in a given mixture.

All 20 of the common amino acids [standard amino acids] are a-amino acids. They have a carboxyl group and an amino group bonded to the same carbon atom (the α- carbon). They differ from each other in their side chains, or R groups, which vary in structure, size, and electric charge. The interaction of the amino acids with the stationary phase like silica varies depending on their 'R' groups. The amino acid that interacts strongly with silica will be carried by the solvent to a small distance, whereas the one with less interaction will be moved further. By running controls [known compounds] alongside, it is possible to identify the components of the mixture.

Since amino acids are colourless compounds, ninhydrin is used for detecting them. To identify this,after development, the TLC plate is sprayed with ninhydrin reagent and dried in an oven, at 105°C for about 5 minutes. Ninhydrin reacts with α- amino acids that results in purple coloured spots [due to the formation of the complex - Rheuman's purple] [http://vlab.amrita.edu/?sub=3&brch=63&sim=156&cnt=1]. Rf values can be calculated and compared with the reference values to identify the amino acids. [The Rf value for each known compound should remain the same provided the development of plate is done with the same solvent, type of TLC plates, method of spotting and in exactly the same conditions].

PRACTICAL 9: STERILIZATION TECHNIQUES

Materials Required: Autoclave, spirit lamp, glass beadsterilizer, filters, ethanol, laminar air hood.

Principle and procedureWet heat (Autoclaving)

The method of choice for sterilisation in most labs is autoclaving; using pressurised steam to heat the material to be sterilised. Sterilisation of plant media and autoclavable plastic wares, can normally be achieved in 15 minutes by autoclaving at 121.6° C and15 psi pressure. This method is also useful for sterilization of glassware, cotton, forceps, scalpels etc

Dry heat (Flaming, Glass bead sterilization)

Inoculation loop can be sterilized by passing an ethanol dipped loop over the flame for a few seconds. Glass bead sterilization is used to keep the metal instruments such as scalpels, scissors, forceps sterilized in the laminar air flow. Temperature of the glass bead sterilizeris around 250°C- 265°C.

Filtration

Filtration is a great way of quickly sterilizing solutions without heating. Filters, of course, work by passing the solution through a filter with a pore diameter that is too small for microbes to pass through.Filters can be scintered glass funnels made from heat-fused glass particles or, more commonly these days, membrane filters made from cellulose esters. For removal of bacteria, filters with an average pore diameter of 0.2um is normally used.

But viruses and phage can pass through these filters so filtration is not a good option if these are a concern.

Solvents

Ethanol is commonly used as a disinfectant, although since isopropanol is a better solvent for fat it is probably a better option.Both work by denaturing proteins through a process that requires water, so they must be diluted to 60-90% in water to be effective. Although ethanol and IPA are good at killing microbial cells, they have no effect on spores.

Radiation

UV, x-rays and gamma rays are all types of electromagnetic radiation that have profoundly damaging effects on DNA, so make excellent tools for sterilization.The main difference between them, in terms of their effectiveness, is their penetration.UV has limited penetration in air so sterilisation only occurs in a fairly small area around the lamp. However, it is relatively safe and is quite useful for sterilising small areas, like laminar flow hoods.X-rays and gamma rays are far more penetrating, which makes them more dangerous but very effective for large scale cold sterilization of plastic items (e.g. syringes) during manufacturing.

Laminar air hood

Laminar airflow hoods are used in commercial and research tissue culture settings. A horizontal laminar flow unit is designed to remove particles from the air. Room air is pulled into the unit and pushed through a HEPA (High Efficiency Particulate Air) filter with a uniform velocity of 90 ft/min across the work surface. The air is filtered by a HEPA filter so nothing larger than 0.3 micrometer, which includes bacterial and fungal spores, can pass through. This renders the air sterile. The positive pressure of the air flow from the unit also discourages any fungal spores or bacteria from entering. Depending on the design of the hood, the filters are located at the back or in the top of the box. A UV lamp is attached on the top and initial sterilization for 15- 20 min is carried out using UV lamps followed by air flow through HEPA filter.

Practical 10: Preparation of MS nutrient medium

Materials required: glassware, chemicals, pH meter, distilled water, autoclave.

Principle:

The basic nutritional requirements of cultured plant cells as well as plants are very similar. However, the nutritional composition varies according to the cells, tissues, organs and protoplasts and also with respect to particular plant species. The appropriate composition of the medium largely determines the success of the culture. A wide variety of salt mixtures have been reported in various media. A nutrient medium is defined by its mineral salt composition, carbon source, vitamins, growth regulators and other organic supplements.
When referring to a particular medium, the intention is to identify only the salt composition unless otherwise specified. Any number and concentration of amino acids, vitamins, growth regulators and organic supplements can be added in an infinite variety of compositions to a given salt composition in order to achieve the desired results.

Procedure:

1. Dehydrated M S Basal medium (Murashige and Skoog 1962) is used after dissolvingin deionized water, to which agar (0.75%) is added.
2. pH of the media to be adjusted between 5.6-5.8 using 1N HCl and 1 N NaOH.
3. For liquid cultures agar is not added.
4. Approximately, 30 ml of the media is poured in 300 ml capacity glass bottles withpolypropaline cap each and autoclaved at 121.6°C and 15 PSI pressure for 16 minutes.

Composition of M S Medium	
Ingredients	**milligrams/litre**
Potassium nitrate	1900.00
Ammonium nitrate	1650.00
Calcium chloride.$2H_2O$	440.00
Magnesium sulphate	180.69
Potassium phosphate monobasic	170.00

Manganese sulphate.H_2O	16.90
Boric acid	6.20
Potassium iodide	0.83
Molybdic acid (sodium salt).$2H_2O$	0.25
Zinc sulphate.$7H_2O$	8.60
Copper sulphate.$5H_2O$	0.025
Cobalt chloride.$6H_2O$	0.025
Ferrous sulphate.$7H_2O$	27.80
EDTA disodium salt.$2H_2O$	37.30
myo - Inositol	100.00
Thiamine hydrochloride	0.10
Pyridoxine hydrochloride	0.50
Nicotinic acid (Free acid)	0.50
Glycine (Free base)	2.00
Sucrose	30000.00
TOTAL gm/litre	34.54

Precautions:

- Regular stirring is to be done while dissolving the agar.
- Media should be dissolved in lower volume of around 800 ml and then volume shouldbe made up to 1000ml.

Practical 11: Callus induction from various explants, micro-propagation, hardening andacclimatization.

Materials required: Prepared media with phytohormones, laminar air flow, scalpel and forceps, ethanol.

Principle:

For successful initiation of callus culture and micro propagation, three important criteria should be accomplished:

1. Aseptic preparation of plant material,
2. Selection of suitable nutrient medium supplemented with appropriate ratio of auxinsand cytokinins and
3. Incubation of culture under controlled physical condition.

Procedure:

1. Wipe down and turn on the laminar air flow 15 minute before doing work in the hood.Flames –sterilize the instruments.
2. The young, actively growing nodal segments (2-3 cm), apical buds or leaves are takenas explants and washed thoroughly under running tap water.
3. Later surface sterilised in 0.1% bavistin (systemic fungicide) solution(w/v) for 15minfollowed by rinsing 3-4 times with distilled water.
4. Finally, the explants are surface sterilized under laminar air flow cabinet, with 0.1%$HgCl_2$ (w/v) for 2-5 min and thoroughly rinsed with sterilized distilled water.
5. This sterilized nodal explants are inoculated in plantgrowth medium (ex-Murashigeand Skoog, 1962) solidified with agar (0.75%) and supplemented with different combinations of phytohormones (generally cytokinins for shoot multiplication and a combination of cytokinins and auxins for callus induction). pH of the media is adjusted between 5.6-5.8 and autoclaved at 121.6°C and 15 PSI pressure for 16 minutes.
6. Cultures are maintained in the growth chamber with 16h/8h (light/dark) photoperiodat 25 ± 2 °C with light intensity of 25 μmol m^{-2} s^{-1} by cool-white fluorescent lamps.
7. Rooting of in-vitro multiplied shoot lets are induced in plant growth medium supplemented with different concentrations of auxins.

8. The plantlets thus generated were hardened primarily in portray containing vermicompost: coco peat (1:1) inside a poly tunnel for 8- 10 days followed by secondary hardening for 20 days in poly bags containing FYM: garden soil: sand(1:1:1) inside green house.

Result:

Precautions:

- Wear a lab coat and keep long hair tied back.
- Wipe down working surfaces and hands with ethanol.
- Use sterile equipment.
- Stay as organized as possible—label everything and set up all of your materials beforegetting started.
- Inspect all equipment and media for visible contamination before use.
- If you must completely remove a lid from a tube, plate or bottle, set it down within the hood with the open surface facing up. Otherwise, keep tubes, plates or bottles closed as much as possible.
- Do not pass your hands/arms over any open bottle, plate or tube.

PRACTICAL 12: DEMONSTRATION ON ISOLATION OF DNA

Materials Required: Extraction buffer, Microfuge tubes, Mortar and Pestle, Liquid Nitrogen, Absolute Ethanol (ice cold), 70 % Ethanol (ice cold), 7.5 M Ammonium Acetate 65° C water bath Chloroform : Iso Amyl Alcohol (24:1) Water (sterile), TE Buffer (10 mM Tris, pH 8, 1 mM EDTA).

Composition of extraction buffer (For 5 ml)

component	Stock concentration	Final Concentration	Volume taken from stock
Tris	1M	0.1 M	500 µl
EDTA	0.5M	20 mM	200 µl
NaCl	5M	1.4 M	1.40 ml

+ CTAB 0.1g (2%) + PVP 0.1g (2%) (Heatat 65° Ctill dissolved)+ β-mercaptoethanol 10µl (0.2%)

Principle:

Isolation of DNA using CTAB exploits that polysaccharides and DNA have different solubilities in CTAB depending on the concentration of sodium chloride. At higher salt concentrations, polysaccharides are insoluble, while at lower concentrations DNA is insoluble. Consequently, by adjusting salt concentration in lysates containing CTAB, polysaccharides and DNA can be differentially precipitated.Polyphenols are compounds that contain more than one phenolic ring (e.g., tannin), a structure that binds very efficiently to DNA. They are naturally occurring in plants, but are also generated when plants have tissue damage (browning). Upon the homogenization of plant tissues, polyphenols are synthesized by liberated polyphenol oxidase. The addition of polyvinyl pyrrolidone prevents the interaction of DNA and phenolic rings by binding up the polyphenols.

Procedure:

1. Weigh 200 mg leaves.
2. Crush with liquid nitrogen.
3. Add 1 ml extraction buffer.

4. Mix well and incubate at 65oC for 30 min.
5. Cool down to room temperature.
6. Add equal volume of chloroform : isoamylalcohol (24:1).
7. Centrifuge at 10000 rpm for 10 min. at 4˚C.
8. Take out upper aqueous phase in fresh tube.
9. Add 0.6 volume of chilled isopropanol.
10. Incubate at -20o C for 1 hour.
11. Centrifuge at 12000 rpm for 15 min. at 4˚C.
12. Discard supernatant and add 1ml 70% ethanol.
13. Centrifuge at 10000 rpm for 10 min. at 4˚C.
14. Discard supernatant and air dry pellet at room temperature.
15. Add 50 µl of TE (10:1) and store at 4˚C for overnight.

Result:

Precautions:

- Material finely ground in liquid nitrogen should be immediately transferred into theextraction buffer.
- In chloroform : isoamyl alcohol extraction, the aqueous phase should be carefully removed and organic phase re-extracted to ensure full recovery of DNA. If no separation is observed between the two phases, may be due to high concentration of DNA and /or cell debris in aqueous phase, dilution with more digestion buffer and re-extraction is the solution.
- Care should be taken to do the operations as gently as possible. Vortexing, pipettingusing fine tips etc. should be avoided to prevent the shearing of DNA.
- DNA should not be over dried as resuspension in TE become difficult.
- All the glassware, plastic ware, pestles and mortars etc. should be decontaminatedproperly. Care should be taken to prevent cross-contamination.
- Blank extraction controls are carried out along with normal extractions to check forany contamination.

Practical 13: Demonstration of gel electrophoresis techniques.

Materials required: TAE buffer, Agarose gel (1% in TAE buffer), loading dye, castingtray, gel electrophoresis unit, trans illuminator, ethedium bromide solution, distilled water.

Principle:

Agarose gel electrophoresis is a routinely used method for separating proteins, DNA or RNA. Nucleic acid molecules are size separated by the aid of an electric field where negatively charged molecules migrate toward anode (positive) pole. The migration flow is determined solely by the molecular weight where small weight molecules migrate faster than larger ones.In order to visualize nucleic acid molecules in agarose gels, ethidium bromide or SYBR Green are commonly used dyes. Illumination of the agarose gels with 300-nm UV light (under trans illuminator) is subsequently used for visualizing the stained nucleic acids.

Procedure:

1. Prepare a 1 % solution of agarose by melting 1 g of agarose in 100 mL of 1X TAEbuffer in a microwave for approximately 2 min.
2. Allow to cool for a couple of minutes then add 2.5 µl of ethidium bromide, stir to mix.
3. Cast a gel using a supplied tray and comb. Allow the gel to set for a minimum of 20min at room temperature on a flat surface.
4. Load the following into separate wells - 10 µL 1kb ladder, 5 µL sample + 1 µL 6x Loading dye.
5. Run the gel for 30 min at 100 V.
6. Expose the gel to UV light (under transilluminator) and photograph.
7. Confirm DNA quality, presence of a highly resolved high molecular weight band indicates good quality DNA, presence of a smeared band indicates DNA degradation.

Result:

Precautions:

- Temperature of the agarose solution should be around 55-65° C at the time of casting. Avoid pouring it very hot or cold.
- Ethidium bromide is a mutagen and should be handled as a hazardouschemical (Always wear gloves while handling).

PRACTICAL 14: DNA FINGERPRINTING

Materials Required:PCR unit, Gel electrophoresis Unit, Sourthern Blot Unit. DNA extraction chemicals and kit, restriction enzyme.

Principle:

Nearly 0.1% of DNA is unique to the individual that makes all the difference between two individuals. These differences are a consequence of mutations during evolution. As single change in nucleotide may make a few more cleavage site of a given nucleotide or might abolish some existing cleavage site. Thus, if DNA of any individual is digested with a restriction enzyme, fragments pattern (sizes) will be produced and will be difference in cleavage site position. This is the basics of DNA fingerprinting. DNA Fingerprinting is used by scientists to distinguish between individuals of the same species using only samples of their DNA. The process of DNA fingerprinting was invented by Sir Alec Jeffrey at the University of Leicester in 1985.

Procedure:

Unless they are identical twins, individuals have unique DNA. DNA fingerprinting – The name used for the unambiguous identifying technique that takes advantage of differences in DNA sequence. The process of DNA fingerprinting begins by isolating DNA from – blood, semen, vaginal fluids, hair roots, skin, skeletal remains, or elsewhere.

After we isolate the DNA and amplify it with PCR. Treat the DNA with restriction enzymes – cut DNA at specific sequences – Everyone's DNA is different, so everyone's DNA will cut at different sites .This results in different sized fragments. The different sized fragments are called restriction fragment length polymorphisms, or RFLPs. We can observe the different sized fragments in an experiment that separates DNA based on fragment size called Gel Electrophoresis.

Fragments of DNA from restriction enzyme cleavage are separated from each other when they migrate through a support called an agarose gel. The size-based separation of molecules of DNA separate based on size when an electric current is applied to an agarose gel.

The separated DNA fragments are then drawn out of the gel using a nylon membrane. The nylon membrane is treated with chemicals that break the hydrogen bonds in DNA and separate the strands. The single stranded DNA is cross linked to the nylon membrane – By heat or UV light. Incubate the nylon membrane with a radioactive probe of single stranded DNA complementary to the VNTRs.The radioactive probe shows up on photographic film –

Because as it decays it gives off light – The light leaves a dark spot on the film. Different individuals have different patterns of bands – these make up the fingerprint.

FUNDAMENTALS OF PLANT PATHOLOGY- AGS-206

PRACTICAL 1: ACQUAINTANCE WITH VARIOUS LABORATORY EQUIPMENTS AND MICROSCOPY.

1. Compound Microscope

It is used for microscopic examination of the samples in the laboratory.

i) Construction: A compound microscope consists of 3 major parts i.e. body, lens system and illumination system

a) The body of a microscope consists of the base, arm, body tube, stage, iris diaphragm and fine/coarse adjustment knobs etc. The base provides firm support for the rest of the microscope. Arm is used for carrying the microscope and supporting the optical/lens system. Coarse adjustment knob moves the body tube and objectives to bring the specimen roughly into focus. The fine adjustment knob brings the specimen into exact focus. Upper body tube encloses light path between objective and ocular. Lower body tube provides movable section for focusing objectives. Revolving nosepiece holds the objectives, which can be rotated into light path. The stage provides a platform with an opening in the centre on which a slide is placed which contains the specimen to be observed. The clamps or a movable slide holder is used to hold the slide in place. Condenser collects the light rays and focuses them on the specimen. Iris diaphragm is an assembly of thin metal leaves controllable by a lever to produce variable sized openings.

b) The lens system consists of eye piece, objectives and condenser

Eyepiece or the ocular of the compound microscope is composed of 2 or more lenses; the upper compartment or the eye lens is the magnifier and the lower compartment is called the field lens. The commonly used eyepieces are available with magnifications like 1X, 2X, 5X and 10X etc.

Objectives are considered to be the most important part of a compound microscope since they affect the quality of image formation. Most compound microscopes are equipped with 3 objectives having different magnifying powers. They are low power (10X), high power (40X) and oil-immersion (100X) objectives. Low power objective is the shortest and oil-immersion is the longest.

The primary function of a condenser is to supply sufficient cone of light to fill the objective aperture for getting maximum resolving power. Generally, condensers also incorporate iris diaphragm and filter holder. Iris diaphragm is used to control the light intensity.

c) Proper illumination is essential for the efficient utilization of the magnification and resolution of a microscope. The readily available source of illumination is ordinary daylight. As the intensity of daylight varies greatly, artificial light sources (generally a tungsten lamp) are more often used. The most precise of such light sources control the intensity, colour and size of the light beam. The size of the light cone differs with each objective. As the magnification of objective lens increases, the working distance decreases, and the angle of aperture of the objective increases. Therefore, with increasing magnification a larger cone of light must enter the objective.

ii) **Principle of working:** A compound microscope works on the basic principle of magnification, resolving power and illumination.

Magnification is obtained by a series of 2 lens system, the lens system nearest the specimen called objective, magnifies the specimen and produces a real image. The ocular or eye lens system magnifies the real image, yielding a virtual image that is seen by the eye. The total magnification is equal to product of the ocular magnification and the objective magnification.

Resolving power of a lens is its ability to show two closely adjacent points on the object as distinct and separate. This characteristic of a microscope is a function of the wave length of the light used and a characteristic of the lens system known as its numerical aperture:

Wave length

Resolving power = Diameter of the smallest structure visible =

Numerical aperture

The above relationship between the wavelength of light used and numerical aperture in determining resolving power holds good only for parallel light rays. When the specimen is illuminated with oblique rays in addition to direct light rays, the relationship becomes:

Wavelength

Resolving power=

2 x Numerical aperture

Use of compound microscope

- Place a slide on the stage with specimen side up, and centre the section to be examined as accurately as possible over the hole in the centre of the stage.

- Adjust the light source until it passes the maximum amount of light through the specimen. With low power objective in position, lower the body tube by means of the coarse adjustment until the objective is about 5-6 mm from the slide.
- Look through the eyepiece and slowly raise the objective with the coarse adjustment until the specimen is in approximate focus. Never focus downward while looking through the eyepiece. Bring the specimen into sharp focus with the fine adjustment. Adjust the iris diaphragm and sub-stage condenser until the light intensity is optimum, being neither glaring nor dull.
- After examining the specimen with the low-power objective, shift to the high power dry objective by rotating the nosepiece until the objective clicks into place, first making certain that the portion of the specimen you wish to view is exactly centered in the field of the low power objective.
- Look through the eyepiece and slowly raise the body tube with the coarse adjustment until the specimen comes into approximate focus. Then bring the image into final accurate focus by using the fine adjustment.
- Focusing of the oil-immersion objective requires more care than that of the other objectives, but the procedure is essentially the same. First use the low power objective to locate the portion of the specimen to be examined. Raise the body tube and then rotate the nosepiece until the immersion oil such as clove oil or cedar oil on the portion of the slide directly under the objective. Watching the objective form the side, carefully lower it into the oil. Do not allow the objective to touch the slide. Look through the ocular and slowly focus upward with the fine adjustment until the image appears.

Precautions

- Never touch the lenses. If the lenses become dirty, wipe them gently with lens paper.
- Never leave a slide on the microscope when it is not in use.
- Always remove oil from the oil immersion objective after its use. If by accident oil gets on either of the lower power objectives, wipe it off immediately with lens paper.
- Keep the stage of the microscope clean and dry.
- Do not tilt the microscope when working with the oil-immersion system.
- When the microscope is not in use, keep it covered and in the microscope compartment.
- Never allow an objective lens to touch the cover glass or the slide.
- Never force the microscope. All adjustments should work freely and easily.

Fig.- Compound Microscope

2. Autoclave

An autoclave is used for sterilization of media.

Principle: The principle of autoclave is that the water boils at about 100oC, depending upon the vapour pressure of the atmosphere. If the vapour pressure is increased, the temperature will be increased.

Construction: An autoclave is basically a double walled metallic vessel made of thick stainless steel or copper, one end of which has an opening fitted with a tightly closed lid. The lid is provided with pressure gauze to measure the steam pressure and a safety valve. There is also an exhaust valve below to let the steam escape from the bottom of the inner chamber. Autoclave may be jacketed or non-jacketed types. In Jacketed types, the duration of heating is less than in non-jacketed types, however, in non-jacketed ones, water does not condense on objects and the steam is dry, i.e. it does not contain particulate water.

Working: For most purposes, sterilization in autoclave is done for 15 minutes at 121oC temperature which is achieved at 1.05 kg/cm2 pressure.

Precautions

- Autoclave should not be overloaded.
- All the air must be removed from within the autoclave before closing the exhaust valve by keeping the outlet valve open until a jet of continuous air comes out of it.

- Sterilization time must be counted not from the time it is switched on but from the time the required pressure is built up.
- Ensure that there is sufficient water in the autoclave before switching on.
- At the end of the sterilization period, allow the steam pressure to drop to zero and only then open the lid.

Fig.- Autoclave

3. Laminar Air Flow

The laminar air flow system is used for reducing the chances of contamination of cultures while isolation and purification of microorganisms.

Principle: The laminar air flow system works on the principle of application of fibrous filters in air filtration. In this system, air of a closed cabinet is made to pass through high efficiency particulate air filters (HEPA) which filter the air and do not allow any suspended particle above 0.3 mm dimension to go out and as such the air is free of all suspended particles. The air is blown out at uniform velocity and in parallel flow line. Under operational conditions, it

provides class 100 air cleanliness levels (3.5 particles of 0.5 µm or larger size per litre) in the work area.

orking

- Just prior to using the working station, the working surface should be wiped clean with isopropyl alcohol or spirit.
- UV lamp should be turned on at least 15 minutes before work is performed.
- Air supply must be turned on while working on the station.

Precautions

- UV light should be switched off before starting work on the station.
- HEPA filters should be checked after every six months and should be changed when a filter is ruptured or so much loaded with contaminants that it will not produce the proper velocity across the working surface.

Fig.- Laminar Air Flow

4. Hot Air Oven

Hot air oven is a sterilizer using dry heat and is primarily used for sterilization of glassware.

Principle: The hot air oven is used for sterilizing glassware which are not burnt or damaged by high temperature. The action of dry heat is an oxidation process resulting from heat conduction from the contaminated object. Thus the entire object must be heated to a temperature for a sufficient length of time to destroy contaminants.

Construction: Hot air oven consists of an insulated double walled cabinet heated by electricity and can withstand high temperature. The walls of the oven are made up of stainless steel or aluminum and are designed to prevent heat conduction from inside the chamber. There is a motor and fan fitted either at the bottom or back to circulate hot air inside the chamber. This hot air increases the temperature inside the chamber thus sterilizing it. A thermostat regulates the temperature at the desired level and a thermometer is fitted for recording the temperature. The shelves within the hot air oven are perforated to allow proper air circulation.

Working: Generally, the oven is operated at a temperature of 160-180oC for 1 to 1½ hour. The time required for sterilization is inversely correlated to temperature. Commonly used time for different temperature regimes is 1 hour at 180oC, 2 hours at 170oC, 4 hours at 140oC, 12 to 16 hours at 120oC.

Precautions

- Exposure time is counted from the time when objects to be sterilized have reached the desired temperature in the oven.
- Calibrated glass should not be sterilized in hot air oven since the expansion and contraction can cause changes in the graduations.
- Rubber goods and culture media should not be sterilized in the hot air oven.
- After the sterilization process, the oven and its contents should be allowed to reach the ambient temperature before opening the door to prevent breakage and recontamination by cool air rushing into the chamber.

Fig.- Hot air oven

5. Incubators

Incubators are important equipments required for providing an optimum temperature condition for growing micro-organisms including plant pathogens. They are of many types.

i) Bottom heated incubator: A temperature from ambient to 60o C can be maintained in such incubator. Heating elements are provided at the bottom of the equipment, which is fitted with a thermostat, temperature regulatory knob and power on and off indicators.

ii) B.O.D. incubator: Also called cooling incubator, it is designed for the control of incubation/storage temperatures. Mostly, it consists of a cast iron wheel mounted cabinet which is double walled with inner chamber made of stainless steel and outer body made of mild steel duly powder coated. The chamber is provided with two/three removable shelves, made of stainless steel for complete flexibility in use. At the back of the chamber a special compartment is formed which is fitted with a refrigeration evaporator, heater and two powerful centrifugal air circulators for creating a positive air flow throughout the inner chamber for temperature uniformity. A temperature ranging from 5 to 60oC can be maintained by a thermostat.

ii) Orbital shaking incubator: It is especially designed to combined advantage of a closely controlled incubator/ storage temperatures with orbital shaking system necessary for many laboratory procedures. Such a set up is more useful for growing cultures of microorganisms and tissue cells under controlled conditions of temperature and agitation. The variable speed

platform containing samples is set into the circular motion in a horizontal plane, which ensures uniform mixing, minimizes accretion of material on the sides of flasks and provides good transfer of gas to the culture, and avoids undue buffeting of the organisms and cells. It consists of the same cabinet as of BOD incubator and can maintain a temperature ranging from 5 to 60oC.

Examination and record

1. Draw and label the various parts of these equipments.
2. Understand their principle and working, and record the same.

PRACTICAL 2: PREPARATION OF MEDIA, ISOLATION AND KOCH'S POSTULATES

Culture media

Microorganisms can be cultured in the laboratory on artificial substrates called culture media which contain a variety of substances. Most microorganisms require about one per cent of a carbon source in the form of sugar, less than 0.5 per cent of a nitrogen source as salt or yeast extract and small quantities of phosphate, sulphur, potassium, magnesium and traces of calcium, iron, zinc, manganese and molybdenum. These nutrients are supplied to the organisms in different inorganic forms, depending upon their capacity to utilize them.

The culture media commonly used in the laboratory are classified into non-synthetic (natural) and synthetic or organic and inorganic media. Non-synthetic media include organic substances with complex or simple molecules, while synthetic media consist of chemicals of known molecular structure and composition. Such media may be in liquid, solid or semi-solid form. In order to solidify the liquid medium containing the various ingredients one to two per cent agar agar or 10-20 per cent gelatin is added. In a Plant Pathology Laboratory, different synthetic media are prepared, e.g. Richard's, Czapeck-Dox etc. along with some specific media required for specific purposes. However, a non-synthetic potato-dextrose-agar (PDA) medium is routinely prepared and used.

Preparation of synthetic media

Dissolve weighed quantities of chemicals in measured quantities of water. Adjust the pH to the required level by adding N/10 NaOH or N/10 HCl. Filter the contents through absorbent cotton wool or a double layer of cheese cloth. Dispense filtrate into test-tubes or flasks. Plug them with cotton, and sterilize the medium in the test tubes and flasks in an autoclave at the

right temperature and steam pressure. For preparing solid agar medium, half the quantity of water is used for dissolving the chemicals and the remaining half for dissolving the agar agar powder. The agar is dissolved in water by slow heating with constant stirring. The melted agar is added to the other half of the medium containing the chemicals. Usually the pH is adjusted before mixing with agar. The mixture is then dispensed into tubes or flasks as desired, the mouth plugged with non-absorbent cotton and sterilized in an autoclave.

Preparation of potato-dextrose-agar medium

Composition

Peeled potatoes – 250 g

Dextrose (D-glucose) – 20 g

Agar agar – 20 g

Distilled water- 1000 ml

Procedure

Boil the peeled and sliced potatoes (250 g) in water for 30 minutes and then strain the broth through double-layered cheesecloth. Make the volume to one litre by adding distilled water. Add dextrose (20g) and agar agar powder (20 g) and sterilize the media in an autoclave.

Sterilization

Sterilization is the process of freeing a substance from living organisms. Several sterilization processes can be adopted in the laboratory, depending upon the material to be sterilized. Sterilization can be accomplished by direct heating over a flame, exposure to dry heat in a hot air oven or steam heat under pressure in an autoclave, filtration through suitable microbiological filters, chemical treatments and exposure to UV light, X-ray and radioactivity as follows:

i. The inoculating needle, scalpel, scissors and other metallic instruments used in handling microorganisms and the host tissues in the laboratory are usually surface sterilized by rectified spirit, and by heating over a flame for a few seconds.

ii. Glassware such as Petri dishes and pipettes are sterilized by heating at about 100oC for 2 hours in a hot air oven.

iii. Since moist heat is efficient in penetrating materials, it is used for sterilizing laboratory media. Usually test tubes or flasks containing media are autoclaved at 10 p.s.i. pressure (115.5oC) for 30 minutes, 15 p.s.i. pressure (121.6oC) for 15 minutes or 20 p.s.i. pressure (126.6oC) for 10 minutes, depending upon the substance contained in the medium.

iv. In case high temperature is likely to spoil the chemical composition of the medium, pasteurization can be adopted. It is a process by which the flasks or tubes containing the medium are steam heated at 60oC for one hour, on three successive days.

v. Materials which are destroyed even by minimum heating are sterilized either by treating with chemicals or by exposure to UV or X-ray.

vi. Sterilization through a sintered glass filter or Seitz filter or Millipore filter helps to preserve the qualities of the media which are likely to be destroyed by heat or exposure to different light rays.

KOCH'S POSTULATES: Three rules for experimental proof of the pathogenicity of an organism were presented in• 1883 by the German bacteriologist, Robert Koch; a fourth was appended by E. F. Smith (1905). Briefly, these rules state:

1. The suspected causal organism must be constantly associated with the disease.

2. The suspected causal organism must be isolated from an infected plant and grown in pure culture.

3. When a healthy susceptible host is inoculated with the pathogen from pure culture, symptoms of the original disease must develop.

4. The same pathogen must be re-isolated from plants infected under experimental conditions. These rules of proof are often referred to as Koch’s Postulates.

PRACTICAL 3: GENERAL STUDY OF DIFFERENT STRUCTURES OF FUNGI

1. Rhizomorphs: (rhiza=root, morph=shape)

Thick strands of somatic hyphae in which the hyphae loose their individuality and form complex tissues that are resistant to adverse conditions and remain dormant until favourable conditions return. The structure of growing tip of rhizomorphs resemble that of a root tip, hence the name rhizomorph. Eg. *Armillariella mellea.*

2. Sclerotium: (skleron=hard) pl.sclerotia:

It is a hard, round (looks like mustard seed)/ cylindrical or elongated (Claviceps) dark coloured (black or brown)resting body formed due to aggregation of mycelium, the component hyphae loose their individuality , resistant to unfavourable conditions and remain dormant for a longer period of timeand germinate on the return of favourable conditions. Eg. *Sclerotium, Rhizoctonia*

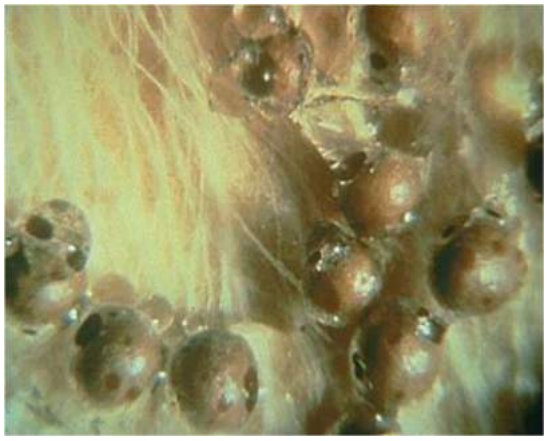


Fig.- *Sclerotium, Rhizoctonia*

3. Stroma: (stroma=mattress) pl.stromata.

A stroma is a compact, somatic structure or hyphal aggregation similar to a mattress or a cushion, on which or in which fructifications of fungi are usually formed. They may be of various shapes and sizes.Hyphal masses like acervuli, sporodochia, pionnotes etc. are the fertile stromata, which bearsporophores producing spores. Eg.*Claviceps, Cercospora*

4. Haustorium: (hauster=drinker) pl.haustoria. It is a outgrowth of somatic hyphae regarded as special absorbing organ produced on certain hyphae by parasitic fungi for obtaining nourishment. They may be knob like (Albugo), elongated (*Erysiphe, Uncinula*), finger like (*Peronospora*).

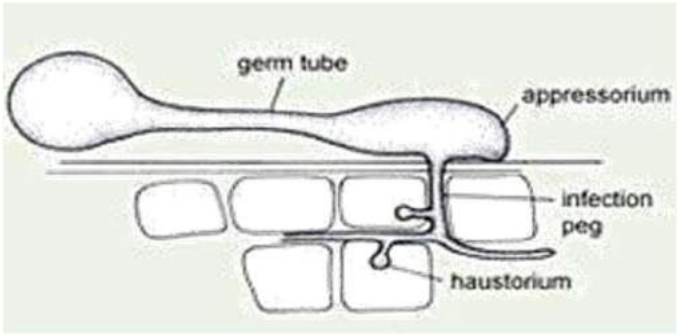


Fig. - Haustorium

5. Rhizoids: (rhiza=root, oeides=like)

These are slender root like branched structures found in the substratum produced by some fungi whichare useful for anchoring the thallus to substratum and for obtaining nourishment from the substrate. Eg. Rhizopus stolonifer.

6. Appresorium: (apprimere=to press against) pl.appressoria

A flattened tip of hyphae or germ tube acting as pressing organ by attaching to the host surface and gives rise to a minute infection peg which usually grows and penetrates the epidermal cells of the host. Eg. Puccinia, Colletotrichum, Erysiphe.

PRACTICAL 4: STUDY OF SYMPTOMS OF VARIOUS PLANT DISEASES

The changes in the host plant which serve to recognise the disease are called the signs and symptoms of the disease. The sign of a disease is the external appearance of some portion of the pathogen of the host. It may be some portion of the mycelium of the parasite or some spore stage.

The best examples are the rusty uredosori and black teleutosori of the Stem Rust of wheat, the smut spore stage of Ustilago and white blisters of white rusts (Albugo). These stages of the pathogens are at first covered but become exposed as the spores mature.

The symptoms are the visible effects which the parasite induces on the host plant. Any visible deviation on the host plant (both physiological and morphological) from the normal in structure and function is called a symptom. Generally the symptoms are growth responses.These are induced by the causal agent operating on the host. They furnish clues to find out the nature of the disease. Symptoms may affect the whole plant or be restricted to a particular organ or parts of an organ. They are, as a matter of fact, the danger signals of a disease. The moderm plant pathologists do not make any distinction between the two terms (signs and symptoms). They club them together as symptoms of a disease and define the term as external signs on the host plant which are characteristic of a given disease. These are the result of interaction between the host and the pathogen.

They divide the symptoms of a disease into two categories:

A. Symptoms due to the external appearance of the pathogen or some portion of the pathogen on the host (signs):

The somatic phase in most of the pathogens is usually invisible. It lies within the tissues of the host plant. During the disease cycle some portion of the parasite such as the reproductive or resting structures become visible by rupturing the overlying tissues of the host.

Some pathogens, however, are ectoparasites. In their case both the somatic and reproductive structures are visible externally and furnish a clue to the diagnosis of the disease. Common examples of such symptoms (signs) are:

1. Mildews: These are a group of important fungal diseases of seed plants in which the parasite is seen as a superficial growth on the host surface (leaves, green stems and fruits) in

the form of patches of varying sizes and colours. The mildews are of two kinds, downy mildews and powdery mildews. The downy mildews are all internal obligate parasites. They are characterised by superficial downy growth consisting of conidiophores and conidia on the host lesions in damp and warm weather.The powdery mildews are external parasites in which the mycelium forms whitish patches on the surface of the leaves of the host plant. The patches appear dusty or powdery with the formation of numerous white conidia which form a coating on the host surface.

2. Smuts: These are the fungal diseases of cereals and other members of grass family that cause the ears (particularly the ovaries) to turn black or sooty. The smut symptoms may as well appear on other parts (leaves, stem or roots) of the host plant.

3. Rusts: These are fungal diseases of grasses and other plants which appear on the host surface as small, coloured pustules-red, brown, yellow, orange, or black in colour.

4. Sclerotia: The conspicuous phase of some fungal diseases such as Ergot of rye is the formation of sclerotia in the position of kernels in the spike. The sclerotium is a compact, hard mass of dormant fungal hyphae. It may be black, greyish violet, dark brown or purplish in colour.

5. White blisters: These are white, shining, blister-like pustules found on the leaves of cruciferous plants caused by Albugo Candida, the white rust. These pustules expose the white, powdery masses of spores.

B. Symptoms which are the visible effects induced by the parasite on the host plant:

These are grouped under three categories necrosis, hypoplasia and hypertrophy. The other important symptoms are wilts, and damping off.

1. Necrosis: Death or killing of the host tissues induced by the attack of a pathogen is called necrosis. It may be caused by rots, blights, wilts, die back and cankers and may be general or local, gradual or rapid. When necrosis is general it is called decay and rotting.The causal organism may be a fungus or a bacterium. Rotting is universal in plants. It takes a heavy toll of useful plants. The rots are of many kinds. The common ones are dry rots, wet rots, soft rots, hard rots, brown rots, and black rots. These rots cause general necrosis and progress slowly or rapidly.

a) Lesions: In many cases necrosis is limited in extent. It is confined to small areas and is called local necrosis. It results in localised spots of diseased tissues. These are called the lesions. The tissue in the spot or the lesion area is dead. Examples of local necrosis are leaf and fruit spots. They are local lesions caused by fungi, bacteria and viruses. The spots are minute, circular or sub-circular and sometimes angular in outline. They are of different colours such as brown, dark, orange, red or white. The colour of the spot depends upon the nature of fungal spores present in the area. The leaf spot causes reduction in leaf surface and sometimes defoliation. The dead tissue of the leaf spot may fall out leaving circular or irregular perforations. These are called shot holes. The leaf shot holes are sharply bordered.

b) Cankers: Local necrosis also results in open wounds often sunken in stems and surrounded by living tissue. These are called the cankers. Canker is a dead lesion. Sometimes it is surrounded by a raised margin. Cankers may be due to slow rots of the outer parts of herbaceous and woody stems. They are thus usually limited in size. They may also be restricted in extent due to the formation of cork around and edges of the wound in the woody plants. When the canker partly encircles the branch its growth is subnormal. The branch is underdeveloped. If the canker completely encircles the branch the part of the branch beyond the canker dies.

(c) Blight: In many cases the leaves, stems or twigs, in response to the attack of the pathogen undergo rapid discolouration followed by death. The dead parts become dark or brown in colour. This condition of the affected organ is called the blight.

(d) Rot: It is a disease in which the affected tissues die and undergo decay. The rot diseases may be classified on the basis of the organ attacked such as leaf, stem or root rots, bud rot and fruit rot. On the basis of type of dissolution brought about by the causal agent, the rots may be classified as soft rot, dry rot, black rot and wet rot.

2. Hypoplasia: It is a subnormal cell production in response to the attack of the pathogen. It results in the subnormal growth of the parts attacked resulting in stunted growth and dwarfing of the host plant.

3. Hypertrophy (overgrowths): It is an abnormal increase in size of one or more organs of a plant in response to the attack of a pathogen. It results in abnormal growth causing distortions, swellings, leaf curls and galls. The excessive growth may be due to two

processes, hyperplasia and hypertrophy. The former consists in rapid cell division and thus increase in the number of cells of which the organ is composed. Hypertrophy leads to abnormal increase in the size of cells only.

4. Wilts: The drooping of the entire plant due to loss of turgidity is a common symptom of disease. The causes may be varied. The wilting due to a disease is permanent and eventually leads to death of the plant.

5. Damping off: Species of Pythium and Rhizoctonia are most important among the fungi which cause damping off disease of seedlings. Either the stem is attacked near the soil level or the crown of roots. The attacked region becomes weak and thus is not able to bear the load of the upper portion of the seedling. Consequently the seedling collapses. It topples down and dies. The seedling of many plants such as chilli, tobacco, tomato and mustard are prone to the damping-off disease.

PRACTICAL 5: STAINING OF PLANT PATHOGENIC BACTERIA

Gram Staining is the common, important, and most used differential staining techniques in microbiology, which was introduced by Danish Bacteriologist Hans Christian Gram in 1884. This test differentiate the bacteria into Gram Positive and Gram Negative Bacteria, which helps in the classification and differentiations of microorganisms.

Principle of Gram Staining

When the bacteria is stained with primary stain Crystal Violet and fixed by the mordant, some of the bacteria are able to retain the primary stain and some are decolorized by alcohol. The cell walls of gram positive bacteria have a thick layer of protein-sugar complexes called peptidoglycan and lipid content is low. Decolorizing the cell causes this thick cell wall to dehydrate and shrink, which closes the pores in the cell wall and prevents the stain from exiting the cell. So the ethanol cannot remove the Crystal Violet-Iodine complex that is bound to the thick layer of peptidoglycan of gram positive bacteria and appears blue or purple in colour. In case of gram negative bacteria, cell wall also takes up the CV-Iodine complex but due to the thin layer of peptidoglycan and thick outer layer which is formed of lipids, CV-Iodine complex gets washed off. When they are exposed to alcohol, decolorizer dissolves the lipids in the cell walls, which allows the crystal violet-iodine complex to leach out of the cells. Then when again stained with safranin, they take the stain and appears red in color.

Reagents Used in Gram Staining

- Crystal Violet, the primary stain
- Iodine, the mordant
- A decolorizer made of acetone and alcohol (95%)
- Safranin, the counterstain

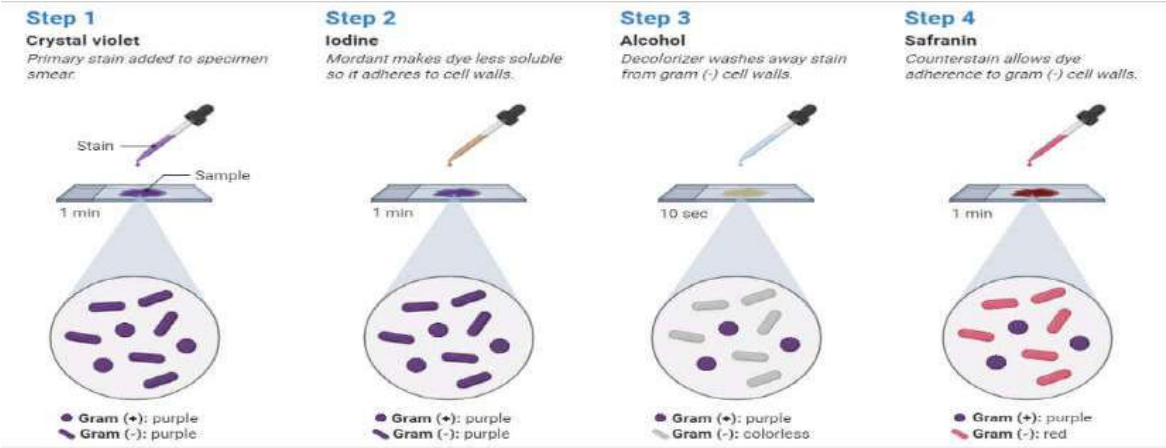
Procedure of Gram Staining

1. Take a clean, grease free slide.
2. Prepare the smear of suspension on the clean slide with a loopful of sample.
3. Air dry and heat fix
4. Crystal Violet was poured and kept for about 30 seconds to 1 minutes and rinse with water.
5. Flood the gram's iodine for 1 minute and wash with water.

6. Then ,wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water.
7. Add safranin for about 1 minute and wash with water.
8. Air dry, Blot dry and Observe under Microscope.

Gram Positive: Blue/Purple Color
Gram Negative: Red Color

Gram Positive Bacteria: *Actinomyces, Bacillus, Clostridium, Corynebacterium, Enterococcus, Gardnerella, Lactobacillus, Listeria, Mycoplasma, Nocardia, Staphylococcus, Streptococcus, Streptomyces* ,etc. Gram Negative Bacteria: *Escherichia coli (E. coli), Salmonella, Shigella, and other Enterobacteriaceae, Pseudomonas,Moraxella, Helicobacter, Stenotrophomonas, Bdellovibrio, acetic acid bacteria, Legionella* etc.



Preparation of slide of bacterial smear and differentiate gram negative to gram positive bacterial cells.

PRACTICAL 6: STUDY OF PHANEROGAMIC PLANT PARASITE

"The plants which produce flower and subsequently bear seeds in fruits and parasitized on other plants known as Phanerogamic plant parasite"

General Properties of Parasitic Plants

- Nutrients and water are transported via a Physiological bridge called the Haustorium.
- A parasite connects its vascular system (at least one of the tissue) to that of the host plant.
- The parasite may totally discard its own photosynthesis.
- Parasites may be mostly exposed at the surface of the host (Epiparasite).
- Mostly parasite hidden within the host organ (Endoparasite).
- The endoparasitic portion is composed of thread-like haustoria permeating the host tissue with a sinker, a single structure that becomes embedded in the host tissue.

Types of Phanerogamic Plant Parasites

There are two types of flowering plants

1. Stem Parasite
2. Root Parasite

Stem Parasite

- **Holoparasite:** Entirely Dependent

For Example: Dodder on gardens, ornamentals and hedge plants.

- **Semi-Parasite:** Partially Dependent

For Example: *Loranthus*on fruits, wasteland, roadside and forest trees.

Root Parasite

- **Holoparasite**: Entirely Dependent

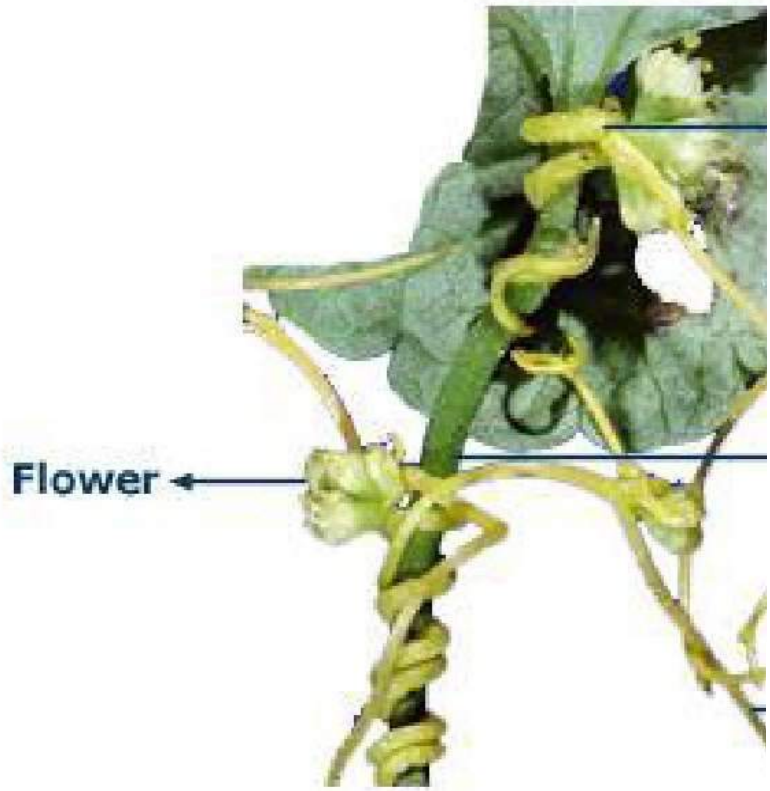
For Example: *Orobanche* on tobacco, mustard, Brinjal tomato, cabbage, cauliflower, turnip and many other solanaceous and cruciferous plants.

- **Semi-Parasite:** Partially Dependent

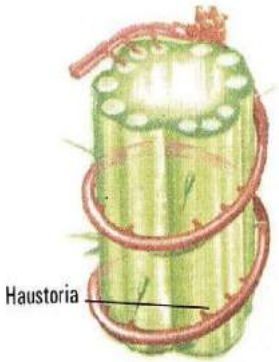
For Example: Striga on Sugarcane, Cereals, maize and Millets.

Dodder (Love vine, Amarbel)

- Scientific name*: Cuscuta* spp.
- It is a non chlorophyllous, leaf less parasitic seed plant
- It is yellow pink or orange in color and attached to the host.
- They do not bear leaves but bear minute functionless scale leaves
- Produces flower and fruits. Flower are white, pink or yellowish in color and found in clusters.
- Seeds are form in capsules. A single plant may produce 3000 seeds.



- When stem of parasitic plant comes in contact with host, the minute root like organsi.e. haustoria penetrates into the host and absorbs.



Mechanism

- The haustoria penetrate the stem or leaf and reach into the cortical region just outside the pericycle like an adventitious root.
- The haustoria secrete enzymes into the plant tissues that hydrolyze reserved food stuffs, such as, starch, and thus make them available to the dodder plant.

- These hydrolyzed substances and water are absorbed by the haustoria and are transported to the dodder stem where they are utilized for further growth and reproduction.

Overwintering and Dissemination

- As impurity of the crop seed.
- As seeds and stem pieces moved by irrigation water.
- As stem pieces present on the dry straw from infested fields.
- As seeds in the manure.
- As stem pieces transported by cattle and farm implements.
- As stem pieces carried by birds or strong winds.

Symptoms on host plants

- Host suffers from malnutrition
- Vitality of the host plant is reduced
- Plant may be dwarf
- Yellowing of the leaves with less flowers and fruiting
- In case of severe attack, the whole plant of the affected part may die.

Mistletoes/Banda

- Scientific name: *Lorunthussp/Dendrophthae* sp.
- Semi-parasitic plant of fruit and forest treee.g. Mango
- Dense cluster of twigs
- Smooth green leaves
- Tubular flower
- No root system
- Develop haustoria
- Synthesized carbohydrates
- It depends on host only for water and minerals but do not totally depend on host

Overwintering and dissemination

Overwinters as seeds.

- Spread by dispersal of its seed mostly through hybrids and to some extent by other animals.
- Birds are attracted by brilliant color of the fruit.
- Droppings of birds containing seeds also help in dissemination of the parasite

Development of parasitic relationship with host

- Seed lands on and becomes attached to the bark of a twig of host
- Germinates and produces a germ tube or radicle. This grows along the bark surface until it meets a bud or a leaf base, at which the radicle becomes broad and flattened on the side of the bark.
- Haustorium is produced and penetrate the bark directly and reaches the phloem and cambium.

Symptoms on host plant

- Stunted growth of the host
- Reduce the leaves size and may show unhealthy green color.
- Flowering and fruiting may be hampered.
- Tumor may form on the in1inch area.
- Quality and yield of fruit is considerably lowered.

Parasitic plant: Broom-rape

- Scientific name: *Orobanche* spp.
- Total root parasites
- Has a stout, fleshy, stem, 10-15 inches long.
- The stem is pale yellow or brownish red in color and is covered by small thin and brown scaly leaves.
- Flowers are white and tubular. The seeds are very small and black in color and may remain viable in the soil for several years.
- They lack chlorophyll and hence any green coloration and their leaves are vestigial.
- Above-ground stems are produced only for the

purpose of flowering and setting seed; in perennial species the plant may persist below ground, unseen for a number of years.

- Most species are highly host-specific, sometimes restricted to a single host species or genus. Others are capable of parasitizing a number of unrelated plants, but usually still show strong regional preferences.
- The seeds germinate when in contact with host roots, triggered by chemical recognition. The fine root of the broomrape grows into the host root, reaching and entering the vascular tissue. An underground tuber develops, from which, eventually, the flowering stems may develop.
- Broomrapes are thermophilic (warmth-loving) and often highly demanding in their habitat preferences.
- Frequently they require dry, open, often nutrient-poor grasslands but they are vulnerable to agricultural 'improvement', scrub development or other types of habitat loss.

How does it persist and disseminate?

The parasite overwinters as seed in the 1 ½ inches depth of soil. Dissemination of seeds are carried out by rain water, birds and even animals.

STRIGA

- Commonly known as witchweed, is a genus of28 species of parasitic plants that occur naturally in parts of Africa and Asia.
- The genus is classified in the family Orobanchaceae although older classifications place it in the Scrophulariaceae.
- Although most species of Striga are not pathogens that affect human agriculture, some species have devastating effects upon crops, particularly those planted by subsistence farmers.
- Three species cause the most damage: *Strigaasiatica, S. gesnerioides, and S. hermonthica.*
- Hosts: corn (maize, Zea) grasses, particularly sorghum and pearl millet, cowpea (Vigna unguiculata).

PRACTICAL 6: STUDY OF FUNGICIDES AND THEIRFORMULATIONS

Fungicides – definition

The word „fungicide‟ originated from two latin words, viz., fungus‟ and "caedo‟. The word „caedo‟ means „to kill.‟ Thus the fungicide is any agency/chemical which has the ability to kill the fungus. According to this meaning, physical agents like ultra violet light and heat should also be considered as fungicides. However, in common usage, the meaning is restricted to chemicals only. Hence, fungicide is a chemical which is capable of killing fungi.

Fungistat

Some chemicals do not kill the fungal pathogens. But they simply arrest the growth of the fungus temporarily. These chemicals are called fungistat and the phenomenon of temporarily inhibiting the fungal growth is termed as fungistatis.

Antisporulant

Some other chemicals may inhibit the spore production without affecting the growth of vegetative hyphas and are called as „Antisporulant‟. Even though, the antisporulant and fungistatic compounds do not kill the fungi, they are included under the broad term fungicide because by common usage, the fungicide has been defined as a chemical agent which has the ability to reduce or prevent the damage caused to plants and their products. So, some of the plant pathologists prefer the term „Fungitoxicant‟ instead of fungicide.

Characters of an ideal fungicide

1. It should have low phytotoxicity
2. It should have long shelf life
3. Stability during dilution
4. It should be less toxic to human being, cattle, earth worms , microorganisms etc.
5. It should be a broad spectrum in its action
6. Fungicide preparation should be ready for use
7. It should have compatibility with other agrochemicals

8. It must be cheaper one

9. It should be available in different formulations

10. It should be easily transportable.

Classification of Fungicides

Fungicides can be broadly grouped based on their (i) mode of action (ii) general use and (iii) chemical composition.

I. BASED ON MODE OF ACTION

Protectant

As the name suggests, protectant fungicides are prophylactic in their behaviour.Fungicide which is effective only if applied prior to fungal infection is called a protectant, eg., Zineb, Sulphur.

Therapeutant

Fungicide which is capable of eradicating a fungus after it has caused infection and there by curing the plant is called chemotherapeutant. eg. Carboxin, Oxycarboxin antibiotics like Aureofungin. Usually chemo therapeutant are systemic in their action and affect the deep-seated infection.

Eradicant

Eradicant are those which remove pathogenic fungi from an infection court (area of the host around a propagating unit of a fungus in which infection could possibly occur). eg. Organic mercurials, lime sulphur, dodine etc. These chemicals eradicate the dormant or active pathogen from the host. They can remain effective on or in the host for some time.

I. Based on general uses

The fungicides can also be classified based on the nature of their use in managing the diseases.

1. Seed protectants : Eg. Captan, thiram, organomercuries carbendazim, carboxin etc.

2. Soil fungicides (preplant) : Eg. Bordeaux mixture, copper oxy chloride, Chloropicrin,Formaldehyde Vapam, etc.,

3. Soil fungicides : Eg. Bordeaux mixture, copper oxy (for growing plants) chloride, Capton,PCNB, thiram etc.

4. Foliage and blossom : Eg. Capton, ferbam, zineb, protectants mancozeb, chlorothalonil

etc.

5. Fruit protectants : Eg. Captan, maneb, carbendazim, mancozeb etc.

6. Eradicants : Eg. Organomercurials, lime sulphur, etc.

7. Tree wound dressers : Eg. Boreaux paste, chaubattia paste, etc.

8. Antibiotics : Eg. Actidione, Griseofulvin, Streptomycin, Streptocycline, etc.,

9. General purpose spray and dust formulations.

II. Based on Chemical Composition

The chemical available for plant disease control runs into hundreds, however, all are not equally safe, effective and popular.Major group of fungicides used include salts of toxic metals and organic acids, organic compounds of sulphur and mercury, quinines and heterocyclic nitrogenous compounds. Copper, mercury, zinc, tin and nickel are some of the metals used as base for inorganic and organic fungicides. The non metal substances include, sulphur, chlorine, phosphorous etc. The fungicides can be broudly grouped as follows and discussed in detail.

Groups of Fungicides – Copper Fungicides, Sulphur Fungicides and Mercury Fungicides

Copper Fungicides

The fungicidal action of copper was mentioned as early as 1807 by Prevost against wheat bunt disease *(Tilletia caries),* but its large scale use as a fungicide started in 1885 after the discovery of Bordeaux mixture by Millardet in France. The mixture of copper sulphate and lime was effective in controlling downy mildew of grapevine caused by *Plasmopara viticola* andlater, late blight of potato *(Phytophthora infestans).*

Some other copper sulphate preparations later developed were Borduaux paste, Burgandy mixture and Cheshnut compound which are all very effectively used in the control of several plant diseases. In addition some preparations of copper oxy chloride preparations arev also mused. These are all insoluble copper compounds very successfully used in managing several leaf diseases and seeding diseases in nursery. Some of the important diseases controlled by copper fungicides are listed below.

COPPER SULPHATE PREPARATIONS

Bordeaux mixture

In 1882, Millardet in France (Bordeaux University) accidently observed the efficacy of the

copper sulphate against the downy mildew of grapes caused by *Plasmopara viticola.* When copper sulphate was mixed with lime suspension, it effectively checked the disease incidence. The mixture of copper sulphate and lime was named as "Bouillie Bordelaise" (Bordeaux Mixture). The original formula developed by Millardet contains 5 lbs of CuSO4 + 5lbs of lime + 50 gallons of water. The chemistry of Bordeaux mixture is complex and the suggested reactionis:

$CuSO_4 + Ca(OH)_2$ $Cu(OH)_2 + CaSO_4$

The ultimate mixture contains a gelatinous precipitate of copper hydroxide and calcium sulphate, which is usually sky blue in colour. Cupric hrdroxide is the active principle and is toxic to fungal spores. In metric system, to prepare one percent Bordeaux mixture the following procedure is adopted:

One kg of copper sulphate is powdered and dissolved in 50 litres of water. Similarly, 1 kgof lime is powdered and dissolved in another 50 litres of water. Then copper sulphate solution is slowly added to lime solution with constant stirring or alternatively, both the solutions may be poured simultaneously to a third contained and mixed well.

The ratio of copper sulphate to lime solution determines the pH of the mixture. The mixture prepared in the above said ratio gives neutral or alkaline mixture. If the quality of the used is inferior, the mixture may become acidic. If the mixture is acidic, it contains free copper which is highly phytotoxic resulting in scorching of the plants. Therefore, it is highly essential to test the presence of free copper in the mixture before applied. There are several methods to test the neutrality of the mixture, which are indicated below:

(i) Field Test: Dip a well polished knife or a sickle in the mixture for few minutes. If reddishdeposit appears on the knife/sickle, it indicates the acidic nature of the mixture.

(ii) Litmus paper test: The colour of blue litmus paper must not change when dipped in themixture.

(iii) pH paper test : If the paper is dipped in the mixture, it should show neutral pH.

(iv) Chemical test: Acid a few drops of the mixture into a test tube containing 5 ml of 10%potassium ferrocyanide. If red precipitate appears, it indicates the acidic nature of the mixture.

If the prepared mixture is in the acidic range, it can be brought to neutral or near alkaline condition by adding some more lime solution into the mixture. Bordeaux mixture preparation is cumbersome and the following precautions are needed during preparation and application.

(i) The solution should be prepared in earthen or wooden or plastic vessels. Avoid using

metal containers for the preparation, as it is corrosive to metallic vessels.

(ii) Always copper sulphate solution should be added to the lime solution, reverse the addition leads to precipitation of copper and resulted suspension is least toxic.

(iii) Bordeaux mixture should be prepared fresh every time before spraying. In case, the mixture has to be stored for a short time or a day, jaggery can be added at the rate of 100kg/100 litres of the mixture.

(iv) Bordeaux mixture is sometimes phytotoxic to apples, peaches, rice varieties like IR8 and maize varieties like Ganga Hybrid 3.

Bordeaux paste

Bordeaux Paste consists of same constituents as that of Bordeaux mixture, but it is in the form of a paste as the quantity of water used is too little. It is nothing but 10 percent Bordeaux mixture and is prepared by mixing 1 kg of copper sulphate and 1 kg of lime in 10 litres of water. The method of mixing solution is similar to that of Bordeaux mixture. It is a wound dresser and used to protect the wounded portions, cut ends of trees etc., against the infection by fungal pathogens.

Burgundy mixture

It is prepared in the same way as Bordeaux mixture, except the lime is substituted by sodium carbonate. So it is called as „Soda Bordeaux". It was developed Burgundy (France) in 1887 by Mason. The usual formula contains 1 kg of copper sulphate and 1 kg of sodium carbonate in 100 litres of water. It is a good substitute for Bordeaux mixture and used in copper-sensitive crops.

Cheshunt compound

It is compound usually prepared by mixing 2 parts of copper sulphate and 11 parts of ammonium carbonate. This formula was suggested by Bewley in the year 1921. The two salts are well powdered, mixed thoroughly and stored in a air tight container for 24 hours before being used. The ripened mixture is used by dissolving it in water at the rate of 3 g/litre. The mixture is dissolved initially in a little hot water and volume is made up with cold water and used for spraying.

I.COPPER CARBONATE PREPARATION

CHAUBATTIA PASTE

Chaubattia paste is another wound dressing fungicide developed by Singh in 1942 at Government Fruit Research Station, Chaubattia in the Almora district of Uttar Pradesh. It is

usually prepared in glass containers or chinaware pot, by mixing 800g of copper carbonate and 800g of red lead in litre of raw linseed oil or lanolin. This paste is usually applied to pruned parts of apple, pear and peaches to control several diseases. The paste has the added advantage that itis not easily washed away by rain water.

I. Copper carbonate preparation

III. Cuprous oxidePreparation	Fungimar, Perenox, Copper Sandoz, Copper 4% dust, Perecot, Cuproxd, Kirt i copper.	Cuprous oxide is a protective fungicide, used mainly for seed treatment and for foilage application against blight, downy mildew and rusts.
IV. Copper oxychloride Preparation.	Blitox, Cupramar 50% WP, Fytolan, Bilmix 4%, Micop D-06, Micop w-50, Blue copper 50, Cupravit, Cobox, Cuprax, Mycop.	It is a protective fungicide, controls *Phytophthora infestans* on potatoes and several leaf spot and leaf blight pathogens in field.

Sulphur fungicides

Use of sulphur in plant disease control is probably the oldest one and can be classified as inorganic sulphur and organic sulphur. Inorganic sulphur is used in the form of elemental sulphuror as lime sulphur. Elemental sulphur can be either used as dust or wettable sulphur, later being more widely used in plant disease control. Sulphur is best known for its effectiveness against powdery mildew of many plants, but also effective against certain rusts, leaf blights and fruit diseases.

Sulphur fungicides emit sufficient vapour to prevent the growth of the fungal spores at a distance from the area of deposition. This is an added advantage in sulphur fungicides as compared to other fungitoxicants.

Organic compounds of sulphur are now widely used in these days. All these compounds, called as „carbamate fungicides", are derivatives of Dithiocarbamic acid, Dithiocarbamates are broadly grouped into two, based on the mechanism of action.

Dithiocarbamates

Monoalkyl Dithiocarbamates Eg. Zineb, Maneb, Eg. Thiram, Ziram, Mancozeb, Nabam, Vapam Ferbam	Dialkyl Dithiocarbamates

List of sulphur fungicides and the important diseases controlled by them are tabulated below:

Trade Name	**Diseases Controlled**	
Inorganic Sulphur 1. Elemental Sulphur (i) Sulphur dust	Sulphur dust Cosan, Wetsulf, Microsul	Sulphur is a contact and protective fingicide, normally applied as sprays or as dust. It is generally used to control powdery mildews of fruits, vegetables, flowers and tobacco. This is also effective against apple scab (Venturia inaequallis) and rusts of field crops.
2. Lime Sulphur (Calcium poly sulphide)	It can be prepared by boiling 9 Kg or rock lime and 6.75Kg of sulphur in 225 litres of water.	Lime Sulphur is effective against powdery mildews as a protective fungicide.
Organic Sulphur	Hexathane 75% WP,	It is used to protect

(Dithiocarbamates) a. Monoalkyl	Dithane Z-78, Funjeb, Lonocol, Parzate C,	foliage and fruits of a wide range of crops
dithiocarbamate 1. Zineb (Zinc ethylene bisdithiocarbamate)	Du Pant Fungicide A,Polyram.	against diseases such as early and late blight of potato and tomato, downy mildews and rusts of cereals, blast of rice, fruitrot of chilly etc.
2. Maneb (Manganese ethylene bisdithiocarbamate)	Dithane M22, Manzate WP, MEB	These two are protective fungicide used to control many fungal diseases of field crops, fruits, nuts, ornamentals and vegetables, especially blights of potatoes and tomatoes, downy mildews of vines, anthracnose of vegetables and rusts of pulses.
3.Mancozeb (Maneb + Zinc ion)	Dithane M45, Indofil M45, Manzeb.	
4. Nabam (DSE) (Di Sodium ethylene bisdithiocarbamate)	Chembam, Dithane A-40, Dithane D-14, Parzate Liquid	Nabam is primarily used for foilar application against leaf spot pathogens of fruits and vegetables. Soil

		applications were also reported to have a systemic action on *Pythium, Flusarium* and *Phytophthora.* It is also used to control algae in paddy fields.
5. Vapam (SMDC) (Sodium methyl dithiocarbamate)	Vapam, VPM, Chemvape, 4-S Karbation, Vita Fume.	It is a soil fungicide and nematicide with fumigant action. It is also reported to have insecticidal and herbicidal properties. It is effective against damping off disease of papaya and vegetables and wilt of cotton. It is also effective against nematode infestation in citrus, potato and root knot nematodes in vegetables.
b. Dialkyl Dithiocarbamate 1. Ziram (Zinc dimethyl dithiocarbamate)	Cuman L. Ziram, Ziride 80 WDP, Hexaazir 80% WP, Corozate, Fukiazsin, Karbam white, Milbam, Vancide 51Z, Zerlate, Ziram, Ziberk, Zitox 80% WDP.	Ziram is a protective fungicide for use on fruit and vegetables crops against fungal pathogens including apple scab. It is non phytotoxic except to zinc sencitive plants. It is highly effective

		against anthracnose of beans, pulses, tobacco & tomato, and also against rusts of beans etc.
2. Ferbam (Ferric dimethyl dithiocarbamate)	Coromat, Febam, Ferberk, Femate, Fermate D, Fermicide, Hexaferb 75% WP, Karbam Black, Ferradow.	Ferbam is mainly used for the protection of foliage against fungal pathogens of fruits and vegetables including *Taphrina deformans* of peaches, anthracnose of citrus, downy mildew of tobacco and apple scab.
3. Thiram (Tetra methyl thiram disulphide)	Thiride 75 WDP, Thiride 750, Thiram 75% WDP, Hexathir, Normerson, Panoram 75, Thiram, TMTD, Arasan, Tersan 75, Thylate, Pomarsol, Thiuram.	It is used for seed treatment both as dry powder or as a slurry. It is a protective fungicide also suitable for application to foilage to control *Botrytis spp.* on lettuces, ornamental, soft fruits and vegetables, rust on ornamentals and *Venturia pirina* on pears. It is also effective against soilborne pathogens like *Pythium, Rhizoctonia* and *Fusarium.*

Mercury Fungicides

Mercury fungicides can be grouped as inorganic and organic mercury compounds. Both the groups are highly fungitoxic and were extensively used as seed treatment chemicals against seed borne diseases. Ignorance compounds show bactericidal property also. However, due totheir residual toxicity in soil and plants and their extreme toxicity nature to animal and human beings, the use of mercury fungicides is beings discouraged. In most of the countries, the use of mercury fungicides is banned and in countries like India, the use of mercury fungicides is restricted only in seed treatment for certain crops. The list of diseases against which mercury fungicides used are listed below

Common Name	Trade Name	Diseases Controlled
I. Inorganic Mercury 1. Mercuric chloride	Merfusan, Mersil	It is used for treating potato tubers and propagative materials of other root crops
2. Mercurous chloride	Cyclosan, M-C Turffungicide.	Mercurous chloride is limited to soil application in crop protection use because of its phytotoxicity.
II. Organomercurials Methoxy ethyl mercury Chloride Phenyl mercury chloride	Agallol, Aretan, Emisan, Ceresan wet (India) Ceresan Dry (India),Ceresol, Leytosan.	These are used mainly for treatment of seeds and planting materials. These fungicides are used for seed treatment by dry, wet or slurry method. For seed treatment 1% metallic mercury is applied at 0.25% concentration

Ethyl Mercury Chloride Tolyl mercury acetate	Ceresan (USA)Agrosan GN.	

Heterocyclic Nitrogen Compounds, Quinones and Miscellaneous Fungicides

Heterocyclic Nitrogen Compounds

Heterocyclic nitrogen compounds are mostly used as foliage and fruits protectants. Some compounds are very effectively used as seed dressers. Some of the commonly used fungicidesare listed below.

Common Name	**Trade Name**	**Diseases Controlled**
1.Captan (Kittleson‟s Killer) (N-trichloromethyl thio-4- cyclohexence-1,2-dicarboximide)	Captan 50W, Captan 75 W, Esso Fungicide 406, Orthocide 406, Vancide 89, Deltan, Merpan, Hexacap.	It is a seed dressing fungicide used to control diseases of many fruits, ornamental and vegetable crops against rots and damping off.
2. Captafol (Cis-N-1,1,2,2-tetra chloro hexane 1,2- dicarboximide)	Foltaf, Difolaton, Difosan, Captaspor, Foleid, Sanspor.	It is a protective fungicide, widly used to control foliage and fruit diseases of tomatoes, coffee potato.

3. Glyodin	Glyoxaliadine, Glyoxide,	It has a narrow specrum of
	Glyodin, Glyoxide Dry, Glyodex 30% liquid and 70% WP.	activity. As a spray, it controls apple scab and cherry leaf spot.
4.Folpet (Folpet) [N-(trichloromethyl-thi)] phthalimide	Phartan, Acryptan, Phaltan, Folpan, Orthophaltan.	It is also a protective fungicide used mainly for foliage application against leaf spots, downy and powdery mildews of many crops.

Benzene compounds

Many aromatic compounds have important anti-microbial properties and have been developed as fungicides. Some important benzene compounds commonly used in plant disease control are listed below.

Common Name	**Trade Name**	**Diseases Controlled**
1. Quintozene (PCNB)	Brassicol, Terraclor, Tritisan 10%, 20%, 40% D and 75% WP, PCNB 75% WP.	It is used for seed and soil treatment. It is effective against *Botrytis, Sclerotium, Rhizoctonia* and *Sclerotinia spp.*
2. Dichloran	Botran 50% WP and 75%WP, Allisan.	It is a protective fungicide and very effective against *Botrytis, Rhizopus and Sclerotinia* spp.
3. Fenaminsosuplh (Sodiumpdimethylamino benzenediazosulphonate	Dexon 5% G and 70% WP	It is very specific in protecting germinating seeds and growing plants from seeds as well as soil-borne infection of

		Phythium, Aphanomyces and *Phytophthora* spp.
4.Dinocap (2,4-dinitro-6-octyl phenylcrotonate)	Karathane, Arathane, DNOPC, Mildex, Crotothane, Crotothane 25% WP, Crotothane 48% Liq.	It is a non-systemic acaricide and control fungicide recommended to control powdery mildews on various fruits and ornamentals. It is also used for seed treatment.

Quinone Fungicides

Quinone areresent naturally in plants and animals and they exhibit anti-microbial activity and some compounds are successfully developed and used in the plant disease control. Quinones are very effectively used for seed treatment and two commonly used fungicides are listed below:

Common Name	**Trade Name**	**Diseases Controlled**
1. Chloranil (2,3,5,6-tetrachlora-1,4-benzoquinone)	Spergon	Chloronil is mainly used as a seed protectant against smuts of barely and sorghum and bunt of wheat.
2. Dichlone (2,3-dichloro-1,4-napthoquinone)	Phygon, Phygon XL WP.	Dichlone has been used widely as seed protectant. This is also used as a foliage fungicide, particularly against apple scab and peach leaf curl.

Organo – Phosphorous		It has a specific action
fungicide Ediphenphos (Edifenphos)(O-ethyl-SS-diphenyldithiophosphate)	Hinosan 50% EC and 2%D.	against *Pyricularia oryzae* (Rice blast). It is also effective against *Corticium sesakii* and *Cochliobolus miyabeanus* in rice.

Organo Tin compounds

Several other organic compounds containing tin, lead, etc. have been developed and successfully used in plant disease control. Among them, organo tin compounds are more popular and effective against many fungal diseases. These compounds also show anti bactericidal properties. Some of the organo tin compounds commonly used are listed below.

Common Name	**Trade Name**	**Diseases Controlled**
1. Fentin hydroxide (TPTHTiphenyltin hydroxide)	Du-Ter WP 20% or 50% WP. Du-Ter Extra-WP, Farmatin 50 WP, Du- Terforte WP, Tubotin.	It is a non-systemic fungicide recommended for the control of early and late blight of potato, leaf spot of sugar beet, blast of rice and tikka leaf spotof ground nut.
2. Fentin acetate (TPTATriphenyl tin acetate)	Brestan WP 40% and 60% WP.	It is a non systemic fungicide recommended to control *Ramularia* spp.on celery and sugar beetanthracnose and downy mildew It is effective against Cercospora leaf spot of

3. Fentin Chloride (TPTC-Triphenyl tinchloride)	Brestanol 45% WP,Tinmate.	sugarbeet and paddy blast

Systemic Fungicides and AntibioticsSystemic Fungicides

Since the late 1960s there has been substantial development in systemic fungicides. Any compound capable of being freely translocated after penetrating the plant is called systemic. A systemic fungicide is defined as fungitoxic compound that controls a fungal pathogen remote from the point of application, and that can be detected and identified. Thus, a systemic fungicide could eradicate established infection and protect the new parts of the plant.

Several systemic fungicides have been used as seed dressing to eliminate seed infection. These chemicals, however, have not been very successful in the cases of trees and shrubs. On the basis of chemical structure, systemic fungicides can be classified as Benzimidazoles, Thiophanates, Oxathilins and related compounds, pyrimidines, morpholines, organo-phosphorus compounds and miscellaneous group.

I. Oxathilin and related compounds

Oxathalins were the earliest developed compounds. This group of systemic fungicide is also called as carboxamides, carboxyluc acid anillides, carboxaanillides or simply as anillides which are effective only against the fungi belong to *Basidiomycotina* and *Rhizoctonia solani.* Some of the chemicals developed are (i) Carboxin (DMOC: 5,6 - dithydra-2-methyl-1, 4-oxathin-3-carboxanillide) and (ii) Oxycarboxin (DCMOD- 2,3-dihydro-5-carboxanillido-6-methyl-1, 4 oxathilin-4, 4, dioxide). The diseases controlled by these chemicals are listed below.

Common Name	**Trade Name**	**Diseases Controlled**
1. Carboxin (5,6-dinydro- 2-methyl-1-4-oxanthin-3-carboxanlido)	Vitavax 10% D, Vitavax 75% WP, Vitavax 34% liq. Vitaflow.	It is systemic fungicide used for seed treatment of cereals against bunts and smuts, especially loose smut of wheat

2. Oxycarboxin (5,6- dihydro-2-methyl- 1,4- oxathin-3-carboxianilid-4,4- dioxide)	Plantvax 5G, Plantvax5% liq. Plantvax 1.5 EC, 10% dust, 75 WP.	It is a systemic fungicideused for the treatment ofrust diseases of cereals, pulses, ornamentals, vegetables and coffee
3.Pyracarbolid (2-methyl- 5,6-dihydro- 4H-Pyran-3- carboxylic acid anilide).	Sicarol.	It controls rusts, smuts of many crops and *Rhizoctonia solani,* but is slightly more effective than carboxin

II. Benzimidazoles

The chemicals of this group show a very broad spectrum activity against a variety of fungi. However, they are not effective against bacteria as well as fungi belongs to *Mastigomycotina*. Two types of fungicidal derivates of benzimidazoles are known. The first type of derivates includes fungicides such as thiabendazole and fuberidazole. The fungicidal moiety of the second type is methyl-2-benzimidazole carbamate (MBC). The fungicides of this group may be simple MBC such as carbendazim or a complex from such as benomyl, which transforms into MBC in plant system. Some of the important diseases controlled by these compounds are shown below:

Common Name	**Trade Name**	**Diseases Controlled**
1.Benomyl (Methyl - 10 (butly carbomyl)-2 benzimidazole carbamate)	Benlate 50 WP, Benomyl. Bavistin 50 WP, MBC, Dersol, B.Sten 50, Zoom, Tagstin, Agrozim,	It is a protective and eradicative fungicide with systemic activity, effective against a wide range of fungi

2. Carbendazim (MBC) (Methyl -2- benzimidazole carbamate)	Jkenstin.	affecting field crops, fruits and ornamentals.It is very effective against rice blast, apple scab, powdery mildew of cereals, rose, curcurbits and apple and Diseases caused by *Verticillium and Rhizoctonia.* It is also used as pre-and postharvest sprays of dips for the control of storage rots of fruits and vegetables. Carbendazim is a systemic fungicide controlling a wide range of fungal pathogens of field crops, fruits, ornamentals and vegetables. It is used as spray, seedling dip, seed treatment, soil drench and aspost harvest treatment of fruits. It is very effective against wilt diseases especially, banana wilt. It controls effectively the sigatoka leaf spot of banana, turmeric leaf spot and rust diseases in many crops.
3. Thiabendazole (TBZ) (2,4-thiazoyl benzimidazole)	Thiabendazole, Mertect,Tecto, Storite.	It is a broad spectrum systemic fungicide effectivel againstmany major fungal diseases. Pathogenic fungal control

4.Fuberidazole (2, (2- buryl) -benzimidazole).	Voronit.	includes species of *Botrytis, Ceratocystis, Cercospora, Colletotrichum, Fusarium, Rhizoctonia, Sclerotinia, Septoria and Verticillium.* It is also effective for the post harvest treatment of fruits and vegetables to control storage diseases. It is used for the treatment of seeds against diseases caused by *Fusarium,* Particularly *F.nivale* on rye and *F.culmorum* of peas

III. Thiophanates

These compounds represent a new group of systemic fungicides based on thiourea. They are the derivatives of thioallophanic acid. These fungicides contain aromatic nucleus which is converted into benzimidazole ring for their activity. Hence, thiophanates are often classified under benzimidazole group and the biological activity of thiophanates resembles of benomyl. Two compounds are developed under this group are discussed.

Common Name	**Trade Name**	**Diseases Controlled**

1. Thiophanate(1,2 - bis (ethyl carbonyl-2- thioureido) benzene)	Topsin 50 WP, Cercobin50 WP, Enovit.	It is a systemic fungicide with a broad range of action, effective against
2. Thiophanate - methyl (1,2 bis (3 methoxycarbonyl-2-thioureido) benzene.)	Topsin-M70 WP, Cercobin-M 70 WP,Envovit-methyl, Mildothane.	*Venturia* spp., on apple and pear crops, powderymildews, *Botrytis* and *Sclerotinia* spp. On various crops. It is effective against a wide range of fungal pathogens, including *Venturia spp.* on apples and pears, *Mycosphaerella musicola* on bananas, powdery mildews on apples, cucurbits, pears and vines, *Pyricularia oryzae* onrice, *Botrytis and Cerospora* on variouscrops.

IV. Morpholines

Common Name	**Trade Name**	**Diseases Controlled**
Tridemorph (2-6 - dimethyl-4-cyclo - tridecyl morpholine)	Calixin 75 EC, Bardew, Beacon	It is an eradicant fungicide with systemic action, being absorbed through foilage

		and roots to give some protective action. It controls powdery mildew diseases of
		cereals, vegetables and ornamentals. It is highly effective against *Mycosphaerella, Exobasidium*

V. Pyrimidines, Pyridines, Piperidines and Imidazole

Common Name	**Trade Name**	**Diseases Controlled**
1. Triadimefon (1-(4-chlorophenoxy)-3, 3-dimethyl-1-(1-2-triazol-1yl) butan-2-one)	Bayleton, Amiral	It is very effective against powdery mildews and rusts of several crops.
2. Triadimenol (1-(4-Chlorophenoxyl-3, 3-dimethyl-1(1,2,4-triazol-1-yl) butan-2-ol)	Baytan	It is also very effective against powdery mildews and rusts of severalcrops.

3. Bitertanal (B-(1-1-biphenyl-4-yloxy-a-(1-1-dimethyl-ethyl-1-H-1,2-4- triazole-1-ethanol)	Baycor Terrazole 30% WP, Terrazole 95% WP,	It is highly effective against rusts and powdery mildewof a variety of crops. It is also used against *Venturia*and *Monilinia* on fruits and *Cereospora* leafspots of groundnut and banana.
4. Etridiazole (5-ethaoxy-3-trichloromethyl, 1,2-4-thiadiazole)	Terrazole 25% EC, Koban, Pansol EG, Pansol 4% DP, Turban WP, Terracoat Aaterra.	It is very effective against *Phytophthora* and *Pythium* spp. and seeding diseases of cotton, groundnut, vegetables, fruits and ornamentals

VI. Hydroxy Pyrinidines

Common Name	**Trade Name**	**Diseases Controlled**

1. Ethirimol (5-butyl 2- ethyl amino-4-hydrop-6-methyl pyrimidine)	Milliatem 80 WDP,Milcurb Super, Milgo	It is effective against powdery mildew of cereals and other field crops. It is also effective against powdery mildews of cucumber and ornamentals.
2. Dimethirimol (5-butyl2-dimethylamino-4- hydroxy-6-methy pyrimidine)	Milcurb	It is very effective against powdery mildews of chrysanthemum and cucurbits.
VII. Furan derivatives 1. Furcarbanil (2-5-dimethyl-3-furanilide)		It is used as seed or soil application, It systemically controlled bean rust and is being used as a seed dressing fungicide against loose smut of wheat and barley.
2. Cyclafuramid (N-cyclohexyl-2,5-dimethyl firamide)		It is effective against bunts, smuts and rusts of cereals, coffee rust, blister blight of tea, smut and red rot of sugarcane, *Fusarium wilt* of tomato, *Rhizoctonia* on tomato, potato, groundnut, rice as well as *Armillaria* sp. On rubber.

VIII.Benzanilidederivative 1. Mebenil (2-methyl benzanilide)		It is effective against yellow rust on wheat and barley *(P. striiformis)* and brown rust on barley *(P. hordei)*. It is also having direct fungitoxic activity against *Sclerotium rolfsli* and *Rhizoctonia.*

IX. Organo phosphorous compounds

Common Name	**Trade Name**	**Diseases Controlled**
1. Pyrazophos (2-0-0-	Afugan, Curamil, WP,	It is used to control

Diethylthionophosphoryl) -5- methyl-6-carbethoxy pyrazolo-(1- 5a)pyrimidine)	Missile EC.	powdery mildews of cereals, vegetables, fruits and ornamentals.
2. Iprobenphos (IBP)(S-benyzl-0-0-bisisopropylphosphorothiate)	Kitazin 48% EC, Kitazin17G, Kitazin 2% D.	It is used to control *Pyricularia oryzae* andsheath blight of rice.
X. Piperazine 1.Triforine(N,N-bis-(1-foramido-2,2,2- trichloroethyl-piperazine)	Saprol-EG, Fungitex.	It is effective againstpowdery mildew, scab and other diseases of fruits and rust on ornamentals and cereals.
XI. Phenol derivative 1. Choloroneb (1-4-dichloro-2,5-dimethoxy benzene)	Demonsan 65 WP, TersanSP, Turf fungicide	It is also active against storage diseases of fruits.It is highly fungistatic to *Rhizoctonia* spp., moderately so to *Pythium*spp. and poorly to *Fusarium* spp. It is used as a supplemental seed treatment for beans and soyabeans to control seedling disease

XIII. Other systemic fungicides

Common Name	Trade Name	Diseases Controlled
1. Metalaxyl (methyl-DLN-(2,6-dimethylphenyl-N-)2-methoxyacetyl	Apron 35 SD, Ridomil Ridomil MZ 72 WP (8% Metalaxyl + 64% Mancozeb) Beam, Bim Alliette 80 WP	It is a systemic fungicide and highly effective for specific use as seed dressing against fungal pathogens of the order Peronosporales.
2. Metalaxyl + Mancozeb		It is a fungicide with systemic and contact action and effective against damping-off, root rots, stemrots, and downy mildew of grapes and millets.
3. Tricyclazole (5-methyl-1,2,4 triazole(3,4b)- benzothiazole)		It is a specific fungicide used against paddy blast fungus, *P. oryzae*
4. FosetylAI. (Aluminium - Trisaluminium		It is a very specific Fungicide for Oomycetous fungi, especially against *Pythium* and *Phytophthora*

Antibiotics

Antibiotic is defined as a chemical substance produced by one micro-organism which islow concentration can inhibit or even kill other micro-organism. Because of their specificity of

action against plant pathogens, relatively low phytotoxicity, absorption through foliage and systemic translocation and activity in low concentration, the use of antibiotic is becoming very popular and very effectively used in managing several plant diseases. They can be grouped as antibacterial antibiotics and antifungal antibiotics. Most antibiotics are products of several actinomycetes and a few are from fungi and bacteria.

I. Antibacterial antibiotics

1. Streptomycin sulphate

Streptomycin is an antibacterial, antibiotic produced by streptomyces griseus. Streptomycin are streptomycin sulphate is sold as Agrimycin,-100, Streptomycin sulphate, Plantomycin, Streptocycline, Paushamycin, Phytostrip, Agristrep and Embamycin, Agrimycin - 100 contains 15 per cent streptomycin sulphate + 1.5 percent terramycin (Oxy tetracycline). Agristerp contains 37 percent streptomycin sulphate. Phytomycin contains 20 percent streptomycin. Streptocycline and paushamycin contains 9 parts f streptomycin and 1 part of tetracycline hydrochloride.

This group of antibiotics act against a broad range of bacterial pathogens causing blights, wilt, rots etc. This antibiotic is used at concentrations of 100-500 ppm. Some important diseases controlled are blight of apple and pear *(Erwinia amylovora)*, Citrus canker *(Xanthomonas campestris p.v. citri)*, Cotton black arm *(X.c.* p.v. *malvacearum)*, bacterial leaf spot of tomato *(Pseudomonas solanacearum)*, wild fire of tobacco *(Pseudomonas tabaci)* and soft rot of vegetables *(Erwinia carotovora)*.

In addition, it is used as a dip for potato seed pieces against various bacterial rots and as an disinfectant in bacterial pathogens of beans, cotton, crucifers, cereals and vegetables. Although it is an antibacterial antibiotic, it is also effective against some diseases caused by Oomycetous fungi, especially foot-rot and leaf rot of betelvine caused by *Phytophthora parasitica var. piperina.*

2. Tetracyclines

Antibiotics belonging to this group are produced by many species of Streptomyces. This group includes Terramycin or Oxymicin (Oxytetracycline). All these antibiotics are bacteriostatic, bactericidal and mycoplasmastatic. These are very effective against seed-borne bacteria. This group of antibiotic is very effective in managing MLO diseases of a wide range of crops. These are mostly used as combination products with Streptomycin sulphate in controlling

a wide range of bacterial diseases. Oxytetracyclines are effectively used as soil drench or as root dip controlling crown gall diseases in rosaceous plants caused by Agrobacterium tumefaciens.

II Antifungal antibiotics

1. Aureofungin

It is a hepataene antibiotic produced in sub-merged culture of Streptoverticillium cinnamomeum var. terricola. It is absorbed and translocated to other parts of the plants when applied as spray or given to roots as drench. It is sold as Aurefungin-Sol. Containing 33.3% Aureofungin and normally sprays at 50-100 ppm. The diseases controlled are citrus gummosis caused by several species of Phytophthora, powdery mildew of apple caused by *Podosphaera leucotricha* and apple scab (Venturia inaequalis), groundnut tikka leaf spot, downy mildew, powdery mildew and anthracnose of grapes, potato early and late blight. As seed treatment it effectively checked are *Diplodia* rot of mango, *Alternaria* rot of tomato, *Pythium* rot of cucurbits and *Penicillium* rot of apples and citrus. As a truck application/root feeding, 2 g of aureofungin- sol+1g of copper sulphate in 100 ml of water effectively reduce. Thanjavur wilt of coconut.

2. Griseofulvin

This antifungal antibiotic was first discovered to be produced by *Penicillium griseofulvum* and now by several species of *Penicillium,* viz., *P.patulum, P.nigricans, P.urticae, and P.raciborskii.* It is commercially available as Griseofulvin, Fulvicin and Grisovin. It is highly toxic to powdery mildew of beans and roses, downy mildew of cucumber. It is also used to control *Alternaria solani* in tomato *Sclerotinia fructigena* in apple and *Botrytis cinerea* in lettuce.

3. Cycloheximide

It is obtained as a by-product in streptomycin manufacture. It is produced by different species of *Streptomyces,* including *S.griseus* and S. *nouresi.* It is commercially available as Actidione, Actidione PM, Actidione RZ and Actispray. It is active against a wide range of fungi and yeast. Its use is limited because it is extremely phytotoxic. It is effective against powdery mildew of beans *(Erysiphe polygoni),* Bunt of wheat *(Tilletia spp.)* brownnot of peach *(Sclerotinia fructicola)* and post harvest rots of fruits caused by *Rhizopus* and *Botrytis* spp.

4. Blasticdin

It is a product of *Streptomyces griseochromogenes* and specifically used against blast disease of rice caused by *Pyricularia oryzae.* It is commercially sold as Bla-s.

5. Antimycin

It is produced by several species of *Streptomyces,* especially *S. griseus* and *S. Kitasawensis.* It is effectively used against early blight of tomato, rice blast and seedling blight of oats. It is commercially sold as Antimycin.

6. Kasugamycin

It is obtained from *Streptomyces kasugaensis.* It is also very specific antibiotic against rice blast disease. It is commercially available as Kasumin.

7. Thiolution

It is produced by *Streptomyces albus* and effectively used to control late blight of potato and downy mildew of cruciferous vegetables.

8. Endomycin

It is a product of *Streptomyces endus* and effectively used against leaf rust of wheat and fruit rot of strawberry *(Botrytis cinerea).*

9. Bulbiformin

It is produced by a bacterium, *Bacillus subtills* and is very effectively used against wilt diseases, particularaly redgram wilt.

10. Nystatin

It is also produced by *Streptomyces noursei.* It is successfully used against anthracnose disease of banana and beans. It also checks downy mildew of cucuribits. As a post harvest dip, it effectively reduces brown rot of peach and anthracnose of banana in stroage rooms. It is commercially marketed as Mycostain and Fungicidin.

11. Eurocidin

It is a pentaene antibiotic produced by *Streptomyces anandii* and called as pentaene G. It is effectively used against diseases caused by several species of *Colletotrichum* and *Helminthosporium.*

PRACTICAL 7: METHODS OF PESTICIDE A P P L I C A T I O N AND THEIR

SAFE USE

Proper selection of a fungicide and its application at the correct dose and the proper time are highly essential for the management of plant diseases. The basic requirement of an application method is that it delivers the fungicide to the site where the active compound will prevent the fungus damaging the plant. This is mostly achieved by spray, fog, smoke, aerosol, mist, dust, or granules applied to the growing plant or by seed or soil treatment.

In addition, some trees and shrubs can be protected by injection of fungicide liquid into the trunk or by brushing wounds with fungicide paints or slurries. In the case of sprays, mists, aerosols and fogs, the fungicide is in of droplets of water of another fluid. In the case of smokers, the solid particles of the fungicide are carried by the air. In the case of dusts and granules, the fungicide is straightly mixed with an inert carrier, impregnated into it coated on the particles, which are applied mechanically.

The object of spraying or dusting is to cover the entire susceptible surface of host with a thin covering of a suitable concentration of the fungicide before the pathogen has come into contact with the host. However, these practices may not effectively eradicate the inoculum present on the surface of the seeds or deep-seated in the seed. So, the application of chemicals as seed dressing is highly essential.

In addition, soil harbours several pathogens which cause root diseases in several crop plants. So treatment of soil with chemicals is also highly useful in reducing the inoculum load present in the soil. The fungicidal application varies according to the nature of the host part diseased and nature of survival and spread of the pathogen. The method which are commonly adopted in the application of the fungicides are discussed.

1. Seed treatment

The seed treatment with fungicides is highly essential because a large number of fungal pathogens are carried on or in the seed. In addition, when the seed is sown, it is also vulnerable to attack by many common soil-borne pathogens, leading to either seed rot, seeding mortality or produce diseases at a later stage. Seed treatment is probably the effective and economic method of disease control and is being advocated as a regular practice in crop protection against soil and seed-borne pathogens. Seed treatment is therapeutic when it kills pathogens that infect embroys, cotyledons or endosperms under the seed coat, eradicative when it kills pathogens that contaminate seed surfaces and protective when it prevents penetration of soil borne pathogens into the seedling. There are various types of seed treatment and broadly they may be divided intothree categories (a) Mechanical, (b) Chemical

and (c) Physical.

A. Mechanical method

Some pathogen when attack the seeds, there may be alteration in size, shape and weightof seeds by which it is possible to detect the infected seeds and separate them from the healthy ones. In the case of ergot diseases of cumbu, rye and sorghum, the fungal sclerotia are usually larger in size and lighter than healthy grains. So by sieving or flotation, the infected grains may be easily separated. Such mechanical separation eleminates the infected grains may be easily separated. Such mechanical separation eliminates the infected materials to a larger extent. This method is also highly useful to separate infected grains in the case of „tundu" disease of wheat. Eg. Removal of ergot in cumbu seeds.

Dissolve 2kg of common salt in 10 litres of water (20% solution). Drop the seeds into the salt solution and stir well. Remove the ergot affected seeds and sclerotia which float on the surface. Wash the seeds in fresh water 2 or 3 times to remove the salts on the seeds. Dry the seeds in shade and use for sowing.

B. Chemical methods

Using fungicides on seed is one of the most efficient and economical methods of chemical disease control. On the basis of their tenacity and action, the seed dressing chemicals may be grouped as (i) Seed disinfectant, which disinfect the seed but may not remain active for a long period after the seed has been sown and (ii) Seed protectants, which disinfect the seed surface and stick to the seed surface for sometime after the seed has been sown, thus giving temporary protection to the young seedlings against soil borne fungi. Now, the systemic fungicides are impregnated into the seeds to eliminate the deep seated infection in the seeds. The seed dressing chemicals may be applied by (i) Dry treatment (ii) Wet treatment and (iii) Slurry.

(i) Dry Seed Treatment

In this method, the fungicide adheres in a fine from on the surface of the seeds. A calculated quantity of fungicide is applied and mixed with seed using machinery specially designed for the purpose. The fungicides may be treated with the seeds of small lots using simpleRotary seed Dresser (Seed treating drum) or of large seed lots at seed processing plants using Grain treating machines. Normally in field level, dry seed treatment is carried out in dry rotary seed treating drums which ensure proper coating of the chemical on the surface of seeds. In addition, the dry dressing method is also used in pulses, cotton and oil seeds with the

antagonistic fungus like *Trichoderma vitide* by mixing the formulation at the rate of 4g/kg of the seed. Eg. Dry seed treatment in paddy.

Mix a required amount of fungicide with required quantity of seeds in a seed treating drum or polythene lined gunny bags, so as to provide uniform coating of the fungicide over the seeds. Treat the seeds atleast 24 hours prior to soaking for sprouting. Any one of the following chemical may be used for treatment at the rate of 2g/kg : Thiram or Captan or Carboxin or Tricyclazole.

(ii) Wet seed treatment

This method involves preparing fungicide suspension in water, often at field rates and then dipping the seeds or seedlings or propagative materials for a specified time. The seeds cannot be stored and the treatment has to be done before sowing. This treatment is usually applied for treating vegetatively propagative materials like cuttings, tubers, corms, setts rhizomes, bulbs etc., which are not amenable to dry or slurry treatment.

a. Seed dip / Seed soaking

For certain crops, seed soaking is essential. Seeds treated by these methods have to be properly dried after treatment. The fungicide adheres as a thin film over the seed surface which gives protection against invasion by soil-borne pathogens.

Eg. Seed dip treatment in paddy.

Prepare the fungicidal solution by mixing any of the fungicides viz., carbendazim or pyroquilon or tricyclazole at the rate of 2g/litre of water and soak the seeds in the solution for 2 hrs. Drain the solution and keep the seeds for sprouting.

Eg. Seed dip treatment in Wheat.

Prepare 0.2% of carboxin (2g/litre of water) and soak the seeds for 6 hours. Drain the solution and dry the seeds properly before sowing. This effectively eliminates the loose smut pathogen, *Ustilago nuda tritici.*

b. Seedling dip / root dip

The seedlings of vegetables and fruits are normally dipped in 0.25% copper oxychloride or 0.1% carbendazin solution for 5 minutes to protect against seedling blight and rots.

c. Rhizome dip

The rhizomes of cardamom, ginger and turmeric are treated with 0.1% emisan solutionfor 20 minutes to eliminate rot causing pathogen present in the soil.

d. Sett dip / Sucker dip

The sets of sugarcane and tapioca are dipped in 0.1% emisan solution for 30 minutes. The suckers of pine apple may also be treated by this method to protect from soilborne diseases.

(iii) Slurry treatment (Seed pelleting)

In this method, chemical is applied in the form of a thin paste (active material is dissolved in small quantity of water). The required quantity of the fungicide slurry is mixed with the specified quantity of the seed so that during the process of treatment slurry gets deposited on the surface of seeds in the form of a thin paste which later dries up.

Almost all the seed processing units have slurry treaters. In these, slurry treaters, the requisite quantity of fungicides slurry is mixed with specified quantity of seed before the seed lot is bagged. The slurry treatment is more efficient than the rotary seed dressers.

Eg. Seed pelleting in ragi.

Mix 2.5g of carbendazim in 40 ml of water and add 0.5g of gum to the fungicidal solution. Add 2 kg of seeds to this solution and mix thoroughly to ensure a uniform coating ofthe fungicide over the seed. Dry the seeds under the shade. Treat the seeds 24 hrs prior to sowing.

(iv) Special method of seed treatment

Eg. Acid - delinting in cotton

This is follows in cotton to kill the seed-borne fungi and bacteria. The seeds are treated with concentrated sulphuric acid @ 100 ml/kg of seed for 2-3 minutes. The seeds are then washed 2 or 3 times thoroughly with cold water and shade dried. After drying, they are again treated with captan or thiram @ 4g/kg before sowing.

II. Soil treatment

It is well known that soil harbours a large number of plant pathogens and the primary sources of many plant pathogens happens to be in soil where dead organic matter supports active or dormant stages of pathogens. In addition, seed treatment does not afford sufficient protection against seedling diseases and a treatment of soil around the seed is necessary to protect them.

Soil treatment is largely curativ in nature as it mainly aims at killing the pathogens in soil and making the soil „safe" for the growth of the plant. Chemical treatments of the soil is comparatively simple, especially when the soil is fallow as the chemical is volatile and disappears quickly either by volatilization or decomposition. Soil treating chemicals should be non-injurious to the plants in the soil adjacentto the area where treatment has been carried out because there may be standing crop in adjacent fields. The soil treatment methods involving the use of chemicals are: (i) Soil drenching, (ii) broadcasting, (iii) furrow application, (iv) fumigation and (v) chemigation.

(i) Soil drenching

This method is followed for followed for controlling damping off and root rot infections at the ground level. Requisite quantity of fungicide suspension is applied per unit area so that the fungicide reaches to a depth of atleast 10-15 cm.

Eg. Emisan, PCNB, Carbendazim, Copper fungicides, etc.

(ii) Broadcasting

It is followed in granular fungicides wherein the pellets are broadcasted near the plant.

(iii) Furrow application

It is done specifically in the control of some diseases where the direct application of the fungicides on the plant surface results in phytotoxic. It is specifically practiced in the control of powdery mildew of tobacco where the sulphur dust is applied in the furrows.

(iv) Fumigation

Volatile toxicants (fumigants) such as methyl bromide, chloropicrin, formaldehyde and vapam are the best chemical sterilants for soil to kill fungi and nematodes as they penetrate the soil efficiently. Fumigations are normally done in nursery areas and in glass houses. The fumigant is applied to the soil and covered by thin polythene sheets for 5-7 days and removed. For example, Formaldehyde is applied at 400 ml/100 Sq.m. The treated soil was irrigated and used 1 or 2 weeks later. Vapam is normally sprinkled on the soil surface and covered. Volatile liquid fumigants are also injected to a depth of 15-20 cm, using sub-soil injectors.

(v) Chemigation

In this method, the fungicides are directly mixed in the irrigation water. It is normally adopted using sprinkler or drip irrigation system.

III. Foliar application

A. Spraying

This is the most commonly followed method. Spraying of fungicides is done on leaves, stems and fruits. Wettable powders are most commonly used for preparing spray solutions. The most common diluent or carrier is water. The dispersion of the spray is usually achieved by its passage under pressure through nozzle of the sprayer. The amount of spray solution required for a hectare will depend on the nature of crops to be treated. For trees and shrubs more amount of spray solution is required than in the case of ground crops. Depending on the volume of fluid used for coverage, the sprays are categorised into high volume, medium volume, low volume, very high volume and ultra low volume. The different equipments used for spray application are: Foot-operated sprayer, rocking sprayer, knapsack sprayer, motorised knapsack sprayer (Power sprayer), tractor mounted sprayer, mist blower and aircraft or helicopter (aerial spray).

B. Dusting

Dusts are applied to all aerial parts of a plant as an alternative to spraying. Dry powders are used for covering host surface. Generally, dusting is practicable in calm weather and a better protective action is obtained if the dust is applied when the plant surface is wet with dew or rain drops. The equipments employed for the dusting operation are: Bellow duster, rotary duster, motorised knapsack duster and aircraft (aerial application).

IV. Post – harvest application

Fruits and vegetables are largely damaged after harvest by fungi and bacteria. Many chemicals have been used as spray or dip or fumigation. Post harvest fungicides are most frequently applied as aqueous suspensions or solutions. Dip application has the advantage of totally submerging the commodity so that maximum opportunity for penetration to the infection sites. Systemic fungicides, particularly thiabendazole, benomyl, carbendazim, metalaxyl, fosety-AI have been found to be very effective against storage diseases. In addition, dithiocarbamates and antibiotics are also applied to control the post-harvest diseases. Wrapping the harvested products with fungicide impregnated wax paper is the latest method available.

VI. Special method of applications

1. Trunk Application / Trunk Injection

It is normally adopted in coconut gardens to control Thanjavur wilt caused by *Ganoderma lucidum*. In the infected plant, a downward hole is made to a depth of 3-4" at an angle of 450C at the height of 3" from the ground level with the help of an auger. The solution containing 2g of Aureofungin soil and 1 g of copper sulphate in 100 ml of water is taken in a saline bottle and the bottle is tied with the tree. The hose is inserted into the hole and the stopper is adjusted to allow the solution in drops. After the treatment, the hole is covered with clay.

2. Root Feeding

Root feeding is also adopted for the control of Thanjavur wilt of coconut instead of trunk application. The root region is exposed; actively growing young root is selected and given a slanting cut at the tip. The root is inserted into a polythene bag containing 100 ml of the fungicidal solution. The mouth of the bag is tied tightly with the root.

3. Pseudostem Injection

This method is very effective in controlling the aphid vector *(Pentalonia nigronervosa)* of bunchy top of bannana. The banana injector is used for injecting the insecticide.Banana injector is nothing but an Aspee baby sprayer of 500 ml capacity. In which, the nozzle is replaced by leurlock system and aspirator needle No. 16. The tip of the needle is closed and two small holes are made in opposite direction.

It is for free flow of fluid and the lock system prevents the needle from dropping fromthe sprayer. One ml of monocrotophos mixed with water at 1:4 ratio is injected into the pseudostem of 3 months old crop and repeated twice at monthly intervals. The same injector can also be used to kill the bunchy top infected plants by injecting 2 ml of 2, 4-D (Femoxone) mixed in water at 1:8 ratio.

4. Corn Injection

It is an effective method used to control Panama will of banana caused by *Fusarium oxysporum* f. sp. *cubense.* Capsule applicator is used for this purpose. It is nothing but an iron rod of 7 mm thickness to which a handle is attached at one end. The length of the rod is 45 cm and an iron plate is fixed at a distance of 7 cm from the tip.

The corm is exposed by removing the soil and a hole is made at 45) angle to a depth of 5 cm.One or two gelatin capsules containing 50-60 mg of carbendazim is pushed in slowly and covered with soil. Instead of capsule, 3 ml of 2% carbendazim solution can also be injected into the hole.

5. Paring and Pralinage

It is used to control *Fusarium* wilt and burrowing nematode (*Radopholus similis)* of banana. The roots as well as a small portion of corm is removed or chopped off with a sharpknife and the sucker is dipped in 0.1% carbendazim solution for 5 minutes. Then, the sucker is dipped in clay slurry and furadan granules are sprinkled over the corm @ 40 g/corm.

FUNDAMENTALS OF ENTOMOLOGY- AGS-207

Practical-1:To study the methods of collection and preservation of insects including immature stages

Insect collection is a source of recreation for many people and may be a hobby for thosewho are interested in studying insects. Methods of collection and preservation of insects arethe pre-requisite to study the insects and their various internal and external organs. Aftercollection, it becomes imperative to keep and preserve the insect specimens intact and safefor longer time to further study the characters or to develop the insect collection museum.Let‘s have a look and do the different types methods of collection, devices used for collectionand preservation of insects including immature stages in this practical session.

Nature of insect collection

A good Zoological collection should consist of at least four (4) individual representativeof each of the order of insects. So that the collection comprises the great diversity and itshould reflect thedifferent forms occurs in insect fauna in a certain ecosystem.

Places of insect collection

Insects are omnipresent and abound anywhere and everywhere. A good place to startcollecting insects is a flowering hedgerow or garden where insects can be found on thedifferent parts of plants like flowers, leaves and stems. Besides these, we can also probe thesmall insects in the soil or near the roots of plants, aquatic insects can be collected in water,ponds, streams, rivers, lakes etc. and even do the indoors collection year round.They can be collected from- **Air (**flying insects), **Wate**r (dragonflies, mayflies andstoneflies that hover over water, aquatic insects and sea shore insects), **Home** (fromfurniture, boxes, bookshelves (fleas, bugs, flies, and mosquitoes), flower, fruits andvegetables brought in), **Debris and animal dung** (which acts as food source formanyinsects), and from **domestic animals and birds** (ecto and endo- parasites).

Catching insects: Aerial insects can be caught during flight or after they have alighted bysweeping a net through the air or foliage or by beating the foliage and holding the netbelow.

- The aerial/ sweep nets can catch aerial insects.
- Net forceps, dippers and dredge, can catch aquatic insects.

- Separator and Berlese funnel can catch soil dwelling insects.

Methods of insect collection

1. Hand picking

This method is suitable for catching the large insects like beetles and grasshoppers. It isvery tedious (hard working) method and not suitable for catching the biting and stingingnatured insects.

2. Aerial net or Butterfly net

It is light in weight, useful for catching activefliers like butterflies, moths, dragonflies,wasp, flies etc. The net consists of three partsviz., loop or frame; handle and porous muslinclothe bags. The diameter of hoop and thedepth of the bag should be in the proportionof 1:2.

3. Sweep net

It is heavier than the aerial net. It consists ofshort handle, a large loop and dense clothbag. This is suitable for collectingleafhoppers, grasshoppers and other smallinsects. The net is swept over vegetation.

1. Beating tray

This method is suitable for collectingcrawling insects and those, which rest onbranches. A beating tray is held under abranch, which is then hit sharply with a stick.

2. Aspirator/Potters/Suction tube

It is the device to collect small insects intoglass vials with no damage to the specimens.It is employed to suck in through a rubbertube small and minute insect that is alreadycollected in the net or sitting on wall orfoliage and on the bark of the tree. Usually itis meant for catching more active insects. Toprevent entry of insect in to mouth, a smallcloth piece is kept in between the glass andrubber tube.

3. Berlese (Tullgren) funnel

Soil arthropods can be sorted out by thismethods. Debris including soil arthropodscan be collected by using the light as thesource of heat in berlese funnel method.

4. Traps- Trapping is a method of collecting insects in the absence of collector. This is themost common methods or techniques used by growers in Integrated pest managementprogramme to catch the insects. There are many different types of traps used for collectionof insects. They are pheromone traps or sleeve traps, fruit fly trap, sticky traps, delta traps,water or Wota traps, pitfall trap, wind pan trap, malaise trap and light traps.

5. Pheromone traps

Synthetic sex pheromones are placed in the traps to attract male moths. The rubberizedsepta containing the pheromone lure are kept in the traps designed especially for thispurpose and used in monitoring, mass trapping and mating disruption programmes. Stickytarps, Water pan traps and funnel type models are available for use in pheromone basedinsect-pest control programmes. **Yellow sticky traps:** Aphids, whiteflies, thrips prefer yellow colour. Yellow colour is paintedon tin boxes and sticky material like castor oil/vaseline is smeared on the sticky material.

Probe trap: It is used by keeping them under grain surface to trap stored product pests.

Pitfall traps: Containers such as small plastic buckets, plant pots, glass jars or jam tins aresunk into the ground to trap flightless, ground-living insects and arachnids, especiallybeetles (ground beetles), cockroaches, crickets, spiders, harvestmen and mites. The containershould be placed in a hole with the upper rim flush with the ground surface. A killing agentand preservative, such as ethylene glycol, should be placed in traps that are not emptieddaily. Radiating vanes, such as wooden planks, placed in the substrate will increase theeffective area of the trap. A bait can be added to the trap to increase its effectiveness. Thetype of bait will depend on the specimens one wishes to catch.

Light traps: Light traps are mainly used for attracting moths & other night flying insectswhich are attracted towards the light. The insects are actively caught or encouraged to entera trap. The simplest light trap consists of a light on a cable hanging out in the field forattracting the pests during nights. However, besides a number of species of moths, beetles,flies, and other insects,

most of which are not pests, are also attracted to artificial light. Soidentification of pests and beneficial insects is of prime importance before any controloperation is executed.

Mercury vapour lamp light trap: This trap is the basic model designed by Robinson (1952).This trap produces ultravoilet, blue and green radiation with little red. This is currently usedtowards a wide range of noctuids and other nocturnal flying insects. a mercury lamp (125W)is fixed at the top of a funnel shaped (or) trapezoid galvanized iron cone terminating in a jarcontaining dichlorvos soaked in cotton as insecticide to kill the insect.

Killing insects

Killing should be immediately after capture. Potassium cyanide (KCN), ethyl acetate,carbon tetrachloride and chloroform are commonly used for killing insects. KCN kills theinsects quicklybut deadly poisonous and must be handled with extreme care. Ethyl acetatekills the insects slowly and does not last long. But the dead insects remain in relaxedcondition for a

longer time without becoming brittle and stiff.

Pinching- In this method, thorax is pressedbetween thumb and index finger swiftly andwith jerk.It needs constant practice. e.g., butterfly, grasshopper.

Injecting- Hypodermic injection of fluids.

Drowning- Larvae and insects without scales, hairs or powdery covering can be killed bysubmerging them in water. They die of Auto toxicity when excessive CO2 unable to escapesfrom spiracles and collects in trachea and tissues.

How to prepare Killing Bottle/ Cyanide Bottle?

Steps involved in preparing the killing bottles are given below -

- Take a wide mouthed strong bottle or vial with a tight fitting lid.
- Place a layer of potassium cyanidegranules/pellets (1/4-inch thickness) atthe bottom ofbottle.
- Cover it with a layer of dry plaster ofParis (1/4 -inch thickness)
- Mix plaster of Paris with enoughwater so that it will pour off from theend of spoon. Pour1/2-inch layer ofwet plaster of Paris over the dry layer.
- Tap the bottle lightly on the table toeliminate any bubble in the bottle.
- Leave the lid off for a day to let theplaster dry in a well-ventilated room,completely awayfrom direct sunlight.
- Keep a circular piece of filter orblotting paper on the top of plaster ofParis and avoidcondensation of water droplets on the side of bottom (to check thesweating process).
- Lastly, the bottle should be tightly corked and labeled with the word ‒Poison‖.
- In place of KCN, now a day‘s Ethyl acetate is being used as the replacement in killingbottle.

Insect Collection Box

Storage of insects is done in the insect boxes,which is made up of wood (top and bottomcould be of plywood) and lined on one(bottom) or both (roof also) sides with corksheets covered with white paper. It is light inweight, moisture proof and airtight. General(common) size of insect collection box is45x30x15 cm.

Labeling

Specimen collected should be uniform in size and labeled properly on stiff paper orreferencecard. Labeling consists of following notes i.e., **Host, Date, collector and**

Location.

Setting or stretching boards

Setting is the method that wings antennaeand Occasionally (Hymenopterans) spread legsin full display of their features. This methodneeds a setting or stretching board which havetwo side‘s boards separated by groove. Bothboard and grooves are lined with thin sheet ofcork. The widthof groove varies according to thewidth of insect body.

Methods of preservation Protection of Insect specimens

Collected Insects can be protected for longer time in insect collection box by putting thenephathalene balls on the corner side of box.

Preservation of insects

a) Temporary preservation

b) Permanent preservation: Insects can be permanently preserved either dry, in fluid, oron microscope slides. Arachnids are always preserved in liquid or on microscope slides. Themethod of preservation depends on the type of arthropods. It can be done by the followingmethods-

- **Dry preservation-** Insects that are to be preserved dry are best mounted in ways thatfacilitate study and permanent storage. Specimens should be mounted soonafterkilling, if possible while still soft.
- **Liquid preservation-** It is done in 70 % ethyl alcohol + 4 % formaline solution. **Softscale insects and mealybugs** can be preserved in mixture of 4 parts 90 % ethanol and 1part glacial acetic acid whereas, **thrips** can be preserved in a mixture of 9 parts 60 %ethanol and 1 part glacial acetic acid. It is very important to periodically check and topup containers of a liquid collection.
- **Mounting on a microscopic slide-** Small specimens have to be mounted on microscopeslides so that they can be studied under a compound microscope. These include groupssuch as thrips, aphids, parasitic wasps, scale insects, booklice, lice and mites. Insect andspider body parts (e.g. mouthparts and genitalia), and larvae often have to be slidemounted.Microscope slide mounts may be temporary or permanent, but specimensmaintained in collections require permanent mounts.

Bringing the specimen home or the laboratoryMaterials required

Butterflies and other large-wingedinsects can be stored in folded protectivepaper envelopes. Most arthropod specimenscan be conveniently stored between layers ofabsorbent paper.

Paper envelops(Newspaper, waxpaper) can be used to keepthe specimen and brought it to home orlaboratory (having good absorbent quality)Cellophane and transparent plastics can alsobe used for this purpose.

Relaxing container/Jar

Relaxing is the method / process of re-softening the insects. Relaxing container/Jar –contains a layer of sand (5 cm thick) or any other absorbent materials (basal wood, pith,synthetic sponge) and few drops of formaline or carbolic acid is added to prevent mould/fungal growth and then covered with filter paper. Cleaning- Dust, pollens and dirt can beremoved with a camel hairbrushdipped in water mixed with detergent.

Preparing insects for the insect collection box

Insects longer than about 8 mm are usually mounted on pins pushed through thethorax. Insect pins are longer than ordinary pins, and are made of stainless steel that doesnot rust. A No. 2 or No. 3 entomological pin is suitable for most insects, although those withdelicate bodies may require a size No. 0 or No. 1.

Entomological pins-There is three general series of pins viz.,**English pins:** Sold by weight, range of 18-30 mm in length and stout, used to pinlepidopteran insects, which lies or kept low in the box.

Continental pins: Sold by 100s, Range 35 mm in length,(000,00,0 & 1-7 Nos.), No. 2 & 3 areuseful for general purpose, 38 mm(No. 8-10), 50 mm(No. 11-12), 000 is the thinnest pin andNo. 12 is the thickest pin.

Minute pin: Minutest and finest pins, used for pinning the insects meant to stage, forminute, softest and fragile insects.

Pinning

It is the best and common method to preserve hard bodied insects. They will dry andremain in perfect condition on the pins for long time without any further treatment. Theyare pinned vertically through the body. Depending upon the size of insect‘s pins has to beselected accordingly. Exact place of insertion of the pin varies among different groups ofinsects.

Double mounting

Pinning is troublesome in smallerinsects. Very small insects cannot be pinnedbecause most ofthe body parts of the insectsare lost during pinning. For such insectsdouble mounting can be followed.

Staging

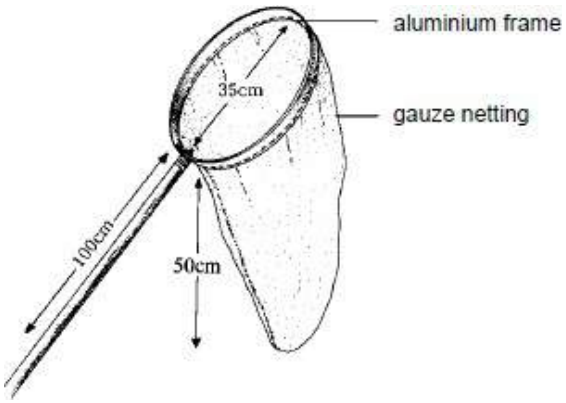
The stage is narrow rectangular piece of cork or pith. The small insect is pinnedcorrectly with amicro pin to the stage. Laterthe stage is pinned in the insect store boxwith a bigger pin.

Carding

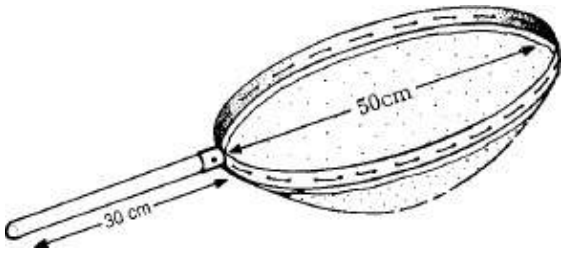
A rectangular white card (**5**x**8 or 5**x**12mm**) may be used as stage. On stage insteadof pinning, the insect specimen is stuck on itby **using glue or adhesive**. After mountingthe insect, card is pinned in the box with alarge pin.

Pointing / gumming

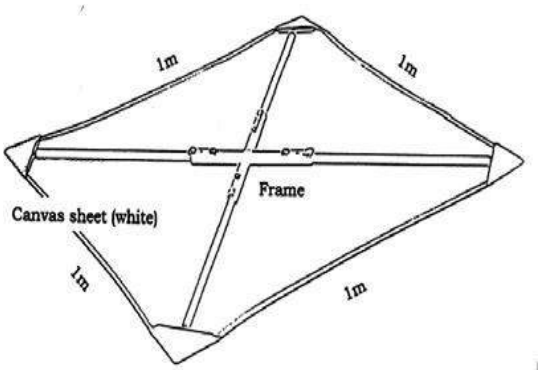
The insect specimen is glued to a cardcut into a triangle of 10 mm height and 5 mmbase. Bend down the tip of card to form asmall surface to which the insect is stuck.Apply a drop of glue or adhesive bytouching the point to the glue and to thethorax of the insects to be mounted.



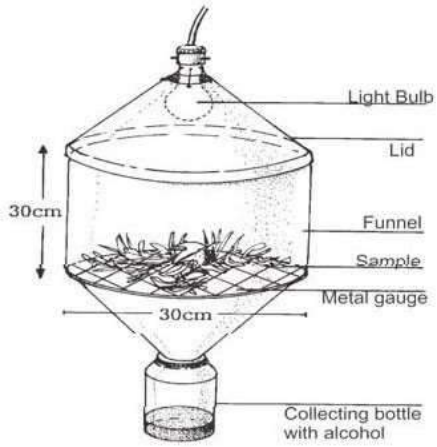
Aerial/Butterfly net



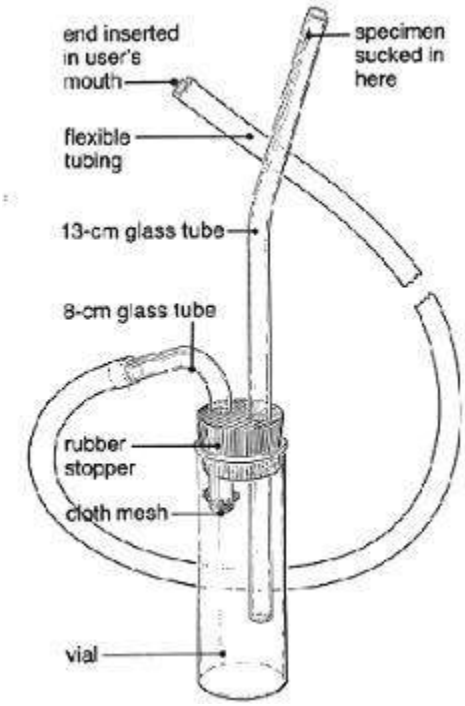
Sweep net

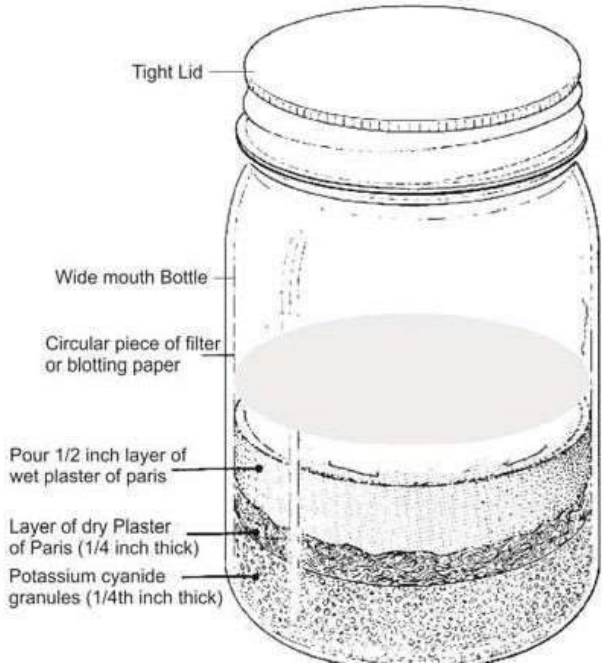


Beating tray

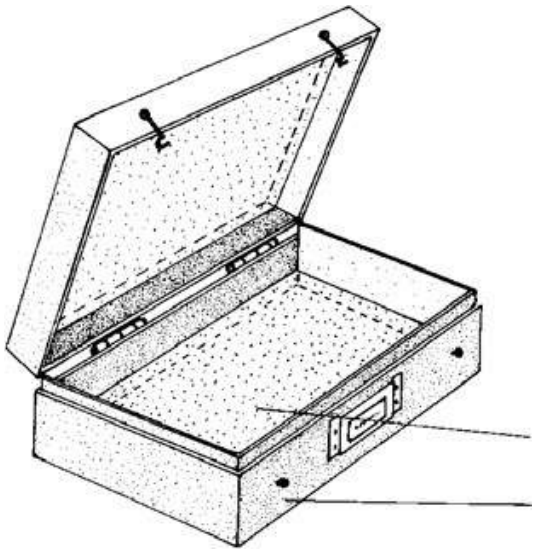


Berlese funnel

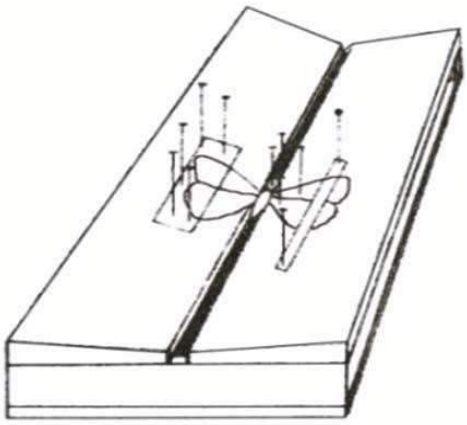




Aspirator/Potters/Suction tube **Insect killing box**

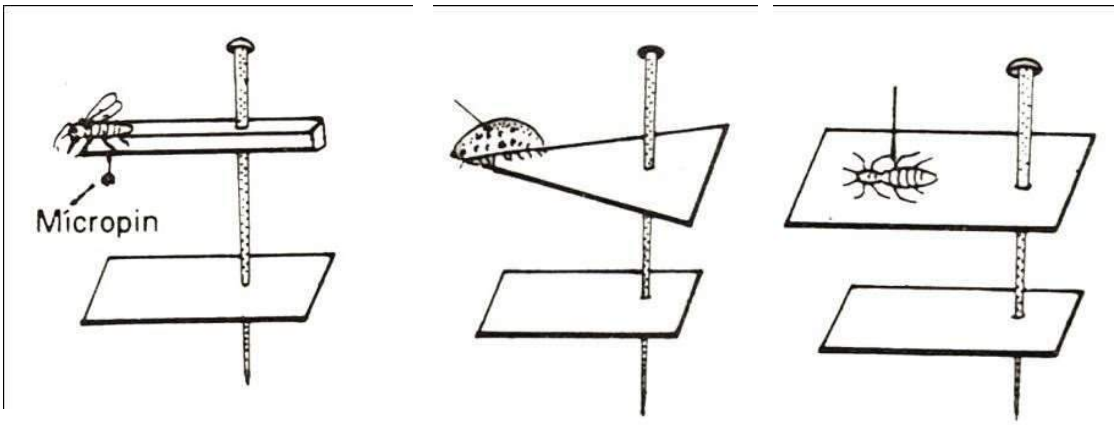


Insect collecting box

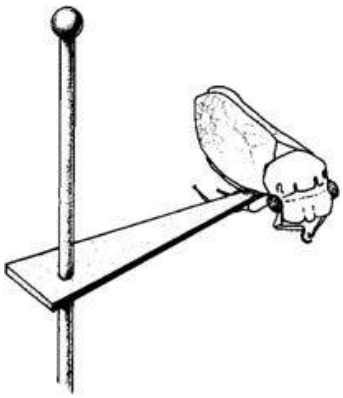


Setting/Stretching box

Double Mounting



Gumming



Practical – 2: To study the External features of Grasshopper

The grasshoppers are widely distributed throughout the country and may be seen inabundance during monsson season. For the generalized morphological description, thisinsect has been considered as the most suitable representative of class insects because itsstructural details are notmuch variable. Apart from this, being larger insize, it can bestudied easily.

The generalized insect body is divided into 3 distinct body regions: a head, a thorax andan abdomen. Grouping of body segments into distinct regions is known as **tagmosis** and thebody regions are called as **tagmata**.

The Head

This is an anterior part of the body formed by the fusion of six segments viz., ocellary,antennal, intercalary, mandibular, maxillary and labial. All these segments are closelyamalgamated to form a hard case or head capsule, the cranium that bears the antennae, eyesand mouthparts. The head is attached to the thorax by means of a flexible membranous neck(cervix) that allows its movement. Head capsule is sclerotized and the head capsuleexcluding appendages formed by thefusion of several sclerites is known as **cranium**.

Sclerites of Head

i. **Vertex**: Summit of the head between compound eyes.

ii. **Frons**: Facial area below the vertex and above clypeus.

iii. **Clypeus**: Cranial area below the frons to which labrum is attached.

iv. **Gena**: Lateral cranial area behind the compound eyes.

v. **Occiput** : Cranial area between occipital and post occipital suture.

Sutures of Head: The linear invaginations of the exoskeleton between two sclerites arecalled assuture (sometimes referred as sulcus).

i. **Epicranial suture/ ecdysial line**: Inverted `Y' shaped suture found medially on the topof head,with a median suture (coronal suture) and lateral sutures (frontal suture).

ii. **Epistomal suture/ Fronto clypeal suture**: Found between frons and clypeus. (epi – above;stoma- mouth parts)

iii. **Clypeo-labral suture**: Found between clypeus and labrum (upper lip).

iv. **Postoccipital suture**: Groove bordering occipital foramen. Line indicating the fusion ofmaxillary and labial segment.

The Thorax: It is a body region situated between head and abdomen. The insect thorax is

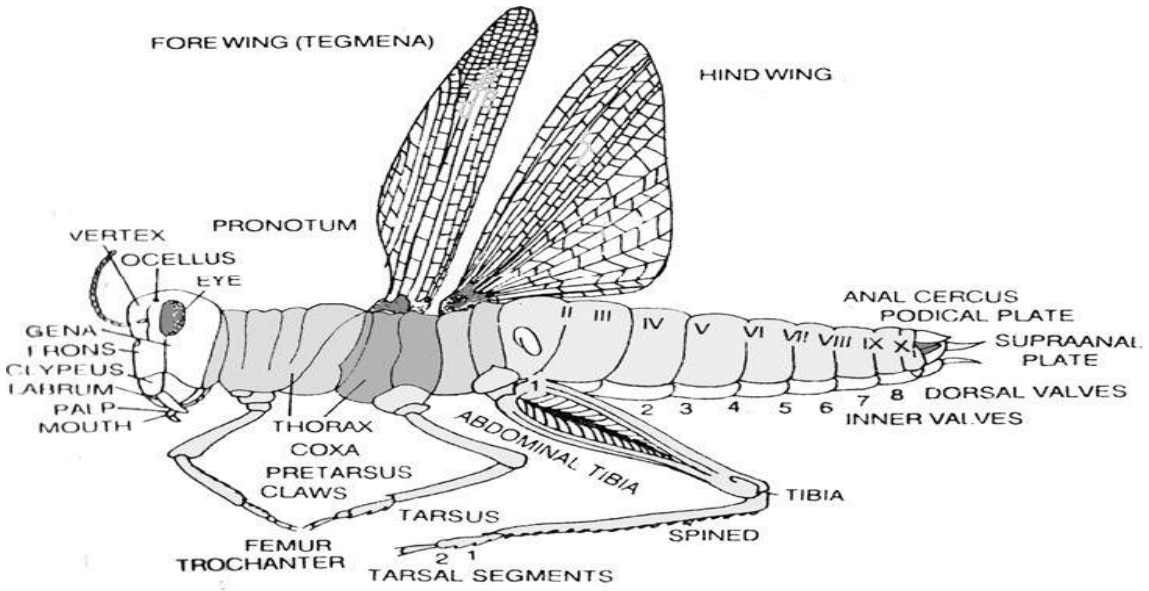
composedof three segments: an anterior prothorax, a middle mesothorax, and a posterior metathorax.Each segment bears a pair of legs. The last two segments often called as pterothorax maybear wings. Meso and metathorax which bear wings are called as **Pterothorax**. Thoracicsegments are made up of three sclerites namely, dorsal body plate **tergum or nota**, ventralbody plate **sternum** and lateral plate **pleuron**

Functions of thorax: Site of locomotion.

Abdomen: Abdominal segments are telescopic in nature, highly flexible and are interconnected bya membrane called **conjunctiva**. Each abdominal segment is made up of only two scleritesnamely dorsal body plate (tergum) and ventral body plate (sternum). In grass hopper

eightpairs of spiracles are present in the first eight segments, in addition to a pair of tympanum inthe first segment. Eight and ninth abdominal segments bears the female genital structureand ninth segment bears male genital structure. Abdominal appendages in adult insects aregenital organs and cerci.

Function: Site of metabolism and reproduction.



Practical -3: To study the types of insect antennae

The collected insect samples can be inspected in laboratory after detaching the antennaand put them under the microscope. They can also be studied through the permanent slidesof different types of antennae by the help of microscope.

Antennae are mobile sensory segmented appendages of the head. They articulate with headin front or between the eyes and arise from antennal socket. The size and shape ofantennae varies in different insects. They used for sensory perception whi ch includesmotion and orientation, odor, sound, humidity, and a variety of chemical cues. Sensilla onantenna acts as tactile, olfaction, carbon dioxide, temperature, wind, humidity, andsound receptors.

Structure of Antenna: Antennae consist of **three parts**:

a) Scape- It is first basal segment of antenna by which the antennae is attached to thehead. It is often distinctly larger than the other succeeding joints. It articulates withthe antennal ridge.

b) Pedicel- The joint immediately followed the scape is pedicel. It is usually smalland contains a special sensory structure known as **Johnston's organ**, which is absentin Diplura, Collembola.

c) Flagellum- It is also known as **clavola**, and is the remaining part of theantenna. Flagellum segments (flagellomeres) increase in number in certain insects. Itis modified according to the surroundings and habits of the insects.

Types of antennae:

1. **Setaceous**: (Bristle like) Size of the segments decreases from base to apex. e.g.Leafhopper,Dragonfly, Damselfly.
2. **Filiform**: (Thread like) Segments are usually cylindrical. Thickness of segments remainssamethroughout. e.g. Grasshopper.
3. **Moniliform**: (Beaded) Segments are either globular or spherical with prominentconstriction inbetween e.g. Termite.
4. **Serrate**: (Saw like) Segments have short triangular projections on one side. e.g.Longicornbettle
5. **Pectinate**: (Comb like) Segments with long slender processes on one side e.g. Sawfly
6. **Bipectinate**: (Double comb like) Segments with long slender lateral processes on boththesides e.g. Silkworm moth

7. Clavate: (Clubbed) Antenna enlarges gradually towards the tip. e.g. Blister beetle

8. Capitate: (Knobbed) Terminal segments become enlarged suddenly e.g. butterfly

9. Lamellate: (Plate like) Antennal tip is expanded laterally on one side to form flat platese.g.lamellicorn beetle

10. Aristate: The terminal segment is enlarged. It bears a conspicuous dorsal bristle calledarista

e.g. House fly

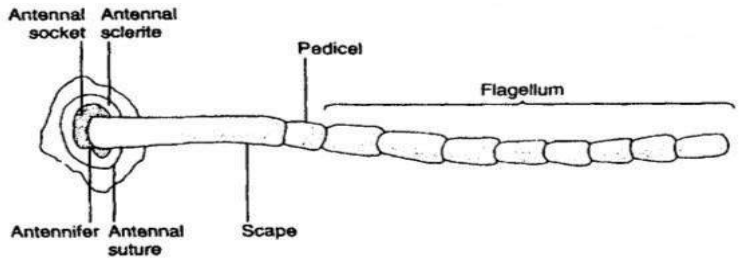
11. Stylate: Terminal segment bear a style like process eg. Horse fly, Robber fly.

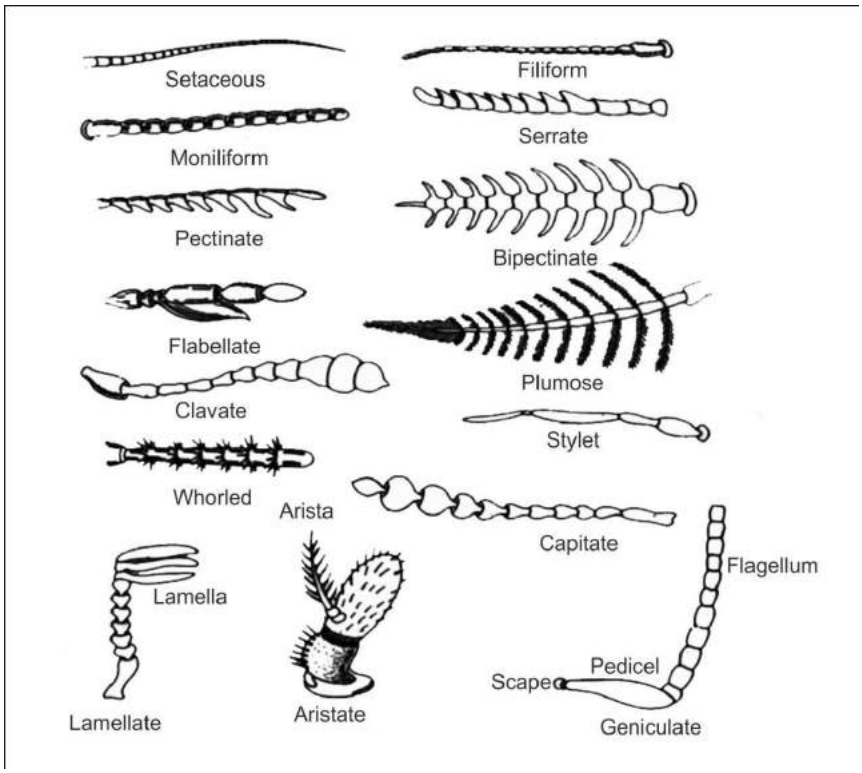
12. Plumose: (Feathery) Segments with long whorls of hairs e.g. male mosquito

13. Pilose: (Hairy) Antenna is less feathery with few hairs at the junction of flagellomeres.e.g.Female mosquito.

14. Geniculate: (Elbowed) Scape is long remaining segments are small and are arranged atanangle to the first resembling an elbow joint. e.g. Ant, weevil and honey bee.

Structure of the antennae





Practical- 4: To study the different types of mouthparts and their modifications

Mouthparts of insects vary among insects of different groups depending upon theirfeeding habits. They are mainly of two types viz., Mandibulate (feeding mainly on solidfood) andhaustellate (feeding mainly on liquid food). Insect mouthparts have becomemodified in variousgroups to perform the ingestion of different types of food and bydifferent methods. Indeed themodifications in the mouthparts to ingest almost all kinds ofthe food material, are one of thefactors for the success of the group.

1. **Biting and chewing type**: e.g. Cockroach & grasshopper.

It is the primitive type of mouth part and consists of the following parts.

i. **Labrum : (Upper lip)** It is flap like, bilobed and attached to the clypeus by an articularmembrane. It is movable. It covers the mouth cavity from above. It helps to pull thefood into the mouth. It holds the food in position so that mandibles can act on it. Itforms the roof ofthe pre oral food cavity.

ii. **Labrum-epipharynx:** Inner surface of the labrum is referred to as epipharynx. It isfrequently membranous and continuous with the dorsal wall of pharnyx. It is an organof taste.

iii. **Mandibles:** There is a pair of mandibles. They are the first pair of jaws. They are alsocalled as primary jaws or true jaws. Mandibles articulate with the cranium at twopoints. They are heavily sclerotised. They are toothed on their inner border. There aretwo types of teeth. Distalare sharply pointed and are called incisor or cutting teeth andproximal teeth are called molar or grinding teeth. They act transversely to bite andgrind the food into small fragments.

iv. **Maxillae:** They are paired and more complicated than mandibles. They are calledsecondary jaws or accessory jaws. At proximal end the first sclerite cardo joins themaxilla to head. The second sclerite is called stipes which articulates with cardo. Stipescarries a lateral sclerite called palpifer which bears a five segmented antenna likemaxillary palp. On the distal end of the stipes, there are two lobes. The outer lobe iscalled galea and inner lobe is lacinia which is toothed. Maxille direct the food into themouth. They hold the food in place when the mandibles are in action. They act asauxillary jaws and assist in mastication of food. Sense organs connected with theperception of touch, smell and taste are abundantly found in palpi.

v. **Hypopharynx :** It is a tongue like organ. It is located centrally in the preoral cavity.Salivary gland duct opens through it.

vi. **Labium /lower lip:** It is a composite structure formed by the fusion of two primitivesegmented appendages. It bounds the mouth cavity from below or behind. It forms thebase of the preoral cavity. It consists of three median sclerites viz., submentum (largebasalsclerite), mentum (middle sclerite) and prementum (apical sclerite). On the lateralside of the prementum there are two small lateral sclerites called palpiger bearing threesegmented labial palpi. Distally prementum bears two pairs of lobes. The other pair oflobes is called paraglossae and inner pair of lobes, glossae. Both pairs when fused arecalled ligula.

2. Piercing and sucking / hemipterous /bug type e.g. Plant bugs.

Labium projects downwards from theanterior part of the head like a beak. Beak isfour segmented and grooved throughoutits entire length. At the base of the labiumthere is a triangular flap like structurecalled labrum. Labium is neither involvedin piercing nor sucking. It functions as aprotective covering for the four stylets(fascicle) found within the groove. Both mandibles and maxillae are modified into long slender sclerotized hair likestructure called stylets. They are lying close together and suited for piercing and sucking. The tips of the stylets may have minute teeth for piercing the plant tissue. The innermaxillary stylets are doubly grooved on their inner faces. When these are closely opposedthey form two canals viz., food canal and salivary canal through sap and saliva areconducted respectively. Saliva contains enzymes or toxins that can distort plant cell wall topermit the stylets to penetrate down and reach phloem for suking the sap. Both palps areabsent.

3. Piercing and sucking / dipterous /mosquito type: e.g. Female mosquito

Mouthparts of female mosquito consistsof an elongate labium which is groovedforming a gutter which encloses six stylets.The stylets are composed of labrum -epipharynx (enclosing the food canal), thehyphophrynx (containing the salivary canal),two maxillae and two mandibles. Both theends of maxillary stylets and mandibularstylets are saw like and suited piercing flesh.The stylets are inserted into host's skin by a strong downward and forward thrust ofbody. Both mandibles and maxillae are reduced in male and they feed on plant nectar andjuices of decaying fruits. Female pierces the skin of human beings into which it injects salivacontaining an anticoagulant (to keep the blood flowing without clotting) and an anesthetic(to keep the victim unaware of the bite) and sucks up the blood. Labium does not pierce butfolds up or back as stylets pierce. Maxillary palpi are present.

4. Chewing and lapping type : e.g.honey bee

Labrum and mandibles are as inbiting and chewing type of mouth parts.But mandibles are bluntand not toothed.They are useful to crush and shape waxfor comb building; ingest pollen grains and other manipulative functions.Maxillolabial structures are modified toform the lappingtongue.The tongue unit consists of two galea of maxillae, two labial palpi and elongatedflexiblehairy glossa of labium. The glossa terminates into a small circular spoon shaped lobecalledspoon or bouton or flabellum which is useful to lick the nectar.

5. Rasping and sucking : e.g. Thrip

Mouth cone consists of labrum, labiumand maxillae. There are three stylets derivedfrom two maxillae and left mandible. Rightmandible is absent. Stylets are useful tolacerate the plant tissue and the oozing sap issucked up by the mouth cone. Bothmaxillary palpi and labial palpi are present.

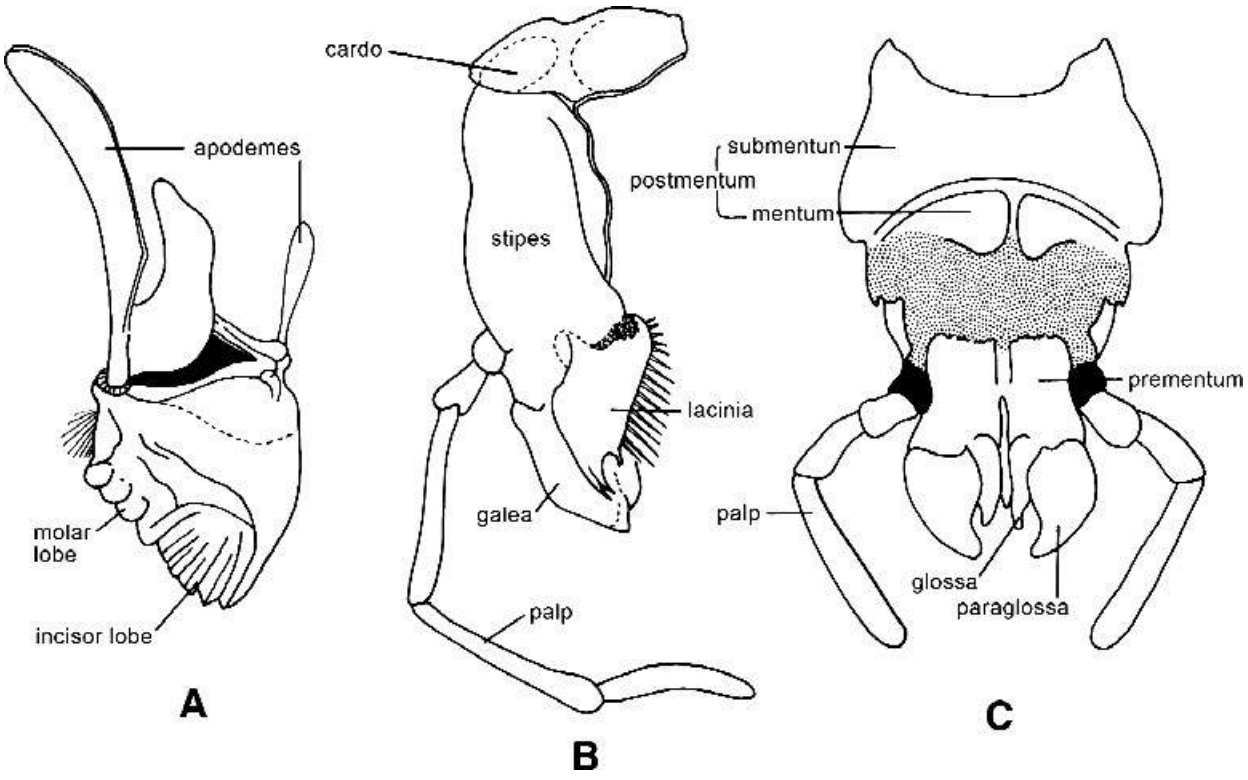
6. Sponging type : e.g. House fly

The proboscis is fleshy, elbowed,retractile and projects downwards fromhead. The proboscis canbe differentiatedinto basal rostrum and distal haustellum.The proboscis consists of labium which isgrooved on its anterior surface. Within thisgroove lie the labrum-epiphraynx (enclosing

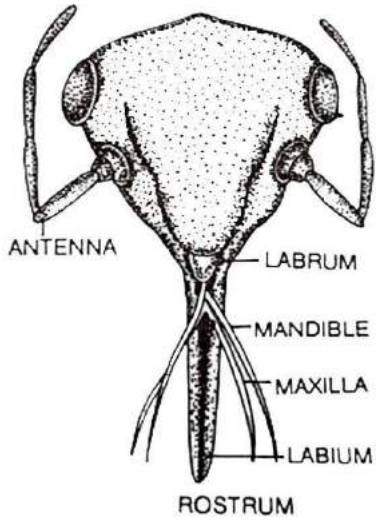
the food canal) and slender hypopharynx(containing the salivary canal).Mandibles are absent. Maxillae are represented by single segmented maxillary palpi.The end of the proboscis is enlarged, sponge like and two lobed which acts as suction pads.They are called oral discs or labella. The surfaces of labella are transvered by capillarycanals called pseudotracheae which collect the liquid food and convey it to the canal. Labellafunction as sponging organs and are capable of taking exposed fluids. These insects oftenspit enzyme containing saliva onto solid foods to liquify them.

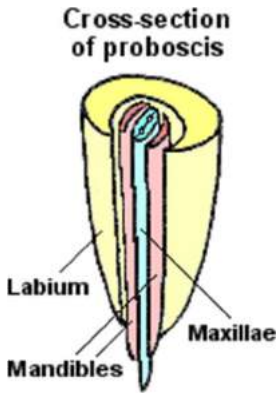
7. Siphoning type : e.g. Moths andbutterflies

Mouth parts consists of elongate sucking tube or proboscis. It is formed by two greatlyelongated galeae of maxillae which are zippered together by interlocking spines and hooks.Galeae are grooved on their inner surface and when they are fitting together closely theyform a suctorial food canal through which the nectar is sucked up. The proboscis is coiled uplike watch springand kept beneath the head when it is not in use. By pumping of blood intogaleae, the proboscis is extended. The other mouth parts are reduced or absent except thelabial palpi and smaller maxillary palpi.



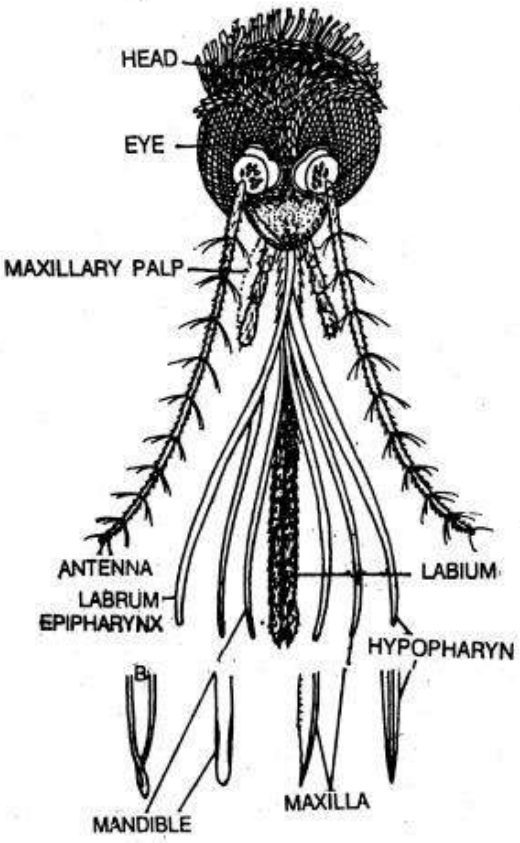
Structure of (A) mandible, (B) maxilla, and (C) labium of a typical chewing insect.

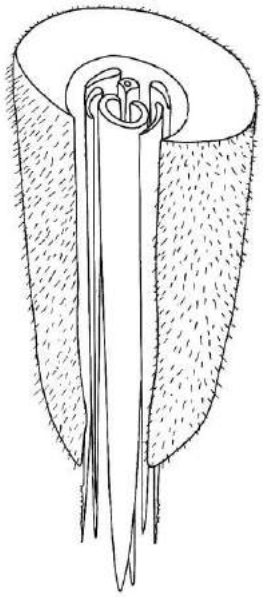




Piercing and sucking type – Bug

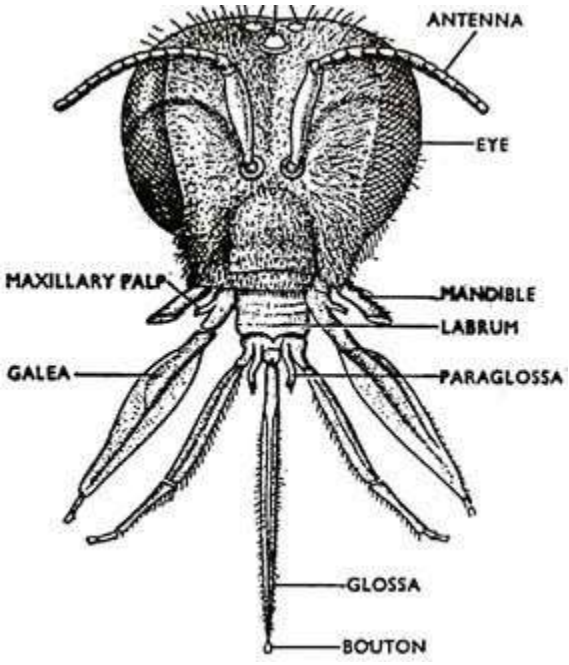
Piercing and sucking type – Mosquito

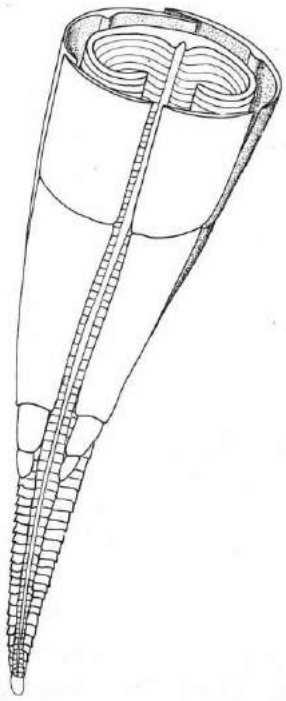




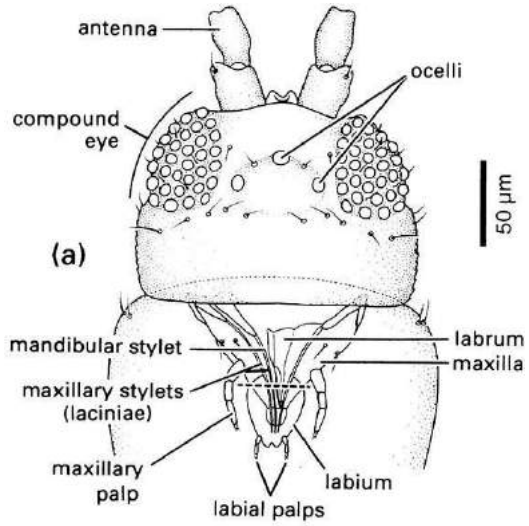
Cross-section of proboscis

Chewing and Lapping type: Honey bee

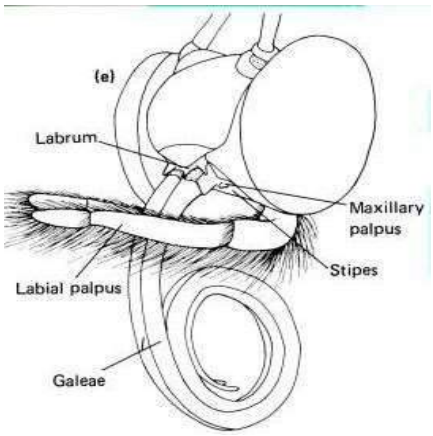


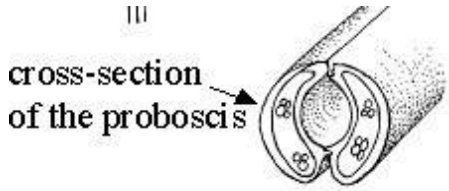


Rasping and sucking type mouth parts

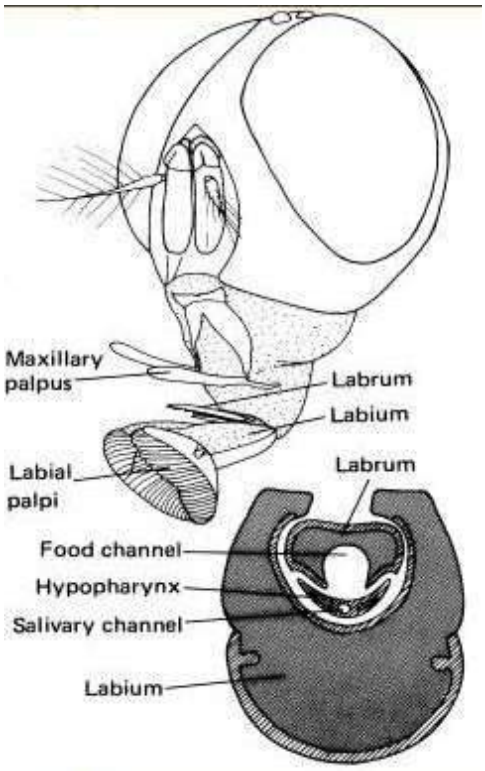


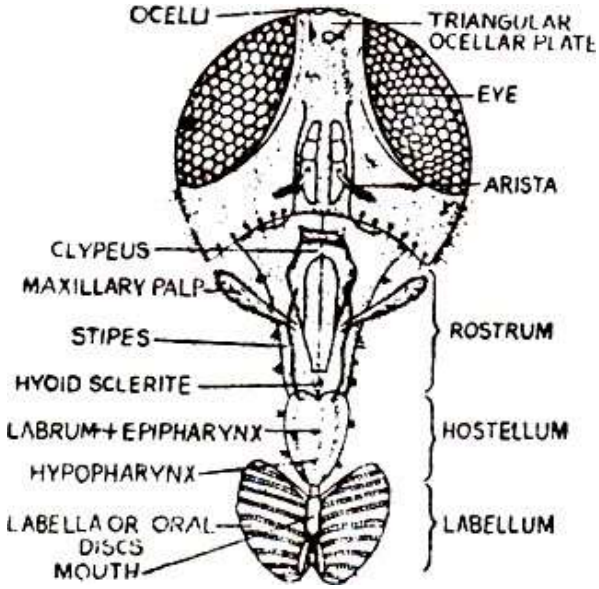
Siphoning type of mouth parts





Sponging type of mouth parts





Practical -5: To study the legs and their modifications

The typical thoracic leg consists of six parts, basal coxa that articulates with the thoraxin the pleural region, small trochanter, femur, tibia, segmented tarsus, and pretarsus. Thecoxa is often divided into two parts, the posterior and the anterior (usually the larger part)being called the meron. The trochanter articulates with the coxa, but usually forms animmovable attachment with the femur. The femur and tibia are typically the longest legsegments. The tarsus, which isderived from a single segment, - is usually sub-divided intoindividual tarsomeres. The pretarsus may consist of a single claw, but it is usually composedof a pair of moveable claws and one or more pads or bristles.Legs are usually lookedupon as the principal organs ofterrestrial locomotion. Theyhave undergone manymodifications and have beenadapted to a wide variety offunctions including swimming,prey capture, pollen collectionand digging.

Ambulatorial (Ambulate - to walk;Walking leg) e.g. Fore leg and middle legof grasshopper. Femur and tibia are long.Legs are suited for walking.

Cursorial: (Cursorial = adapted forrunning : Running leg) e. g. All the threepairs of legs of cockroach. Legs are suitedfor running. Femur is not swollen.

Saltatorial: (Salatorial = Leaping: JumpingLeg) e.g. hind leg of grasshopper.

Fossorial: (Forrorial =Digging; Burrowingleg) e.g. Fore legs of mole cricket.

Natatorial: (Natatorial = pertaining toswimming; Swimming leg) e.g. hing legsof water bug and water beetle.

Raptorial: (Raptorial=predatory; Graspingleg) e.g. Forelegs of preying mantids.

Scansorial: (Scansorial = Climbing;climbing or clinging leg) e.g. all the threepairs of legs of head louse.

Foragial leg: (Forage = to collect foodmaterial) e.g. Legs of honey bee.

i. **Forelegs** : The foreleg has threeimportant structures (Eye brush, Antennacleaner or strigillis and Pollen brush)

ii. **Middle legs**: It has two importantstructures.

(a.) **Pollen brush**: Stiff hairs onbasitarsus form pollen brush which isuseful to collect pollen from middle part oftheir body.

(b.) **Tibial spar**: At the distalend of the tibia, a movable spur is presentwhich is useful to loosen the pellets ofpollen from the pollen basket of hind legsand to clean wings and spiracles.

iii. **Hind legs**: It has three importantstructures viz., pollen basket, pollenpacker and pollen

comb. (a.) **Pollenbasket**: It is also called corbicula. Theouter surface of the hind tibia contains ashallow cavity. The edges of the cavity arefringed with long hairs. The pollen basketenables the bee tocarry a larger load ofpollen and propolis from the field to thehive.
(b.) **Pollen packer**: It is also calledpollen press. It consists of pecten andauricle. Pecten is a rowof stout bristles atthe distal end of tibia. Auricle is a smallplate
Climbing or Sticking leg: e.g. all the threepairs of legs of house fly.

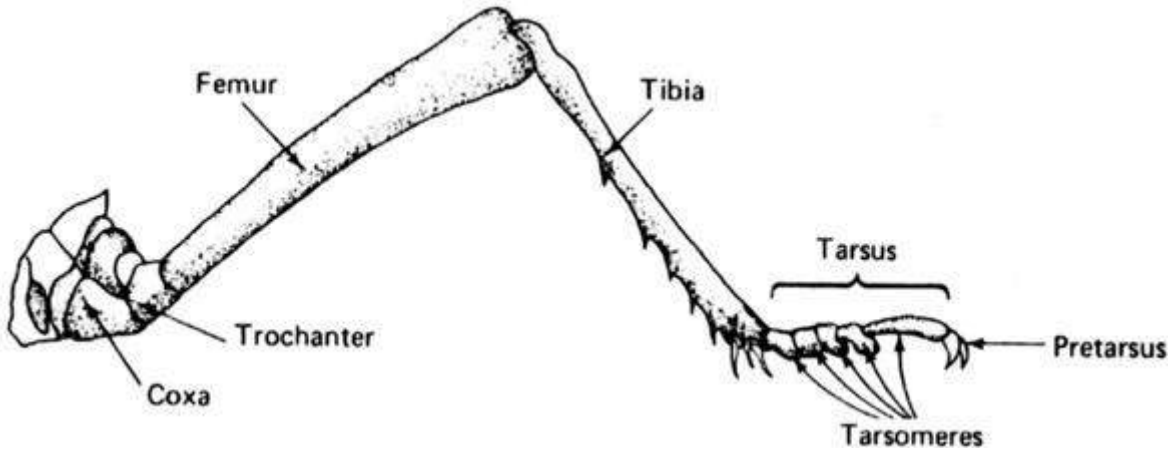
Clasping leg: e.g. Forelegs of male waterbeetle.

Prolegs: Caterpillars‘ posses‘ three pairs of thoracic legs (true-legs) and five pairs of abdominal

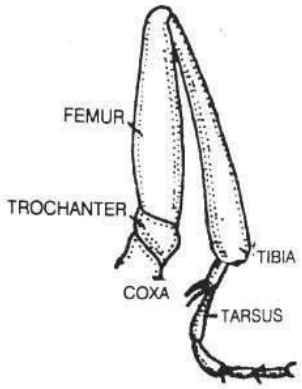
legs (pro-legs) on 3rd, 4th, 5th, 6th, and last abdomenal segments. In some, semi-loopers larvae, prologs on 3rd and 4th abomenal segments absent, and hence while movement, it looks like semi loop, in some, looppers, prologs present only on 6th and last abdominal segments, and hence while movement, it looks like loop.

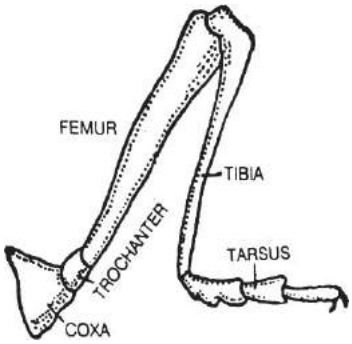
- Thoracic legs are also called the true legs, which are typically jointed and sclerotized.
- Abdominal legs are called prolegs. These are unjointed, short, fleshy with a flat surface at thebottom called planta.
- A number of hooks like structures called crochets are seen arranged in circular or semi cuticular form on the surface of the plants.
- In sawflies of Hymenoptera, the larvae have 3 pairs of true legs in thorax, and 6 or >6 pairs ofprologs in abdomen. This is the unique feature of sawfly larva, but these prologs do not bear crochets, unlike lepidopteran larva.

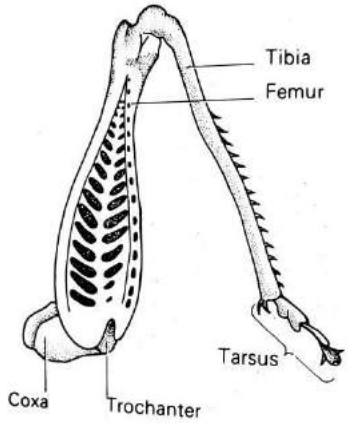
Structure of a typical leg



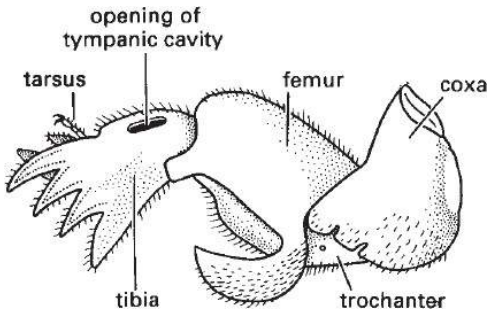
Ambulatorial leg **Cursorial leg** **Saltatorial leg**



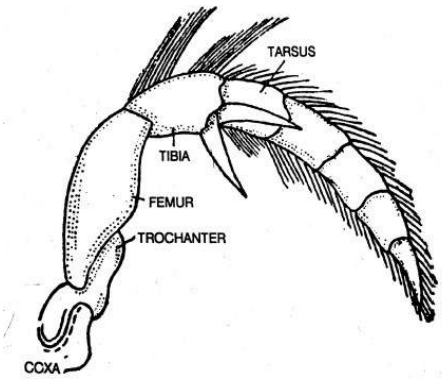




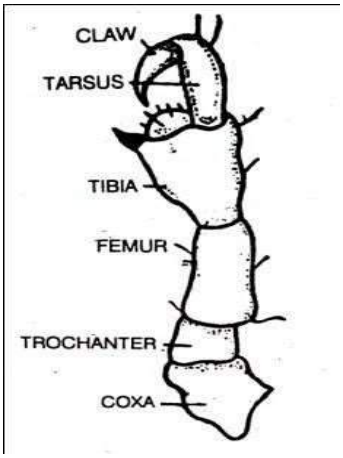
Fossorial leg



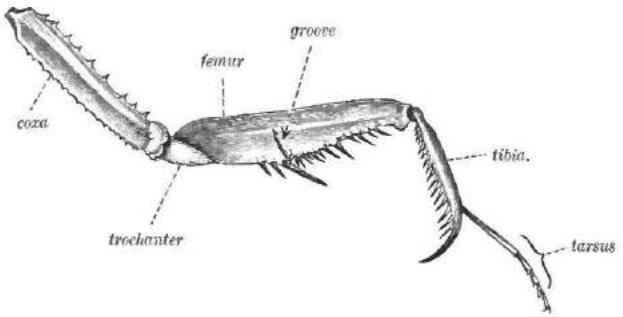
Natatorial leg



Scansorial leg

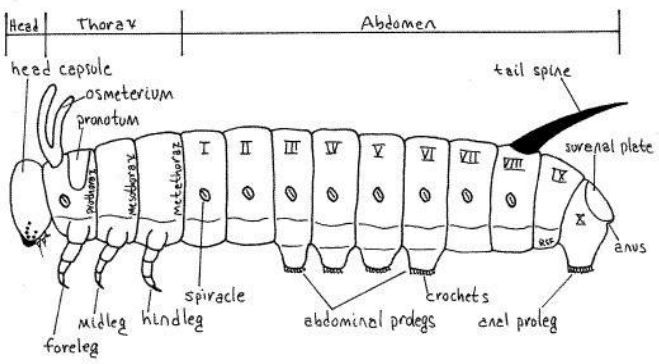


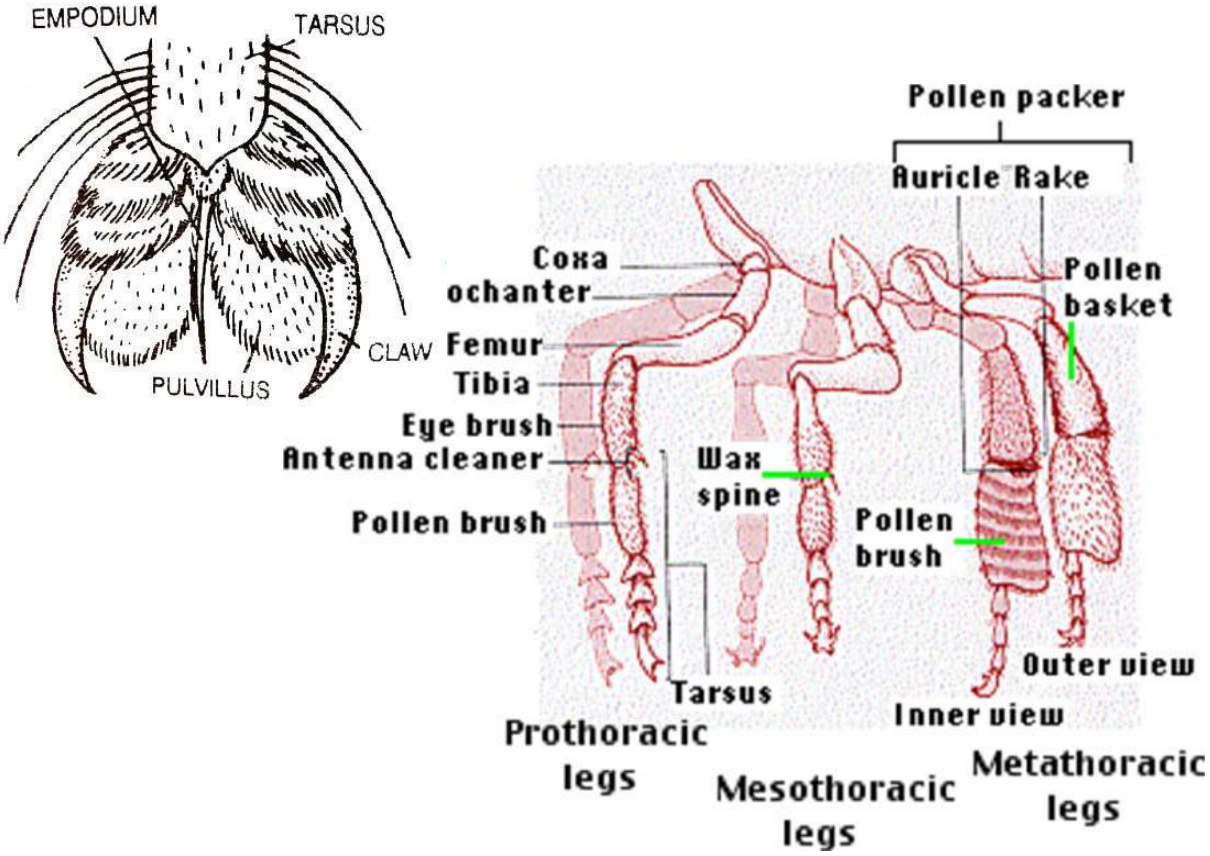
Raptorial leg



Sticking leg

Prolegs

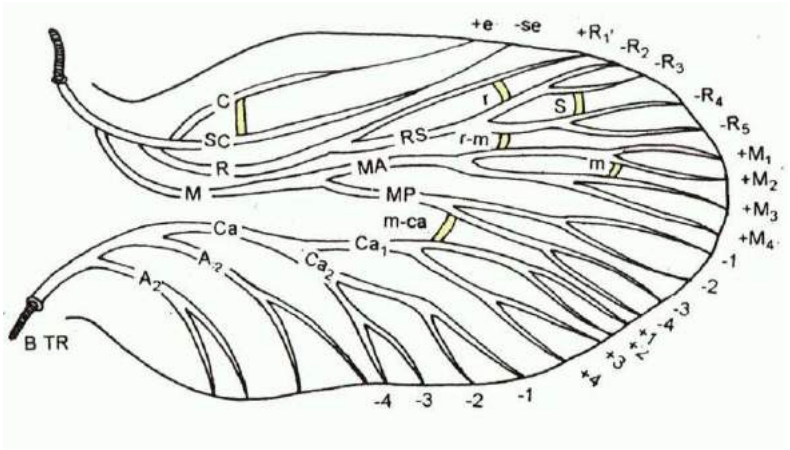




Foraging legs

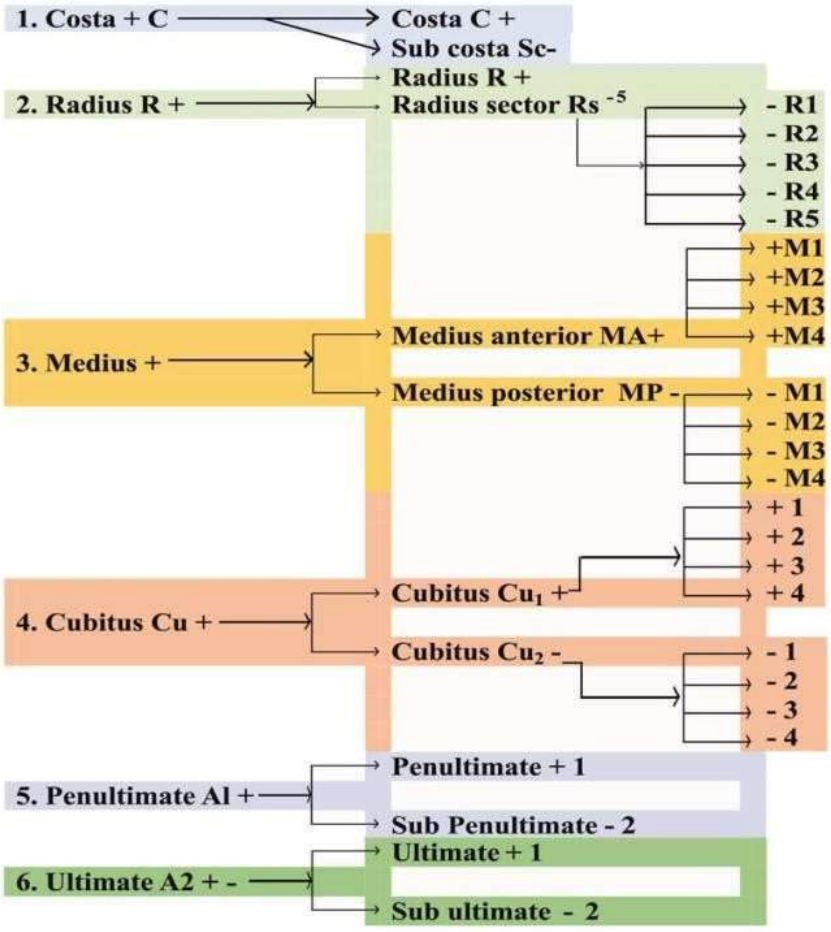
Practical- 6To study about the Wing venation, types of wings and wing coupling apparatus.

The complete system of veins of a wing is termed as venation or neuration. Generally inall the insects there is some similarity in wing venation and therefore, it is presumed that alltypes of wing venation have developed from the common base or the same ancestor. Bymeans of an extensive study of wing venation in different groups of insects, **Comstock andNeedham** constructed a hypothetical type of wing venation from which all other types havepresumely been derived. According to them the primitive wing venation has developedfrom two tracheae which are situated on the anterior and the posterior basal margins ofwings and their branches are spread all over he wing. Each main trachea give rise to threeprincipal veins, thereby forming 6 principal veins namely costa, radius, medius, cubitus,penultimate and ultimate. Each principal vein gives rise to a sub-vein near its base.



Wing venation of a hypothetical wing

The **principal veins are represented by + sign** whereas the **sub veins by - sign**. Thus thewhole wing venation system is represented **by + and the - signs** in alternate as shown in thefigure. The branching of principal veins is represented in the following manner:



Such type of hypothetical wing ventaion is never met in any insect as one or the othervein is invariably found lacking for example the medius vein is absent in order Hemipteraand Ephimereda and submedius is missing in Odonata. Some of the scientists considerprecosta, costa, subcosta, radius, medius, cubitus and anal as the principal veins of the insectwing.

Cross veins

The veins joining the two longitudinal veins are known as cross veins. The importantcross veins along with their symbols are given below :

(i) **Humeral cross vein (h)-** It extends fromcosta to sub-costa near the humeral angle andvein.

(ii) **Radio-medial cross vein (rm)-** It joins the sub radius and the medius veins.

(iii) **Medial cross vein (m)** -The vein joining the m2 and m3 branches of medius is termedasmedial cross vein.

(iv) **Medio-cubital cross vein (m-cu)** - It joins the medius and the cubitus longitudinalveins.

(v) **Radial cross vein (r)** -It extends from R1 to R2.

Wing coupling apparatus

In certain insects special structures have been developed to fasten together the twowings of each side so that it may bring more synchronus action of the fore and hind wings,thereby enabling the insects to fly more swiftly. This action in many insects is ensuredsimply by fore wing overlapping the hind wing. The important coupling device developedin insects‘ wing for adding more efficiency in flying are described below-

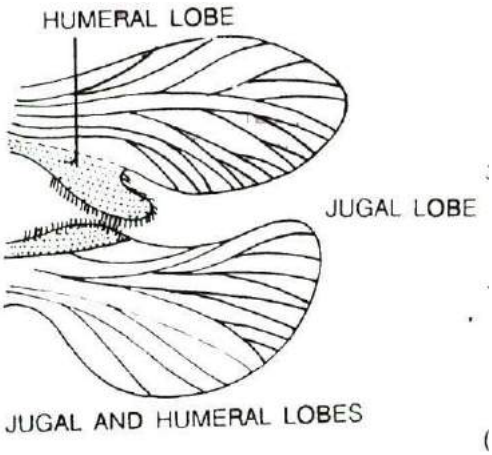
(i) Jugal and humeral lobe- This couplingdevice is commonly found in Lepidoptera,Thchoptera and Mecoptera wherein thewing bases are highly modified. Theposterior end of the fore wing is modifiedinto slender finger like organ which isstiffened by a branch of IIIrd anal vein isknown as the jugal lobe; whereas theanterior margin of the hind wings ismodified in to a small humeral lobe. Thelobes of fore and the hind wings arecoupled with each other during flight.

(ii) Frenulum and Retinaculum -This type ofcoupling apparatus is well illustrated in higherLepidoptera wherein the jugum is lostand the frenulum assumes more importance. In femalebutterflies a number of stoutbristle arise beneath the extended fore wing known as frenulumwhich engages in aretinaculum from a patch of hair near the cubitus of a hind

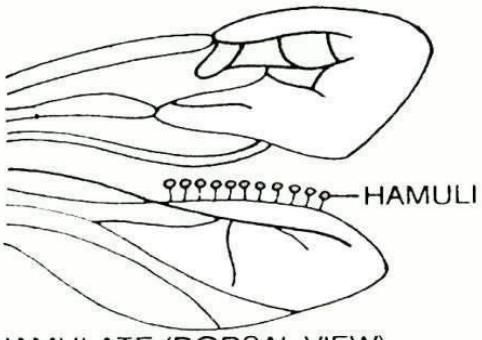
wing. However, inmales thefrenulum bristles are fused into a single stout structure and is held by a curved process from the sub-costal vein of the fore-wing.

(iii) Hamuli -In this modification the costal margin of the hind wings bears a row of smallhooks known as hamuli. These hooks get attached into a fold on the inner margin of thefore-wings. Such coupling apparatus is generally met in Hymenoptera.

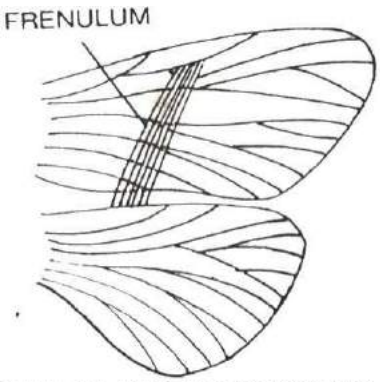
(iv) Amplexiform - This example is commonly met in the insects belonging to familypapilionidac and bombycidae of order Lepidoptera. In this case the wings are coupledsimply by overlapping basally to each other.



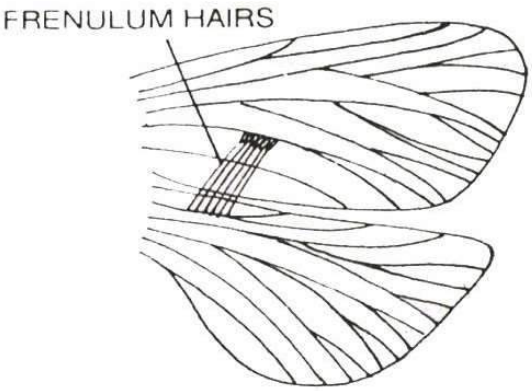
JUGAL AND HUMERAL LOBES



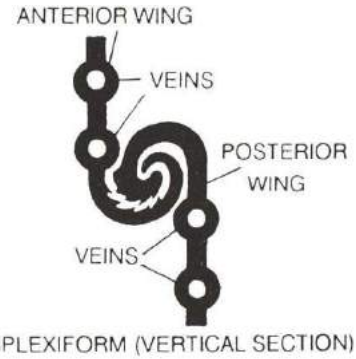
HAMULATE (DORSAL VIEW)



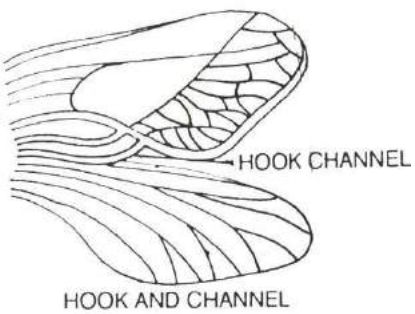
FENULUM AND RETINACULUM
(MALE BUTTERFLY)



FRENULUM AND RETINACULUM
(FEMALE BUTTERFLY)



AMPLEXIFORM (VERTICAL SECTION)



HOOK AND CHANNEL

Practical- 7: To study the types of insect larvae and pupaeMetamorphosis and immature stages

The change in growth and development (form) of an insect during its life cycle frombirth to maturity is called metamorphosis. There are four basic types of metamorphosis ininsects.

Ametabola: (No metamorphosis) e.g. Silver fish.

These insects have only 3 stages in their life cycle namely egg, young ones and adults. It ismost primitive type metamorphosis. The hatching insects resemble the adult in all respectsexcept for the size and called as juveniles. Moulting continues throughout the life.

Hemi-metabola: (Incomplete metamorphosis) e.g. Dragonfly, damselfly and may fly.

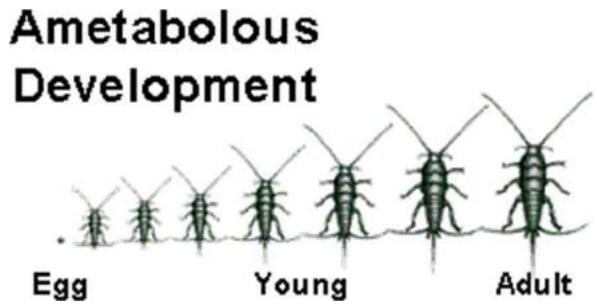
These insects also have 3 stages in their life namely egg, young ones and adults. Theyoung ones are aquatic and are called **naiads**. They are different from adults in habits andhabitat. They breathe by means of tracheal gills. In dragonfly naiad, the lower lip (labium) iscalled **mask** which is hinged and provided with hooks for capturing prey. After final moult, the insects have fully developed wings suited for aerial life.

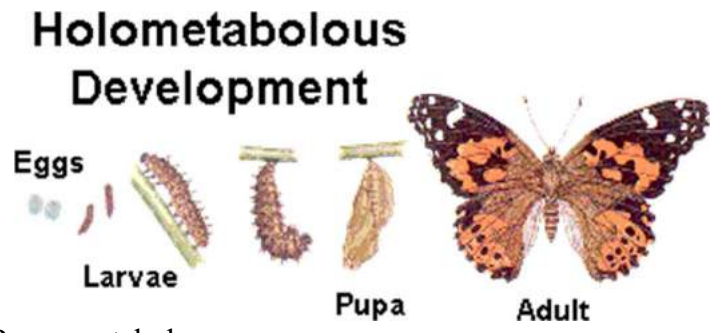
Pauro-metabola: (Gradual metamorphosis) e.g. Cockroach, grasshopper, bugs.

The young ones are called nymphs. They are terrestrial and resembles the adults ingeneral body form except the wing and external genitalia. Their compound eyes and mouthparts are similar to that of adults. Both nymphs and adults share the same habitat. Wingbuds externally appear in later instars. The genitalia development is gradual. Later instarsnymphs closely resemble the adult with successive moults.

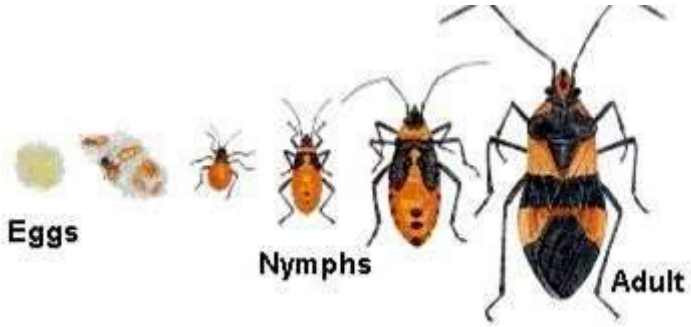
Holo-metabola: (Complete metamorphosis) e.g. Butterflies, moths, fly and bees.

These insects have 4 stages namely egg, larva, pupa and adult. Majority of the insectsundergo complete metamorphosis. Larvae of butterfly is called caterpillar. Larva differsgreatly in form from adult. Compound eyes are absent in larva. Lateral ocelli or stemmataare the visual organs. Their mouth parts and food habits differ from adults. Wingdevelopment is internal. When the larval growth completed it transforms into pupa. It isthe resting and non feeding stage in which the larval tissues disintegrate and adult organsare built up.

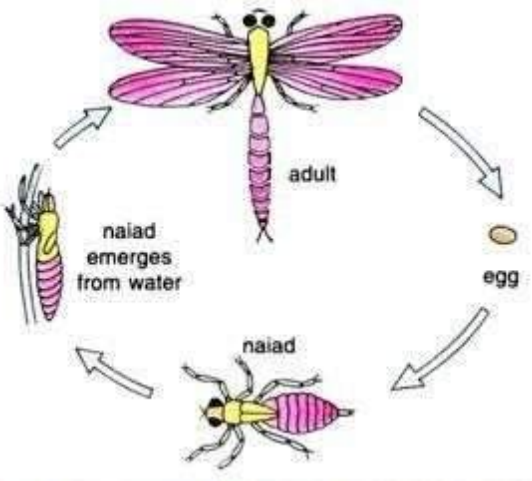




Paura-metabola



Hemi-metabola



Immature stages in insects

Larva: Larval stage is the active growing and immature stage between the egg and pupalstage ofan insect having complete metamorphosis. This stage differs radically from theadults.

Types of larvae:

Type of larva	Modification
I. Oligopod: Thoracic legs are well developed. Abdominal legs are absent.	
i. Campodeiform	They are similar with diplurans genus Campodea. Body is elongate, depressed dorsoventrally and well sclerotized. Head is prognathous. Thoracic legs are long. A pair of abdominal or caudal processes is usually present. Larvae are generally predators and are very active. E.g. grub of antlion or grub of lady bird beetle.

ii. Scarabaeiform	Body is **"C" shaped**, stout and sub-cylindrical. Head is well developed. Thoracic legs are short. Caudal processes are absent. Larva is sluggish, burrowing into wood or soil. e.g. grub of rhinoceros beetle. Body is **"C" shaped**, stout and sub-cylindrical. Head is well developed. Thoracic legs are short. Caudal processes are absent. Larva is sluggish, burrowing into wood or soil. e.g. grub of rhinoceros beetle.
II. Polypod or Eruciform: The body consists of an elongate trunk with large sclerotized head capsule. Head bears powerful mandibles which tear up vegetation. Two groups of single lensed eyes Stemmata found on either side of the head constitute the visual organs. The antenna is short. 3 pairs of thoracic legs and up to 5 pairs of unsegmentedabdominal legs or prolegs or pseudolegs are present. e.g. Caterpillar (larva of moth and butterfly).	
i. Hairy caterpillar	The body hairs may be dense, sparse or arranged in tufts. Hairs may causeirritation, when touched. e.g. red hairy caterpillar.

ii.	**Slug caterpillar**	Larva is thick, short, stout and fleshy. Larval head is small and retractile.
iii.	**Semilooper**	either 3 or 4 pairs of prolegs are present. e.g castor semilooper.
iv.	**Looper**	They are also called measuring worm or inch worm. In this type, only 2 pairs of prolegs are present in 6th and 10th abdominal segments. e.g. Dhaincha looper.
III.	**Apod:** They are larvae without appendages for locomotion. Based on the degree of development and sclerotization of head capsule.	
i.	**Eucephalous**	Larva with well developed head capsule with functional mandibles, maxillae, stemmata and antennae. Mandibles act transversely. e.g. Wriggler (larva of mosquito) and grub of red palm weevil.

ii. Hemicephalous	Head capsule is reduced and can be withdrawn into thorax. Mandibles act vertically. e.g larva ofhouse fly and robber fly.
iii. Acephalous	Head capsule is absent.Mouth parts consists of a pair of protrusible curved mouth hooks and associated internal sclerites.They are also calledvermiform larvae. e.g. Maggot (larva of house fly)

Pupa: It is the resting and inactive stage in all holometabolous insects. During this stage, theinsect is incapable of feeding and is quiescent. During the transitional stage, the larvalcharacters are destroyed and new adult characters are created.

Types of pupae

Type of pupae	Modification
Obtect	Various appendages of pupa viz. Antennae, legs and wing pads are glued to the body by a secretion produced during the last larval moult. Exposed surfaces of the appendages are more heavily sclerotized than the inner surface. e.g. moth pupa.

Chrysali	It is naked obtect type of butterfly. It is angular and attractive coloured. The pupa is attached to the substratum by **hooks** present at the terminal end of the abdomen called **Cremaste**r. The body of chrysalis is attached to the substratum by 2 strongsilken threads called**gridle**.
Tumbler	Pupa of mosquito iscalled tumbler. It is anobtect type pupa. It iscomma shaped withrudimentary appendages. Breathingtrumpets are present inthe cephalic end andanal paddles arepresent at the end ofthe abdomen. Abdomen is capable ofjerkymovementswhich are produced bythe anal paddles. The pupa is **very active**.

Exarate	Various appendages viz. antennae, legs and wing pads are not glued to the body. They are free. All oligopod larvae will turn into exarate pupae. The pupa is soft and pale. e.g. pupaof rhinoceros beetle.
Coarctate	the last larval skin ischanged into a pupal case and the pupa is actually an exarate pupa. The pupal case is dark brown, barrelshaped, smooth withno apparent appendages and called as **puparium**. e.g. fly pupa

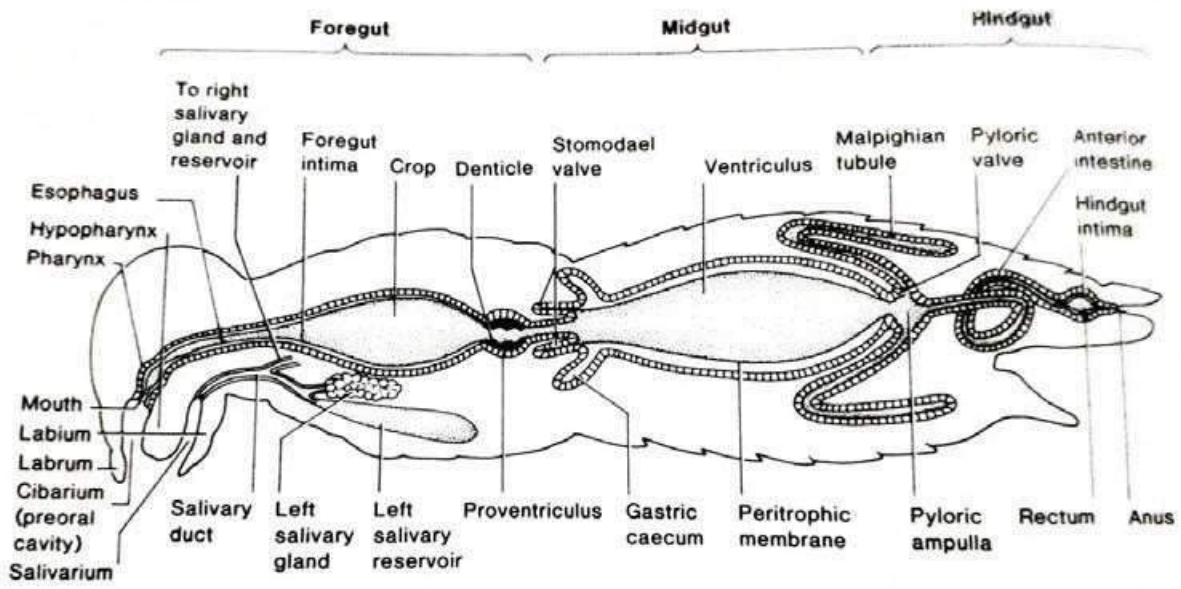
Practical -8: To study the dissection of alimentary canal/nervous system in insects (Grasshopper/Cockroach)

The best learning situation requires one specimen and set of tools per two students forstudies. Students working in pairs have ample opportunity to fully participate in thedissection and to carefully examine the specimen. They are also able to share and discusstheir observations during and after the dissection.

Materials required for dissecting Cockroach

Dissection Kit includes- Surgical scissors, Iris scissors, Tissue forceps, Scalpel, handle,Scalpel blades, Probe with angled tip, Dissection needles, Dropping pipette, Blow pipes,Dissection tray, Dissecting pins, Rigid metal ruler, And case Camel hair brush etc

Diagram of Alimentary canal showing the major subdivisions in a generalized GrasshopperInsect



Digestive system

It includes the organs of ingestion (alimentary canal and its associated glands) and thephysiology of digestion. The organs of ingestion are located in the head and are meant forthe intake of food. The preoral cavity is enclosed by the mouth parts and is divided into twoparts by the hypopharynx, the anterior region in which the alimentary canal opens is termedas cibarium and in which the salivary duct opens is known as salivarium. In the

suckingInsects the cibarium is modified into a sucking pump while salivarium serves as the salivarysyringe.

Alimentary canal

The alimentary canal of grasshopper/cockroach is a simple, hollow and tubular instructure which runs from the buccal cavity to anus. It is distinctly divided into thefollowing three primary regions

1. Foregut or stomodaeum.
2. Mid gut or mesenteron or ventriculus.
3. Hind gut or proctodaeum.

1. Foregut or Stomodaeum

It constitutes the anterior region of the alimentary canal which is primarily an organ ofingestionand shows as a site for storing food. It consists of the following paris

(i) Pre-oral food cavity-It has been described previously and indeed it is not a part ofalimentary canal.

(ii) Pharynx-It is situated in between the pie-oral cavity and the oesophagous and isprovided by the dilateral muscles. These muscles are highly developed in those thoseinsects which pharynx helps in forming the suking pump.

(iii) Oesophagous-It is simple straight tube which runs from the posterior region of thehead to thorax and joins with the crop.

(iv) Crop- It is simple bag like structure and serves as a storage reservoir for the food.Apparently it is a dilated portion of the oesophagous but differs histologically by thepresence of sclerotized ridges which are arranged transversely in the crop. Since itserves as a reservoir for food hence its walls are thin and the muscles are poorlydeveloped.

(e) Gizzard-It is situated in the posterior region of the crop which cannot be apparentlydistinguished from crop but differs internally by having the longitudinal folds into thelumen in which cuticular teeth are attached. Its posterior part is concentric in the internallayer of six 'V' shaped processes are attached which form the cardiac valve with the folds ofgizzard. Its major function is to regulate the passage of food into the mid gut.Histologically, the following layers may be distinguished in the walls of the stomodaeum

(1) Intima - The inner most layer of chitin found in continuation of body cuticle.

(2) Epithelial layer-It is a thin layer secreting the intima.

(3) Basement membrane- Bounding the outer most surface of the epithelium.

(4) Longitudinal muscles—These muscles are less developed than circulatory muscles.

(5) Circulatory muscles - These are well developed.

(6) Peritoneal membrane - It is often difficult to detect and consists of apparentlystructurelessconnective tissue.

2. Mid Gut or Mesenteron

It is relatively a short tube or elongated sac with uniform diameter extends from hepaticcaecae or cardiac valve to Malpighian tubes or pyloric valve. Histologically, the inner wall ofmesentcronor stomach is not made up of chitin, but consists of following layers

(i) Peritrophic membrane (ii) Enteric epithelium

(iii) Basement membrane (iv) circular muscles

(v) Longitudinal muscles (vi) Peritoneal membrane

The enteric epithelium is made up of three types of cells:

(i) The columnar cells whichsecret the enzymes and absorb the digested food,

(ii) the regenerative cells which renew thedestroyed and dead epithelial cells throughsecretion or in the process of degeneration and the goblet cells which are of uncertain functions. Thus, there are following five major function of enteric epithelium:

(i) to make digestiveenzymes

(ii) to absorb the digested food

(iii) to produce new cells

(iv) to absorb the water

(v) to excrete the waste material outside the body.

The inner surface of midgut is sometime lined by a thin membrane known asperitrophic membrane which protects the epithelial cells from the direct contact of foodparticles. Thismembrane is absent in Lepidopterans and hemipterans.

3. Hind Gut or Proctodaeum

It extends from the posterior end of midgut to the anus and is also an invagination ofthe body wall. The hind gut consists of the some layers as the fore gut except that thecircular muscles of its are developed both inside and outside the layer of longitudinalmuscles. The hind gut is externally marked by the insertion of the Malpighian tubes andinternally by the pyloric valve. It may be divided into three distinct regions(i) Ileum or small intestine (ii) Colon or large intestine

(iii) Rectum.

Ileum- It is a small tube which has many folds in its inner wall.

Colon- It is situated on the 5th and 6th segments of the abdomen and is a slender tubewhich, cannot be easily distinguished from the ileum. In some insects it is just like 'S' instructure.

Rectum- Both the ends of the rectum are comparatively slender while the middle portion isthick and large which consists of six rectal papillae internally and six ridges of longitudinalmuscles externally. The rectum opens to exterior through the anus which is situated at thecaudal end ofthe abdomen.

Salivary Glands - The labial glands which are associated with the gnathal appendages arethe salivary glands. A pair of salivary glands is found in the grasshopper which generally liein the thorax and are convoluted tubes often branched and racemose. Both the ducts ofsalivary glands unite together beneath the oesophagous to form a common salivary ductwhich opens into the salivarium.

Physiology of digestion

The grasshopper is phytophagous and eats the leaves and soft parts of the plants whichare holdby the maxillae and, they bring the food near to mandibles where it is broken intosmall particles. These small food particles are sent to the buccal cavity with the help oflabrum and labium. On entering the buccal cavity, it is subjected to the action of salivawhich contains the amaylase enzyme. It acts on the carbohydrates present in the food andchange them into simple sugar i.e., glucose which is absorbed in the crop. Saliva is alsohelpful in moistening the food. This food passes onward to the crop where the secretions ofthe midgut and the hepatic 'caecae mix with it. These secretions are weakly acidic or alkalineand contain maltase, invertase, lactase, protease, lipase, peptidase, erypsin and trypsinenzymes which act on the food. Due to the action of these eyzymes the starch is convertedinto sugars, protein into amino acids and fat into fatty acids. After this the food comes togizzard where it is again masticated then it passes through the cardiac valve intomesenteron where further digestion of the food takes place. The digested food is absorbedby the spongy and thick walls of mesenteron. The undigested food passes to the hind gut(proctodaeum) through pyloric valve where the absorption of water takes place and thenwaste and undigested food expelled out through anus in the form of excreta. The absorbedfood is utilized for the following purposes

(i) In the form of energy required for different life activites

(ii) Some part is consumed in theformation of muscles etc.

(iii) The rest is stored in the fat bodies which are used in emergency.

Filter Chamber:

- A number of cicadids and cercopids suck sap from xylem, which contains amino acidsin

very dilute solution and relatively higher concentration of salts.

- This solution has to be concentrated before absorption so as to avoid excessive dilutionof haemolymph.
- In these insects removal of excessive water is done with the help of ‑the filterchamber‖.
- The filter chamber consists of an expanded thin walled bladder-like anterior midgut,which lies in close association with (or surrounds) the posterior midgut (interior) andproximal ends of the Malpighian tubules (interior) or anterior part of the hindgut.
- The chamber formed within the folds of the anterior gut is called the filter chamber. Itis suggested that the Malpighian tubules produce a hypertonic fluid, which is rich inK+. This establishes an osmotic gradient from the anterior midgut to the filter chamberthen to the Malpighian tubules, so that water passes almost directly to the hindgut andabsorption of nutrients takes place in the more central region of the midgut. Filterchamber of the coccids has parts of midgut invaginated into the rectum.

Practical- 9To study the male and female reproductive systems in insects (Grasshopper)MALE REPRODUCTIVE ORGANS

The male reproductive organs consist of the followings- (i) A pair of testes (ii) A pair ofvasa deferentia (iii) Seminal vesicles (iv) Ejaculatory duct (v) Penis or Aedeagus (vi)Accessory glands (vii) Male genital atrium

The Testes-They are located above the midgut and held in position by the surrounding fatbodies and tracheae. Each testis is a more or less ovoid body partly or completely dividedinto a variable number of follicles or lobes which are cylindrical in shape. Each follicle isconnected with vas deferens by a relatively well developed slender tube known as vaseffcrens. The peritoneal investment of the follicle is developed to the extent of envelopingthe testis as a whole in a common coat known as scrotum. the presence of the sex cells indifferent stages of development.

These zones are as follows

(i) The germarium - It is the region having primordial genii cells or spermatogonia whichundergo multiplication.

(ii) The zone of growth – In this zone the spermatogonia increase in size and undergorepeated mitotic division and develop into spermatocytes.

(iii) The zone of division and reduction-Here the spermatocytes undergo meiosis andproduce spermatids.

(iv) The zone of transformation - The spermatids are transformed into spermatozoa.The

masses of spermatozoa are generally enclosed in the testicular cyst cells from whichthey are released in the vas deferens. In addition, the testes contain large elements knownverson's cells or apicalcells.

Vas deferens- These are the paired canals leading from the testes which are partly or whollymesodermal in origin.

Seminal Vesicles- The Vas deferens vary greatly in length in the majority of insects. EachVas deferens becomes enlarged along its course to form a sac known as seminal vesicle inwhich spermatic fluid is collected.

Ejaculatory duct -Posteriorly, the vasa deferentia unite to form a short common canal whichis continuous with a median ectodermal tube known as ejaculatory duct. The terminal endofejaculatory duct opens in the male genital atrium.

Aedeagus- The terminal end of the ejaculatory duct is enclosed in a finger-like evaginationof theventral body wall which forms the male intromittent organ known as aedeagus. It issituated on9th abdominal sternum of the grasshopper on the conjunctival membrane of theposterior margin. **Accessory glands-** These are one to three pairs in number and usually present in relationwith thegenital ducts opening into seminal vesicle. These are tubular or sac-like in structure.In most ofthe cases their secretions mix with spermatozoa and in some insects glands aredirectly concernedwith the formation of the spermatophores.

THE FEMALE REPRODUCTIVE ORGANS

The female reproductive system consists of the following organs- (i) A pair of ovaries(ii) A pair of lateral oviducts (iii) Spermatheca (iv) Vagina and genital chamber (v) Accessoryglands(Collaterial glands)

The ovaries-These are typically more or less compact bodies lying in the body cavity of theabdomen on either side of the alimentary canal. Each ovary is about 2 cm long andcomposed of a variable number of ovarioles and open into the oviduct. A typical ovariole isan elongated tube in which the developing eggs are disposed one after the other in a singlechain. The oldest oocyte is situated nearer the union with the oviduct. The wall of anovariole is made of follicular epithelium whose cells rest upon a basement membraneknown as tunica propria.

Each ovariole may be differentiated into three zones:

(i) **Terminal filament-** It is the slender thread like apical prolongation of the peritoneallayer. The filaments of the ovary combine to form a common thread termed as terminalfilament. The terminal filament of one ovary units with the filament of the other

ovaryto form a median ligament. It aids in maintaining the ovaries in the position and isattached to the dorsal diaphragm.

(ii) **The germarium**- It is situated below the terminal filament and forms the apex of anovariole. It consists of a mass of cells which are differentiated from the primordial germcells.

(iii) **The region of growth**- It is also called as vitellarium which constitutes the majorportion ofan ovariole. The vitellarium contains the developing eggs (oocytes). Theepithelial layer of thewall of vitellarium grows inwards to enclose each oocyte in adefinite sac known as follicle. Thecells of the follicle secrete the chorion of the egg andin some cases serve to nourish the oocytes. Three types of ovarioles may be recognized on the basis of presence or absence ofnutritive cells.

(a) Panoistic type– Nutritive cells are absent e.g., grasshopper and other insects ofOrthopteraand Isoptera.

(b) Polytrophic type-Nutritive cells are present and arranged inalternate with the oocytes e.g.,Hymenoptera**.**

(c) Acrotrophic type-Nutritive cells arepresent and situated at the apices of the ovarioles e.g.,Hemiptera.

The oviducts– The lateral oviducts are paired canals leading from the ovaries and areformed from the mesoderm. These lateral oviducts form the common oviduct which opensinto the vagina. Each oviduct is an enlarged pouch which stores eggs. The vagina is greatlyenlarged to form a chamber, known as uterus, for the reception of developing eggs.

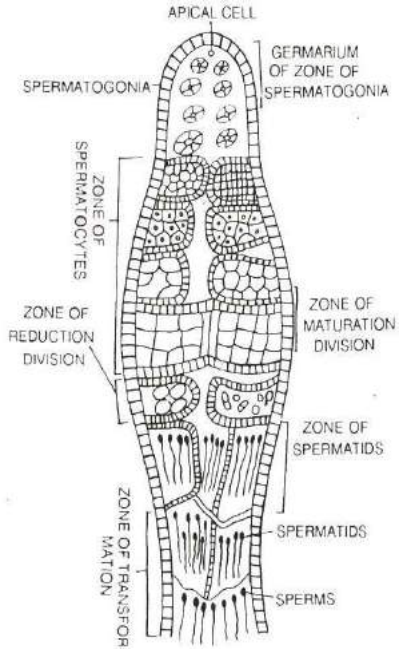
The Spermatheca– This is a pouch or sac for the reception and storage of the spermatozoa(seminal fluid) and is also known as receptaculum seminis. It generally opens by a duct intothe dorsal wall of the vagina which is known as sperm duct. In many insects pairing takesplace only once and since the maturation of eggs may extend after the union of the sexes, theprovision of spermatheca allows for their fertilization from time to time. A specialspermathecal gland opens into the duct of spermatheca and secretes a fluid which lengthensthe life of sperms.

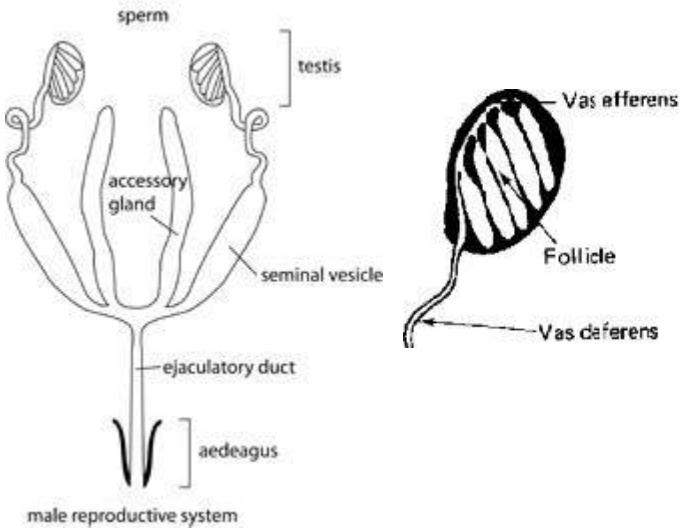
Genital chamber– The vagina opens into the genital chamber on 9th sternum and thischamber iscalled bursa copulatrix which helps in copulation.

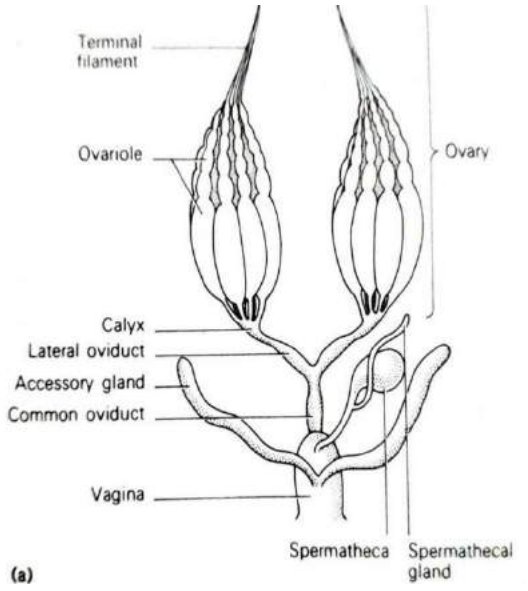
Accessory glands– These are paired structures opening into the distal portion of the vagina.These glands provide material for the formation of egg pod or ootheca.

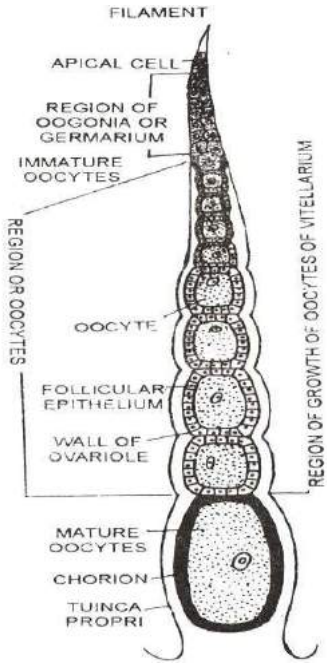
Fertilization– After copulation; the spermatic fluid is received in the spermatheca. The eggcomes down from the oviduct to the vagina which has an opening (micropyle) into its shellfor the entrance of male germ cell (spermatzoan). One or two spermatozoa enter the eggthrough micropyle and only one succeeds in fertilizing the egg. After fertilization theaccessory glands secrete a fluid around the egg
which hardens it.

Male reproductive organ









L. S. of Testicular follicle

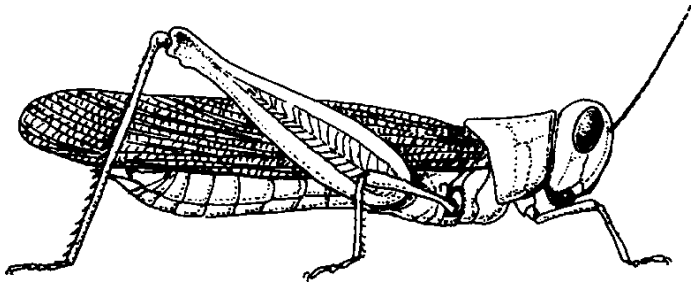
Female reproductive organ **L.S. of an Ovariole**

Practical -10To study the characters of orders Orthoptera, Dictyoptera, Odonata, Neuroptera, Isoptera,Thysanoptera and their families

Order- Orthoptera(Ortho- straight; ptera - wing)

Synonyms : Saltatoria, Saltatoptera, Orthopteroid

Common names : Grasshoppers, Locust, Katydid, Cricket, Mole cricket



Characters

Distribution : Worldwide but mainly in tropicsBody : Medium to large sized

Mouthparts : Chewing and biting type

Eyes : Well developed compound eyes; ocelli 2or 3

Antenna : variable, filiform in most of the insects

Thorax : Large prothorax with shield in many of the insects

Wings : Forewings are called **tegmina** (hard and lathery in texture),Hind wingsare **membranous**

Legs : Hind legs is usually adopted for jumping (saltatorial)

Cerci : Short and unsegmented

Ovipositor : Long and well developed

Specializedorgans : Stridulatory (sound producing) organ and auditory (hearing) organpresentMetamorphosis : Gradual / Paurometabola type

Sub-orders : Caelifera and Ensifera

Family: Acrididae (Caelifera)Characters

Antenna : shorter than the body length

Legs : Hind legs are long and meant for jumping with the help of levatormuscles

Tarsus : three segmented

Ovipositor : Short and horny

Sound production : Tympanum is located on either side of the 1st abdominal segment.Sound is produced by **femoro-alary mechanism** (a row of peg likeprojections found on the innerside of each hind femur which arerubbed against hard radial vein of the tegmen)Examples(Short hornedGrasshopper andLocusts) Dhan ka tidda – *Hieroglyphus banian*

Kharif ka tidda – *H. nigrorepletus*

Ghas ki tiddi – *Chrotogonus sp.*

Locust – *Schistocerca gregaria*

Family: Tettigonidae (Ensifera)Characters

Antenna : Long as long as body or larger

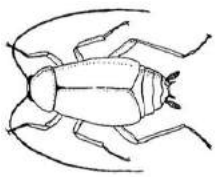
Tarsus : Four segmented

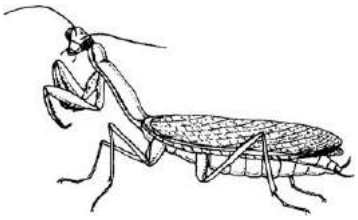
Ovipositor : Sword like

Sound production : Alary type (a thick region on the hind margin of forewing (scraper)is rubbed against a row of teeth on the stridulatory vein (file)present on the ventral side of another forewing which throws theredonant areas on the wing (mirror) into vibrations to producesound)Examples : (Long horned grasshoppers, Katydids and bush crickets)

Order-Dictyoptera(Dictyon = network; ptera=wings)

Synonyms : Oothecaria, Blattiformia Common names : Cockroaches and preying mantids**Characters**





Body : Medium to large sized

Head : Hypognathous

Antenna : Filiform or setaceous

Mouthparts : Chewing type

Thorax : Prothorax usually larger than meso and meta thorax

Wings : Forewings thickened, leathery with a marginal costal vein calledtegmina, Hindwings membranous and folded fanlike

Tarsi : 5 segmented

Cerci : Short and many segmented

Eggs : Contained in Ootheca Metamorphosis : Gradual/paurometabola

Sub-orders : Blattaria (Cockroach) and Mantodea (Preying mantids)

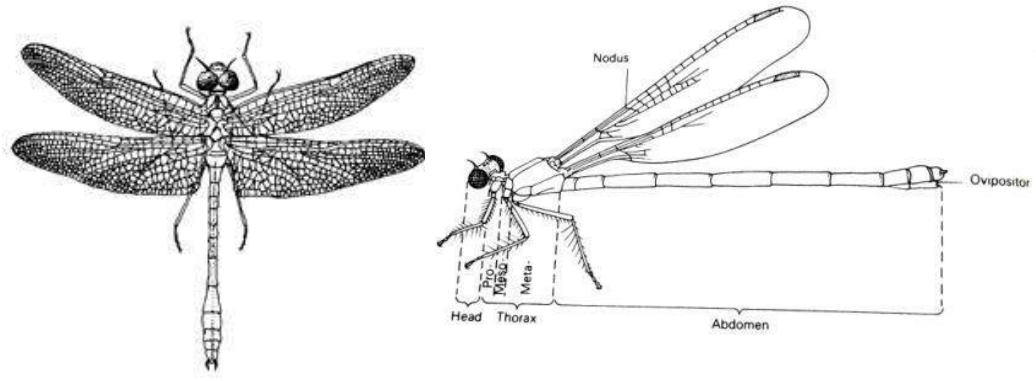
Important Families of Dictyoptera

Characters	Blattidae	Mantidae
Head	Not mobile in all directions	Mobile in all directions
Pronotum	Shield like and cover the head	Elongated, do not cover head
Ocelli	Degenerated- 2 called as fenestra	Three
Body	Flattened, dark coclored	Elongated
sometimescylindrical Legs	Cursorial running type	Forelegs are raptorial,
middleand		
hind legs are ambulatorial	Gizzard	Powerfully armed with
chitinous	NoChitinous teeth	
teeth		
Mating behaviour	Do not devour male during mating	Often (but not always)Ootheca
	Chitinous	Not chitinous
Nymphal charcter	Not cannibalistic	Cannibalistic
Mimicry	Absent	Mimic leaves and flowers
Habitat	Omnivorous	Mostly outdoors
Economic importance	Household pest	Predators on crop pest
Examples	American Cockroach	Preying mantids

Order- Odonata

(Odon = tooth; strong mandibules)

Common names : Dragonflies and damselflies



Characters

Body : Long, cylindrical, medium to large sized, attractively colouredHead
: Globular and constricted behind into a petiolate neck

Antenna : Very short, bristle like, setaceous

Eyes : Compound eyes are large. Ocelli- Three

Mouthparts : Adapted for biting, Mandibles are strongly toothed Lacinia andgalea are fused to form mala which is also toothed

Wings : Membraneous, venation is net work with many cross veins. Wingshave a dark pterostigma towards the costal apex. Sub costa ends innodus. Wing flexing mechanism is absent.

Legs :Basket type arrangement, 3 segmented tarsi, They are suited forgrasping, holding and conveying the prey to the mouth.

Abdomen :Abdomen is long and slender, In male gonopore is present on 9thabdominal segment. But the functional copulatory organ is presenton the 2nd abdominal sternite. Before mating sperms are transferredto the functional penis. Female have gonopore on 8th segment.

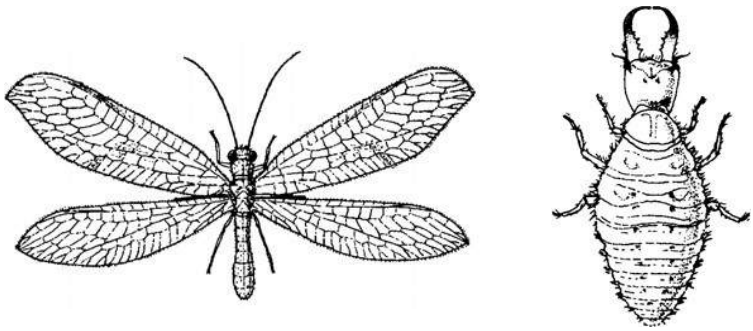
Metamorphosis : Incomplete with three life stages. The Nymphs (called naiad) isaquatic. Labium is greatly elongated, jointed and bears two hooks atapex. It is called mask. It is useful to capture the prey.

Sub-orders : Anisoptera (Dragonfly) and Zygoptera (damselfly)

Importance : Adults are aerial predators. They are able to catch, hold and devourthe prey in flight. Naiads are aquatic predators. Dragonflies anddamselfliescan be collected with an aerial net near streams andponds especially on a sunny day. Naiads can be collected fromshallow fresh water ponds and rice fields.

Order- Neuroptera (Neuro=nerve; ptera=wing)

Common names : Lace wings, Ant lions, Mantispidflies, Owlflies



Characters

Body : Soft bodied insects

Antenna : Filiform, with or without a terminal club

Mouthparts : Chewing type in adults

Wings : Wings are equal, membranous with many cross veins, held in a roof-likemanner over the abdomen, weak fliers

Larva : Campodeiform with mandibulo-suctorial mouthparts

Pupa : Exarate, Pupation takes place in a silken cocoon, Six out of eightMalpighian tubules are modified as silk glands. They spin thecocoons through anal spinnerets.

Sub-orders : Megaloptera and Planipennia

Planipennia : Families

1. **Chrysopidae:** Body pale green in colour, eyes are golden yellowin colour, pedicellate/stalked eggs to avoid cannibalism andpredation, larvae prey on soft bodied insects especially on aphids,exhibits camouflage with debris, biocontrol agents, mass multipliedeasily for pest control in field. (e.g. Green lacewings, Goldeneyes,Stinkflies, Aphid lions)

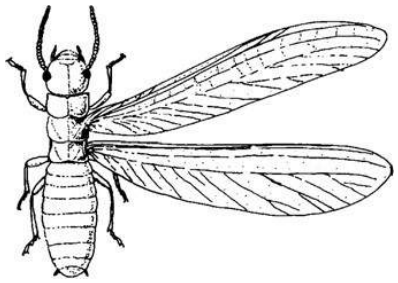
2. **Mantispidae:** Resemble preying mantids, larvae predaceous (e.g.Mantispidflies).

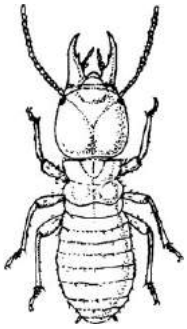
3. **Myrmeleontidae:** Resemble damselfly (Ant lions)

4. **Ascalaphidae:** resemble dragonfly (Owlflies)

Order- Isoptera (Iso=equal; ptera=wing)

Synonyms : Termitina / termitida / SocialiaCommon names : Termites, White ants **Characters**





Body : Minute to large sized and soft

Head : Prognathus, characteristic depression **"Fontanella"** is present on thedorsum of head

Mouthparts : Biting and chewing type

Eyes : Compound eyes present in the winged form; in apterous form itmay ormay not be present; Ocelli 0 – 2

Antenna : Short and moniliform

Wings : Identical in size, form and venation, two pairs, membranous andsemi transparent. Wings are extended beyond abdomen and flexedover abdomen when at rest.

Abdomen : Broadly joined to the thorax without constrictionCerci : Short

Genital organs : Externally lacking in both sexes

Specialities : They are ancient polymorphic, **social insects** living in colonies

Examples Termites- *Odontotermusobesus*, *Eutermusheimi*, *Microtermesanandi*

Caste system is existing in isopteran.

Termite castes

1. Reproductives	**2. Non-reproductives (sterile)**
King	**Workers** (dominate the colony, usually blind and apterous)

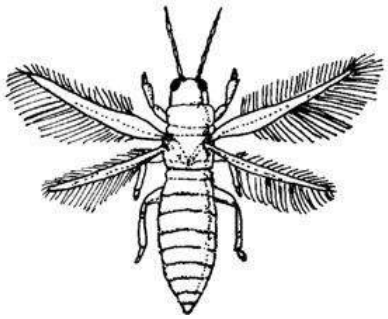
Queen **Soldiers**- (a) mandibulate (b) Nasute (defend the colony)

Order: Thysanoptera (Thysano-fringe; ptera- wing)

Synonyms : Physopoda

Common names : Thrips

Characters



Body : Minute, slender, soft bodied insects

Mouthparts : Rasping and sucking type, Mouth cone is formed by the labrum andlabium together with basal segments of maxilla. There are threestylets derived from 2 maxillae and left mandibles. **Right mandibleis absent**. Hence mouth parts are asymmetrical.

Antenna : Moniliform

Eyes : Compound eyes well developed, ocelli present in alate form

Wings : Either present or absent, when present very narrow and **fringedwith hairs** which increase the surface area, weak fliers and passiveflight in wind is common

Legs : Ambulatorial, Tarsus is with one or two segments, At the apex ofeach tarsus a protrusible vesicle is present.

Abdomen : 11 segmented, pointed. An appendicular ovipositor may be presentor absent

Cerci : absent

Metamophosis : Paurometabola/gradual, Nymphal stage is followed by prepupaland pupalstages which are analogous to the pupae ofendopterygote insects.

Sub-orders : Terebrantia (Important family is **Thripidae**) and tubulifera(ovipositor absent, tubular abdomen, Wing venation is absent)

EconomicImportance : Most of the thrips species belong to the family Thripidae and arephytophagous. They suck the plant sap. Some are vectors of plantdiseases. Few are predators.

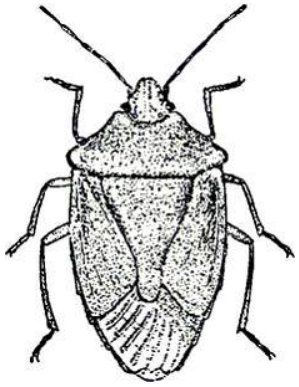
Examples : Rice thrips, *Stenchaetothripsbiformis*and Onion thrips, *Thripstabaci*

Practical -11:To study of characters of order Hemiptera and its families

Order –Hemiptera

(Hemi - half; ptera – wings)

Synonyms : RhynchotaCommon names : True bugs **Characters**



Body : Minute to large sized

Head : Opisthognathous

Mouthparts : Piercing and sucking type, 2 pairs of bristle like stylets which are the modified mandibles and maxilla are present. Stylets rest in thegrooved labium or rostrum

Antenna : Mostly with 4 or 5 segments

Thorax : Mesothorax is represented dorsally by scutellum.

Wings : Forewings are mostly hemelytra basally coriaceous anddistallym

Cerci : Always absent

Metamorphosis : Usually gradual, rarely complete

Alimentary canal Modified into **filter chamber** to regulate liquid food

Sub-orders : Heteroptera (Hetero -different; ptera - wing) andHomoptera (Homo -uniform; ptera - wing)

Characters	Heteroptera	Homoptera
Forewings	heavily sclerotized at the base, hemelytra	Uniformly textured

Wings at rest	Held flat over the abdomen	held roof like over the back
Head	porrect or horizontal	deflexedBases of forelegs do not touch the headtouch the head
Habitat	Both terrestrial and aquatic	Only terrestrial
(Herbivorous)	(Herbivorous, predaceous or bloodsucking)	
Glands present	Odoriferous or scent glands	Wax glands usually present
Honeydew secretion	uncommon	Common
Scutellum (Triangular plate found Betweenthe wing bases)	well developed	Not well developed
Antenna	Relatively long	Short
Ocelli	Dorsal ocelli 0 or 2	2 or 3

Families of Hetroptera sub-order

Family: Coreidae (Squash bugs / Leaf footed bugs)

Members with many branching veins arising from a transverse basal vein. Stink glandsare found inside the metathorax and glands opening are found on the sidesof the thoraxbetween middle andhind coxae. They emits disagreeable / foul pungent odour. Hind tibiaand tarsi are expanded and leaf like. Nymphs and adult suck the sap from the panicles orpods of pulses.

e.g. Rice gundhi bug- *Leptocorisaacuta*, Pod bug- *Riptortuspedestris*

Family: Pyrrhocoridae (Red bug or Stainer)

They are elongate oval bugs. They show warning colouration. They are brightly markedwith red and black. Feeding injury caused by these bugs leads to the contamination by thefungus Nematospora resulting in yellowish brown discolouration of the lint.

e.g. Red cotton bug- *Dysdercuscingulatus*

Family: Reduviidae (Assassin bugs or Kissing bugs)

Predaceous insects, Head is narrow elongated and beak like. The portion behind thecompound eyes is narrow and resembles a beak. The rostrum is short and three segmentedantenna is filiform. Abdomen is broad in the middle. The lateral margins of the abdomen areexposed beyond the margin of the wings. e.g. *Rhynocorismarginatus*-

predators on bees andother pests.

Families of Homoptera sub-order

Family: Jassidae or Cicadellidae (Leaf hoppers and Jassids)

Insects have wedge shaped body with attractive colour. Hind tibiae have a double rowof spines. Ovipositor is modified for lacerating plant tissue. Nymphs and adults have thehabits of running sidewise. They suck the plant sap and also transmit the viral disease.e.g. Green leaf hopper- *Nephotettixvirescence*transmits the Rice tungro virus disease.

Family: Delphacidae (Plant hoppers)

Large mobile flattened spur is present at the apex of hind tibia. It causes hopper burn,transmits viral disease in rice. e.g. Brown plant hopper- *Nilaparvatalugens*

Family: Lophopidae (Aeroplane bugs)

Head is produced into snout. Hind trochanter is directed backward. Hind basitarsus ismoderately long. Both nymph and adult suck the sap and reduce the quality and quantity ofcane juice. e.g. Sugarcane leaf hopper- *Pyrillapurpusilla*

Family: Aleyrodidae (Whiteflies)

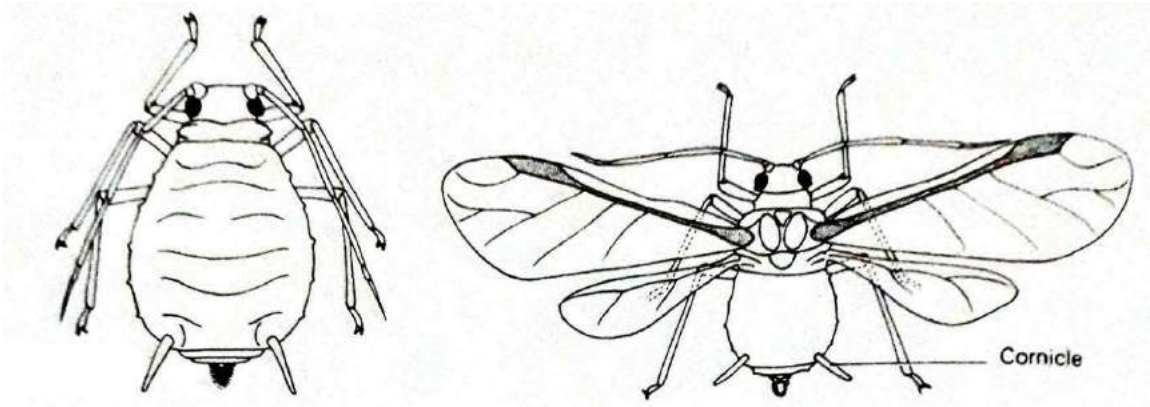
Minute insects, which superficially resemble like tiny moths. Wings are opaque anddusted with mealy white powder wax. Wing venation is much reduced. Vasiform orifice ispresent in the last abdominal tergite. It is conspicuous opening provided with an operculum.Beneath the operculumthere is a tongue- like organ termed ligula. The anus opens at thebase of the ligula through whichthe honey dew is excreted in large amount. Immatureinstars are sessile, scale like, with wax covering. Metamorphosis approaches theholometabolous type due to the presence of a quiescent stage prior to the emergence ofadults. It transmits vein clearing/mosaic disease in Bhendi (Okra).

e.g. Cotton whitefly-*Bemisiatabaci*

Family: Aphididae (Aphids or Plant Lice)

Body is pear shaped. Both apterous and alate forms are found. A pair of cornicles orsiphonculi or wax tube or honey tube is present in the dorsum of 5th or 6th abdominalsegments which secretes wax like substance. The chief constituents are being Myristic acid,sugars and water. They excrete copious amount of honey dew on which ants feed and sootymould fungus grows. Aphids are known for their extraordinary fecundity, short

life cycleand parthenogenitic reproduction. Life cycle is highly complex and it involves alteration ofgeneration. They feed on plant sap and disseminate plant diseases. e.g. Cotton aphid –*Aphisgossypii*



Family: Kerridae or Lacciferidae (Lac Insect)

Females are highly degenerate without legs, wings and antennae. The body is irregularglobular. Body is enclosed in a thick resinous cell. Dermal glands secretion of this insectprovides the stick lac. e.g. Lac insect – *Lacciferlacca*

Family: Pseudococcidae (Mealy bug)

Body is elongate, oval in shape. Body segmentation is distinct. Body is covered by longradiating thread of mealy secretion. Functional legs are present in all instars. Wings areabsent. Nymph and adults suck the sap and affect the growth of spindle leaf. e.g. Coconutmealy bug-*Pseudococcuslongispinus*.

Family: Cicadidae (Cicadas)

Males have sound producing organs at the base of the abdomen. Sound producingorgans consists of a pair of large plates, the opercula covering the cavity containingstructures producing sound.

In the anterior part of the cavity beneath each operculum is ayellowish membrane. A shining mirror is located in the posterior part of the cavity. In thelateral wall of the cavity is an oval shaped ribbed structure, the tymbal. These are vibratedby strong muscles to produce sound. Each species has a characteristics song. Tympanum ispresent in both the sexes. Wings are transparent. Eggs are inserted into the tree twigs by thefemale. Nymphs drop to the ground, enter the soil and feed on root sap. Anterior femurs ofthe nymph are thickened with spines beneath and are suited for digging the soil. Life cycleof periodical cicada lasts for 13- 17 years.

Practical- 12: To study of characters of order Lepidoptera and its families

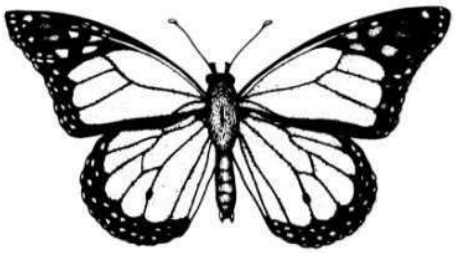
Order: Lepidoptera

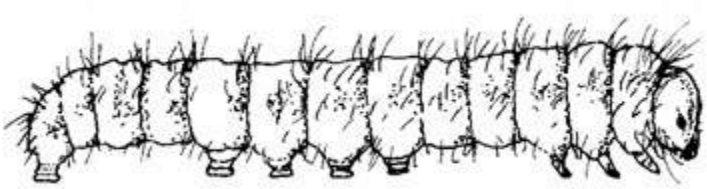
(Lepido- scale; ptera- wings)

Synonyms : Glossata

Commonnames : Butterflies, moths and skippers

Characters





Body : Body, wings and appendages are densely clothed with overlappingscales, which give colour, rigidity and strength. They insulate the bodyand smoothen air flow over the body.

Mouthparts : Mouthparts in adults are of **siphoning type**. Mandibles are absent. The**galeae of maxillae are greatly elongated** and are held together byinterlocking hooks and spines. The suctorial proboscis is coiled up likeawatch spring and kept beneath the head when not in use.

Wings : Wings are membranous and are covered with overlapping **pigmentedscales**. Forewings are larger than hind wings. Wings are coupled byeither frenate or amplexiform type of wing coupling.

Larvae : Larvae are **polypod-eruciform** type. Mouthparts are adapted forchewing with strong mandibles.There are three pairs of five segmented thoracic legs ending in claws.Two to five pairs of fleshy unsegmented **prolegs** are found in theabdomen. At the bottom of the proleg, **crochets** are present.

Pupae : Pupa is generally **obtect**. It is either naked or enclosed in a cocoonmade out of soil, frass, silk or larval hairs.

Sub-orders : **Ditrysia and Monotrysia**

Most of the lepidoptern insects **(97 %)** are grouped under the **suborder Ditrysia** in which thefemale insects have **two pores i.e., the copulatory pore** is located in the **8th** abdominalsternite and the egg pore in the **9th** abdominal sternite. Remaining insects are grouped underthe **suborder Monotrysia** in which the female insects have **one pore.**

Butterfly Family

1. Papilionidae (Swallotail Butterfly)

They are often large and brightly coloured (Fig. on cover page). Prothoracic legs havetibial epiphysis. In many species hind wings has tail like prolongation. Amplexiform type ofwing coupling is present. Larval body is either smooth or with tubercles. RetractileOsmeteria are present on the prothoracic tergum of the caterpillar. e.g. Citrus butterfly,*Papiliodemoleus*

2. Pieridae (Whites and sulphurs)

They are white or yellow or orange coloured with black markings. Larva is green,elongate and covered with fine hairs. Larval body segments have annulets. e.g. Cabbagewhite butterfly,*Pierisbrassicae*

Moth Family

1. Arctidae (Tiger moth)

Wings are conspicuously spotted or banded. They are nocturnal and attracted to light.Larva is either sparsely hairy or densely hairy (wooly bear). e.g. Spotted boll worm, *Eariasvitella*, Sunhemp caterpillar, *Utetheisapulchella*

2. Bombycidae (Silk worm moths)

Antenna is bipectinate. Larva is either with tuft of hairs or glabrous with medio dorsalhorn on8th abdominal segment. Pupation occurs in dense silken cocoon. e.g. Mulberry silkworm, *Bombyxmori*

3. Gelichidae (Paddy moths)

Forewings trapezoidal and narrower than hind wing. Caterpillars bore into the seedstubers and leaves. e.g. Cotton pink boll worm, Pectinophora gossypiella, Angumous grainmoth, *Sitotrogacerealella*, Potato tuber moth, *Pthoremeaoperculella*

4. Noctuidae (Noctua moths)

They are medium sized, stoutly built moths. They are nocturnal and attracted to lights.Labial palpi is well developed. All crochets on the larval prolegs are of same size andarranged in semi circle. Some larvae are semiloopers. They have either 3 or 4 pairs ofprolegs. Larvae

attack the plants during night. Larvae of some species remain concealedbeneath the surface of the ground or litter during day and feed on plants during night. Theyoften cut small seedlings close to the ground and hence they are called cut worms. e.g.Tobacco cut worm, *Spodopteralitura*

5. Pyraustidae/ Pyralidae

Proboscis is vestigial in many species. Labial palp is snout like. Larval habit varies. Itmay live among aquatic plants and bore into the stem or remain in silken web among spunup plants parts. Some larvae are aquatic and gill breathing. e.g. Rice stem borer, *Scirpophagaincertullus*

6. Saturniidae (Moon moth, giant silk worm moth)

They are large sized moths. Antenna is bipectinate. Transparent eye spots are presentnear the centre of each wing. The spots are either circular or crescent shaped. Larva is stoutand smooth with scoli. Cocoon is dense and firm.e.g. Tusor silk worm, *Anthereapaphia*,which yields silk.

Practical- 13: To study the characters of order Coleoptera and its families

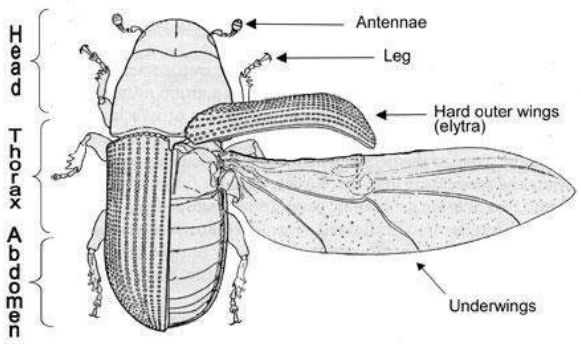
Order: Coleoptera

(Coleo-Sheath; petra- wing)

Synonyms : Elytroptera

Common names : Beetles, Weevils

Characters



Body : Minute to large sized insects

Antenna : 11 segmented

Mouthparts : **Chewing and biting** type, Mandibles are short with blunt teeth

atthe mesal face in phytophagous group, In predators the mandiblesare long, sharply pointed with blade like inner ridge. In pollenfeeders teeth are absent and the mandibles are covered with stiffhairs.

Thorax : Prothorax is large, distinct and mobile. Mesothorax and metathoraxare **fused** with the first abdominal segment. Forewings are heavilysclerotized, veinless, hardened and called **elytra**.

Wings : Forewings are heavily sclerotised, veinless and hardened. They arecalled elytra. Forewings do not overlap and meet mid-dorsally toform a mid-dorsal line. It is not used for flight. They serve as a pairof convex shields to cover the hind wings and delicate tergites ofabdomen. Hind wings are membranous with few veins and areuseful in flight. At rest they are folded transversely and keptbeneath the elytra. In some weevils and ground beetles theforewings are fused and hind wings are atrophied.

Abdomen : Cerci and a distinct ovipositor are absent.

Development : Metamorphosis is complete. Larvae are often called grubs. Pupae

are usually exarate and rarely found in cocoons.

Importance : It is the largest order. It includes predators, scavengers and many

crop pests. They also damage stored products.

Sub-orders : **Adephaga (predators/ devourers) and Polyphaga (eaters of manythings).**

Families of predators :Cicindelidae (Tiger beetle), Carabidae (Ground beetle),

Dytiscidae(True water beetle), Gyrinidae (whirligig beetle),Coccinellidae (Lady bird beetle),Lampyridae (Firefly, glow worm)

Families of scavengers :Scarabaeidae (Scarabs, dung beetle), Hydrophilidae

(waterscavenger beetle)

Families of stored product pests:

Anobiidae (Wood worm/ wood borer) e.g. Cigrette beetle- *Lasiodermaserricorne*

Bostrychidae (Grain borer) e.g. Lesser grain borer -*Rhizoperthadominica* **Families of crop pests:**

1. **Apionidae** e.g. Sweet potato weevil, *Cylasformicarius*, a pest both in the field and in storage.Head is produced into snout. Antennae are not elbowed. Grubs are apodous.

2. Cerambycidae (Longicorn beetles/ Longhorn beetles)

e.g. Mango stem borer, *Batocerarufomaculata*

3. Curculionidae (Weevils/ snout beetles)

Minute to large sized insects. Frons and vertex of the head produced into snout, which iscylindrical and in some species larger than the beetle itself. Mouthparts (mandible andmaxilla) are present at the tip of the snout, It is useful to feed on internal tissues of the

plantand provide a place for egg laying. Antenna is geniculate and usually found in the middle ofthe snout. Grubs are apodous and acephalous. Weevils are important crop pests during bothin field and in storage.

e.g. Coconut red palm weevil, *Rhynchophorusferrugineus*

4. Galerucidae / Chrysomelidae (Pumpkin beetle)

Antenna are closely approximated. Third tarsomere is deeply bilobed. Larvae are rootfeeders. Adults bite holes on plants.Red pumpkin beetles, *Raphidopalpafoevicollis*

5. Melolonthidae (Chaffer beetle, June beetle, White grub)

They are stout beetles with glossy surface. Head is small. Labrum is well sclerotized.Adults are attracted to lights. They feed on tree foliage during night and hide in soil duringday time. Larvae are scarabaeiform and root feeders. e.g. Groundnut white grub, *Holotrichiaconsanguinea*, a serious pest under rainfed conditions.

Families of Predators:

1. Coccinellidae (Lady bird beetle)

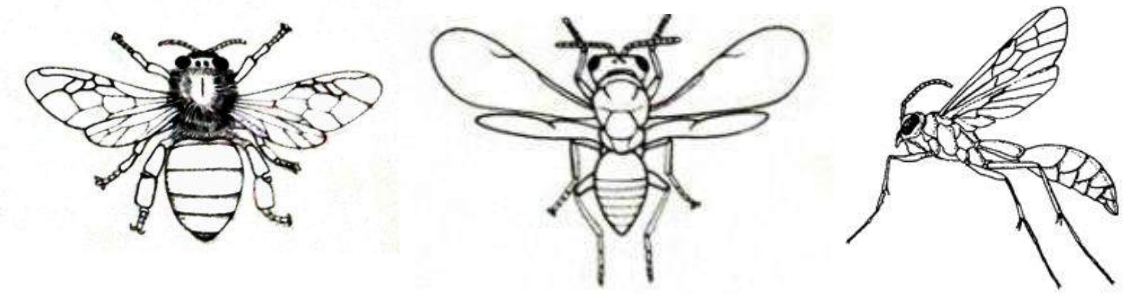
They are hemispherical insects. The body is convex above and flat below. The bodyappearance resembles like a split pea, head is small, turned down ward and received into aprominent notch of prothorax. Elytra is strongly convex, brightly coloured and variouslyspotted. Grubs are campodeiform and spiny. The last larval skin either covers the pupa andgets attached to its anal end. Except the genus Epilachna others are predators on aphids,scales, mites and whiteflies. e.g. *Coccinellaseptupunctata*, *Coccinellavigintioctopunctata*

Practical- 14To study the characters of order Hymenoptera and Diptera and their familiesOrder: Hymenoptera

(Hymen- membrane; ptera- wings), (Marriage on wings)

Common names : Sawflies, ants, bees, and wasps

Characters



Mouthparts : Primarily adapted for chewing, Mandibles are very well

developed.In bees both labium and maxillae are integrated to form the **lappingtongue**.

Thorax : Modified for efficient flight. Pronotum is collar like. Mesothorax isenlarged. Metathorax is small. Both prothorax and metathorax arefused with mesothorax.

Wings :Stiff and membranous. Forewings are larger than hindwings. Wingvenation is reduced. Both forwings and hindwings are coupled by arow of hooklets (**hamuli**) present on the leading edge of thehindwing.

Abdomen :Basally constricted, The first abdominal segment is called**propodeum**. It is fused with metathorax. The first pair of abdominalspiracles is located in the propodeum. The second segment is knownas **pedicel** which connects the thorax and abdomen. Abdomenbeyond the pedicel is called **gaster** or **metasoma**.

Ovipositor :Always present in females. It is variously modified for oviposition

orstinging or sawing or piercing plant tissue.

Metamorphosis : Complete

Larva :Often the grub is apodous and eucephalous. Larva is rarely eruciform.

Pupa : Exarate and frequently enclosed in a silken cocoon secreted fromlabial glands

Sex determination : Fertilized eggs develop into females and males are produced from unfertilized eggs.

Importance : Productive and beneficial insectsSub-orders : **Symphyta and Apocrita**

Suborder Symphyta- Abdomen is broadly joined with thorax, stemmata present, ovipositorissaw like and suited for piercing plant tissues, habits are phytophagous ,e.g. sawflies andhorntails **Family: Tenthrinidae (Saw flies)**

They are wasp like insects. Abdomen is broadly joined to the thorax. Ovipositor is sawtoothed and suited for slicing the plant tissue. Larva is eruciform. It resembles a lepidopterncaterpillar. It has 1 pair od ocelli, papillae (reduced antanna), 3 pairs of thoracic legs and6-8pairs of abdominal legs.Prolegs lack chrochets. They are external feeder on the foliage.Larvae while feeding usually have posterior part of the body coiled over the edge of the leaf.(Mustard Sawfly, Athlia lugens proxima is a defoliator of mustard and cruciferous vegetables.

Suborder Apocrita– Abdomen is petiolated, Stemmata are absent, Legs are absent,ovipositor is not saw like and Suited for piercing or stinging,they are generally parasitic.

Family:Apidae (Honey bee)

Body is covered with branching or plumose hairs. Mouth parts are chewing andlapping type. Mandibles are suited for crushing and shaping wax for building combs. Legsare specialized for pollen collection. Scopa (pollen basket) is present on hind tibia. They aresocial insects with 3 castes viz. Queen, drone and workers.Division oflabour is noticedamong honey bees. Indian honey bee, *Apisindica*

Formicidae (Ants)

They are common widespread insects. Antennae are geniculate. Mandibles are welldeveloped. Wings are present only in sexually mature forms.Petiole may have 1 or 2 spines.They are social insects with 3 castes viz. Queen, males and workers. Workers are the sterilefemales and theyform the bulk of the colony. Exchange of food materials between adultsand immature insects is common. After a mating flight queen alone finds a suitable nestingsite.Many species have associated symbiotic relationship with homopteran insects.

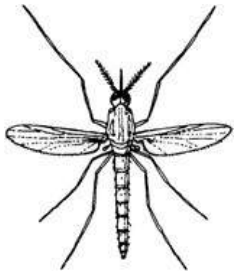
Braconidae (Braconid wasp)

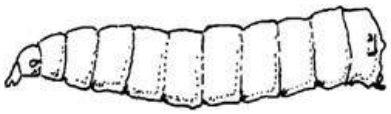
They are small, stout bodied insects. Fore wing has one recurrent vein. Petiole is neithercurved nor expanded at the apex. Gaster is sessile or subsessile. Abdomen is as long as headand thorax together. They parasitize lepidoptern larvae commonly. They are gregariousparasites. In many species, Polyembryony is observed. Bracon brevicornis is mass multipliedand released for the control of black headed coconut caterpillar.

Order- Diptera (Di-two;ptera-wings)

Common names : True flies,Mosquitoes,midges,gnats

Characters





Body : Small to medium sized, soft bodied insects

Antenna : Often hemispherical and attached to the thorax by a slender neck

Mouthparts : Sucking type

Thorax :Fused together. The thoracic mass is largely made up ofmesothorax.

Wings : Single pair of wings. Forewings are larger, membranous andusedfor flight. Hind wings are highly reduced, knobbed at the end andare called **halteres**

Metamorphosis : Complete

Larvae : Larvae of more common forms are known as maggots. They are**apodous** and **acephalous**.

Pupa : Pupa is generally with free appendages, often enclosed in thehardened last larval skin called **puparium**. Pupa belongs tothecoarctate type.

Sub-orders : **Nematocera (Thread-horn), Brachycera (Short-horn) andCyclorrhapha(Circular-crack)**

Families of agricultural Importance:

Syrphidae (Horse flies, Flower flies)

They are brightly coloured and brilliantly stripped. A vein like thickening is present inbetween the radius and median in the forewing. Abdomen has distinct black and yellowmarkings. Maggots prey on soft bodied insects especially aphids. Adults are excellent fliers.They hover over flowers. They feed on pollen and nectar. They aid in pollination.

Tephritidae (Fruit flies)

Sub costa bends apically and fades out. Wings are spotted or banded. Female has asharp and projecting ovipositor. Maggots can hop. They are highly destructive to fruits andvegetables. Cucurbit fruit fly, *Dacuscucurbitae*.

Tachinidae (Tachinid flies)

Arista is completely bare. Abdomen is stout with severalnoticeable bristles.They arenon specificendoparasite on the larvae and pupae of Orthoptera, Hemiptera, Lepidopteraand Coleoptera.

Practical- 15: To study the Insecticides and their formulations.

Insecticide formulations

After an insecticide is manufactured in a relatively pure form (technical grade), it mustbe formulated before it can be applied. Formulation is the processing of the technical gradeby various methods which is done to make the product safer, more effective and moreconvenient to use. Formulation is the final physical condition in which the insecticide is soldcommercially. In a formulation, there are one or more chemicals (formulants) which are theactive ingredients (a.i.) and other ingredients which have no pesticide action (inertingredients). There are mainly three types of pesticide formulations (liquid, solid and gas).

A single pesticide may be sold in more than one formulation. Formulation type depends on several factors:

- toxicology of the active ingredient,
- chemistry of the active ingredient,
- how effective the product is against the pest,
- the effect of the product on the environment (plant, animal or surface etc.),
- how the product will be applied and the equipment needed the application rate.

Characteristics of an Appropriate Insecticides Formulation

- Highly toxic to target insects.
- Not repellent or irritant to target insects
- Long-lasting
- Safe to humans and domestic animals
- Stable during storage and transportation
- Cost-effective

TYPE OF FORMULATIONS

Emulsifiable Concentrates (EC)

- It consists of a technical grade material, organic solvent and a emulsifier.
- Emulsifier makes the water insoluble toxicant to water soluble

- When an emulsifiable concentrate is added to water and agitated (i.e., stirredvigorously), the emulsifier causes the oil to disperse uniformly throughout the carrier(i.e., water) producing an opaque liquid (oil in Water suspension).
- A few formulations are Water in oil suspension. These are opaque and thick, employedas herbicide formulations, because they result in little drift.
- These are easy to transport and store, and require little agitation in the tank. However,care must be exercised in handling the toxic concentrates.
- Shelf life approximately 3 years
- More than 75% of all insecticides formulations are applied as sprays.
- Examples : Quinalphos 25EC, Dimethoate 30EC, Chlorpyriphos 20EC.

Dusts (D)

- Simplest of all formulations and the easiest to apply.
- The technical material (active ingredient) is mixed with an inert diluents carrier suchasclay, organic flour, pulverised minerals.
- In a formulated dust, the following two types of mixtures are usually found :

Undiluted toxic agent, e.g., sulfur dust used for control of mites and powdery mildew and**Toxic** a.i. plus an inert diluent. This is the most common dust formulation sold as 2%,5%, or 10% a.i dust.

- Concentration of dust formulation ranged between 0.1% to 25%
- Particle size of dust particles 1-40 μ pass through 325 mesh sieve.
- Least effective and cause wind drift leading to poor deposit on surface. It has beencalculated that not more than 10-15% of the applied material is retained on the surface.
- Highly toxic to beneficial insects.
- Example : Carbryl 5 D, Malathion 5D.

Granules (G)

- The chemical is in the form of small granules of inert material, either as a coating onthesurface of the inert granules, or as an impregnated toxicant in the granules.
- Consist of small pellets of the active ingredients sprayed on to clay and allowingsolventsto evaporate
- Size: 0.25 – 0.38 mm (20-80 mesh or 30-60 mesh i.e (i.e., the number of grits (granules)per inch of the sieve through which they have to pass).)

- The amount of active ingredient varies from 2-10 per cent.
- Used mainly as systemic insecticides and can be applied on to the soil, or may beplacedin the whorl of leaves depending on the nature of pest control required.
- Granular insecticides may be more economic since precise applications are possiblewiththem.
- Much safer to apply than dusts and are generally less harmful to beneficial insects suchasbees.
- Example : Carbofuran 3G, Phorate 10G, Cratap 4G.

Wettable Powders (WP)

- Concentrated dusts containing a inert diluents (50-75% talc or clay) and a wetting agenttofacilitate mixing the powder with water before spraying.
- Much more concentrated than dusts, containing 15 to 95 per cent active ingredient.
- Do not dissolve washers and rubber hoses;do not damage materials sensitive to organicsolvents
- Leave effective residues in cracks and crevices and are not phytotoxic.
- Require frequent agitation and cause corrosion of valves, nozzles and pumps andsprayers
- Should never be used without dilution.
- These are easy to carry, store, measure, and mix. However, care must be taken toprotectagainst inhalation during handling.
- Example: Carbaryl 50WP, Sulfur 80WP, Bacillus thurnigiensis var. kurstaki 5WP.

Soluble Powders (SP or WSP)

- Contain a finely ground water soluble solid which dissolves readily upon the additionofwater forming true solution.
- Do not require constant agitation and forms no precipitate.
- The amount of active ingredient in soluble powder ranges from 15-95% by weight; itisusually not more than 50%.
- Soluble powder have all the advantages of wettable powders except the inhalationhazardduring mixing.
- Example: Cartap hydrochloride 50SP, Acephate 75SP.

Water Dispersible Granules (WDG)

- Water dispersible granules, or dry flowables is a relatively new type of formulation andbeing developed as safer and more commercially attractive alternatives to wettablepowders and suspension concentrates formulations.
- They are becoming more popular because of convenience in packaging and use, nondusty,free-flowing granules which should disperse quickly when added to water in thespray tank.
- They therefore represent a technological improvement over wettable powders. Thedispersion time in water is a very important property and to ensure that no problemsshould occur during mixing in the spray tank.
- It is necessary for all the granules to disperse completely within two minutes in varyingdegrees of water temperature and hardness.
- Example: Endosulfan 50 WG, Cypermethrin 40 WG, Thiamethaxam 25 WG,Deltamethrin 25 WG.

Suspension Concentrates (SC)

- Pesticide particles maybe suspended in an oil phase, but it is much more usual forsuspension concentrates to be dispersed in water.
- A stable suspension of solid pesticide(s) in a fluid usually intended for dilution withwaterbefore use. Ideally, the suspension should be stable (i.e. not settle out).
- The active ingredient range between 0.1-60%.
- A.I. must be water insoluble with friable crystals, Easy to tankmix (very compatible) -

A.I. tends to settle out over time.

- Farmers generally prefer suspension concentrates to wettable powders because theyarenon-dusty and easy to measure and pour into the spray tank.
- Example: Fipronil 5 SC, Sulphur 52 SC.

Microencapsulation/Capsule Suspensions (CS)

- The polymer membrane, or microencapsulation technique, has become popular inrecent years.
- These are particles of pesticide, either solid or liquid encapsulated by polymericcoatings. Microcapsule solids are suspended in water as a concentrate and dilutedproduct (1:100 to 1:1000) is applied in spray solution to soil or foliar canopy.
- The rate of release of the active ingredient can be controlled by adjusting

themicrocapsule/droplet size, the thickness of the polymer membrane and the degree ofcross-linking or porosity of the polymer.

- Example: Lambda Cyhalothrin 10 CS, Lambda Cyhalothrin 25 CS etc

O/W Emulsions (EW)

- Oil-in-water emulsions are now receiving considerable attention reduced or eliminatedvolatile organic compounds (VOCs) for safer handling.
- They are water based, oil-in-water emulsions can have significant advantages overemulsifiable concentrates in terms of cost and safety in manufacture, transportation anduse.
- The active ingredient must have very low water solubility to avoid crystallizationissues.
- Example: Butachlor 50 EW, Cyfluthrin 5 EW, Tricontanol 0.1 EW etc

Flowable Suspension (FS)

- Flowable suspensions are concentrated 40% to 70% w/w suspensions of micronizedinsoluble active pesticide in water.
- FSs must be formulated for low viscosity and good fluidity, so that transfer to the spraytank is easy and complete. This requires an effective wetting agent and an efficientdispersing agent to ensure adequate dispersion of the pesticide in the water. Since theactive ingredients in FSs are insoluble, good suspension stability is essential.
- If the suspension settles and leaves sediment at the bottom of the container, theapplication of the pesticide may be too weak to be effective.
- A combination of smectite clay (bentonite) and xanthan gum works synergistically toprovide excellent long term suspension stability at low viscosity and at low cost.
- Example: Thiram 40 FS, Thiamethoxam 30 FS, Tebuconazole 5.36 FS

Microemulsions (ME)

- Microemulsions are thermodynamically stable transparent dispersions of twoimmiscibleliquids and are stable over a wide temperature range.
- Involves the incorporation of the insecticide in a permeable covering, microcapsulesorsmall spheres with diameter ranging from 1-50 μ.
- The total concentration of surfactants for a microemulsion can be as high as 10–30%ormore, compared with about 5% for a typical o/w emulsion.
- The insecticides escape through the small sphere wall at a slow rate over an

extendedperiod of time.

- Microemulsions have relatively low active ingredient concentrations, but the highsurfactant content and solubilisation of the active ingredient may give rise to enhancedbiological activity.
- Example: Neemazal 30 MEC, Pyrithiobac Na 5.4 + Quizalofop-P-Ethyl 10.6 ME.

Oil Dispersion Formulations

- One of the latest formulation types is oil dispersions (ODs). This technology allows veryefficient and environmentally friendly agrochemical formulations.
- In ODs the solid active ingredient is dispersed in the oil phase, making it especiallysuitable for water-sensitive or non-soluble active ingredients.
- The oil-phase can comprise different oils such as mineral oils, vegetable oils or esters ofvegetable oils.
- Special attention is needed with the auxiliaries in ODs: suitable oil-compatibledispersing agents and emulsifiers adjusted to the type of oil which forms a stableemulsion after dilution with water.

ZW Formulation of CS & EW

- A mixed formulation of CS and EW is a stable suspension of microcapsules of the activeingredient and fine droplets of active ingredient(s) in fluid, normally intended fordilution with water before use.
- In the case of microcapsules, the active ingredient is present inside discrete, inert,polymeric microcapsules.
- The formulation is intended for dilution into water prior to spray application. Mixturesof active ingredients one of which is encapsulated are used to provide a broaderspectrum of pest control.
- Formulating the active ingredients together eliminates the need for tank mixing (whichcan lead to incompatibilities).
- Example: Lambda Cyhalothrin-25.0 CS + Chloropyriphos-10.0 EW

Flowable Powder (FP)

- The technical material is wet milled with a clay diluent and water with a suspendingagent, a thickener and anti freeze compound forming a thick creamy pudding likemixture which mixes well with water.
- Needs constant agitation to prevent the insecticide from coming out of suspension

andsettling.

Oil solutions

- Formulated by dissolving the insecticides in an organic solvents for direct use in insectcontrol
- Rarely used on crops as they cause severe burning of foliage.
- Effective on livestock, as weeds sprays along roadsides, in standing pools formosquito‘s larvae control, and in fogging machines for adult mosquito control.

Aerosols

- Most common of all formulations for home use
- Consists of toxicant (2%), solvent (10%), knockdown agent (2%) and propellant (86%).
- The active ingredients soluble in volatile petroleum oil is kept under pressure providedbypropellant gas
- When solvent is atomized, it evaporates quickly leaving behind small droplets of theinsecticides suspended in air
- The toxicant is suspended as minute particle (0.1 - 50 w/w) in air as a fog or mist.
- Used for the knockdown and control of flying insects and cockroaches, but they provideno residual effect.
- Caution must be taken when used as they produce droplets well below 10 μ, readilyabsorbed by alveolar tissues in the lungs.

Ultra low volume concentrates (ULV)

- Technical ingredient is dissolved in minimum amount of solvent 0.6 litre to 5.0 litre/hainvery small droplets of 1-15μ.
- Small droplets can better penetrate thick vegetation and other barriers
- Used for insect control in large areas where high volume of water constitutes a technicaldifficulty.

Fumigants

- Gases or low volatile liquids of low molecular weight which readily penetrate thematerialto be protected
- Used for the control of insects in stored products, for soil sterilization.
- Most of the fumigants are liquid and are mixtures of two or more gases.

Fogging concentrates

- Used in control of adult flies and mosquitoes for public health.
- Fogging machines generate droplets of 1-10µ.

Smoke generators

- They are used in the form of coil like strips containing pyrethrum, oxidant and wooddustfor the control of mosquitoes. When ignited, these coils release vapours.

Impregnating materials

- Used in the treatment of woollens for moth proofing and timbers against wooddestroyingorganisms.

Poison bait

- Contains low level of toxicant incorporated in to material such as food stuffs, sugars,molasses etc. that are attractive to target pest.

LABEL INFORMATION

Every pesticide container has a label affixed on it with a leaflet. The label givesinformation of the pesticide in the container. The leaflet contains information on directionsto use warnings, disposal and storage. Both the lable and leaflet are statutorily requiredunder the Insecticide Act, 1968. The following information must be furnished on the label.

- Name of the pesticide (Brand name, Trade name, Common name), Name of themanufacturer and address, Registration number, Kind and name of active ingredientand their percentage, Types of formulation, Net content by weight, Batch number(assigned by manufacturer), Date of manufacture, Expiry date, Antidote statement
- Warming symbols and signal (warming symbol is of diamond shaped consisting of twotriangles with a colour in the lower triangle and a signal in the upper triangle).

Practical -16: To study the Pesticide appliances and their maintenancePlant Protection Equipments

SPRAYERS

Sprayer is a machine used to apply liquid chemicals on plants to control pest anddiseases. It can also be used to apply herbicides to control weeds and to spray micronutrientsto enhance plant growth. The main functions of a sprayer are

- Breaking the chemical solution in to fine droplets of effective size.
- Distributing the droplets uniformly over the plants.
- Applying the chemicals with sufficient pressure for positive reaching the plants
- Regulating the amount of liquid applied on plants to avoid excessive application.

A variety of high volume sprayers are available in the market. Almost all types of highvolume sprayers have some kind of pump to supply pressurised spray liquid to thehydraulic nozzle which breaks the liquid into spray droplets and throws the spray awayfrom it. The high volume sprayersare both manually operated or power operated type.

Principle: The function of sparyer is to atomize the spary fluid in to small droplets and ejectit with some force.

Parts of sprayers: The important parts are tank, agitator, pressure gauge, valves, filters,pressure chamber, hose, spray lance, cut off device, boom and nozzle.

Fig.1. Sprayer components

Nozzle body: It is the main component on which other component of a nozzle fit (Fig. 1a).

Swirl plate: It is the part of a cone nozzle which imparts rotation to the liquid passingthrough it(Fig. 1b).

Spray gun: It is a lance from which spray is readily adjustable during the operation.

Spray boom: It is a spray lance with spray nozzles fitted to a head, mounted at right anglesto

thelance (Fig. 1d).

Filter: It is a component to remove suspended matter larger than a predetermined size fromfluid. **Over-flow pipe**: It is a conduit through which excess fluid from a pump is by-passed bytheaction of a relief valve or pressure regulator.

Relief valve: It is an automatic device to control the pressure of fluid or gas within rangeapredetermined value.

Pressure regulator: It is an automatic device to control the pressure of fluid or gas withinarange of settings.

Cut-off valve: It is a mechanism between the pump and the nozzle to control the flow ofliquidfrom the sprayer. This is operated by hand.

Nozzle disc: It is component containing the final orifice of a nozzle usually acone nozzle.

Nozzle boss: It is a lug on spray boom or spray lance to which a nozzlebody or cap is screwed.**Nozzle tip**: It is component containing the final orifice of a nozzle usually afan nozzle.

Spray lance: A hand-held pipe through which the liquid reaches thenozzle mounted at the free end.

TYPES OF NOZZLE: The three common types of nozzle

a. Hollow cone nozzle: This liquid is fed into a whirl chamber through atangential entry or through a fixed spiral passage to give a rotatingmotion. The liquid comes out in the form of a harrow conical sheetwhich then breaks up into small drops. This is used for insecticide andfungicide spraying.

b. Solid cone nozzle: This nozzle covers the entire area at small range.The construction is similar to hollow cone nozzle with the addition ofan internal jet which strikes the rotating liquid just within the orifice ofdischarge. The breaking of drop is mainly due to impact. This is usedfor herbicide spraying.

c. Fan nozzle: It is a nozzle which forms narrow elliptical spray pattern. In this type theliquid is forced to come out as a flat fan shaped sheet which is then broken intodroplets.

This nozzle is mostly used for low pressure spraying.

a. Hollow cone nozzle b. Solid cone nozzle c. Fan nozzle

TYPES OF SPRAYERS:

A. Manually Operated Hydraulic Sprayers- In this type, the hydraulic pump directly actson the spray fluids and discharge it.

a. Hand syringe

It is a single acting pump working on the principle of cycle pump. it consists of cylinderin to which the spray fluid is drawn during the suction stroke and delivered during thepressure stroke and discharge through nozzle. It is useful to operate only a small area.

b. Hand Sprayers

This is a simple sprayer. It creates hydraulic pressure by forcing spray solution to anozzle by the direct action of hand pumping. The spray solution is filled in a plastic can (5-10 L) which is usually shoulder slung. A dip-tube draws liquid from the tank due to handactuation of the plunger. Held by both the hands the piston pump is worked by slidingaction. The capacity of this sprayer is about 0.5 acre per day. It is useful for small scalespraying in nursery or kitchen gardens and pot plants.

c. Bucket or Stirrup Pump Sprayer:

It consist either of a double acting pump with two cylinders or a single acting pumpwith one cylinder. The other parts of the sprayer are the plunger assembly, foot valueassembly, hose, lance and nozzle, a stirrup and an adjustable foot rest. The suction part ofthe pump is immersed in the spray solution kept on floor in a bucket. The pump is operatedby hand by one person while the other person holding the delivery line, trigger cut-offdevice and lance nozzle sprays pesticide. This sprayer is used both for public healthspraying and agricultural spraying purposes. This type of sprayer is useful for sprayingsmall trees. Area covered per day is 0.5 to 0.8 ha.

d. Knapsack Sprayer

The sprayer is mounded on the back of operator with help of a pair of mounting straps.The pump of the sprayer is actuated by working a hand lever up and down by one hand ofthe operator and the other hand holds the cut off device for spraying purpose. This

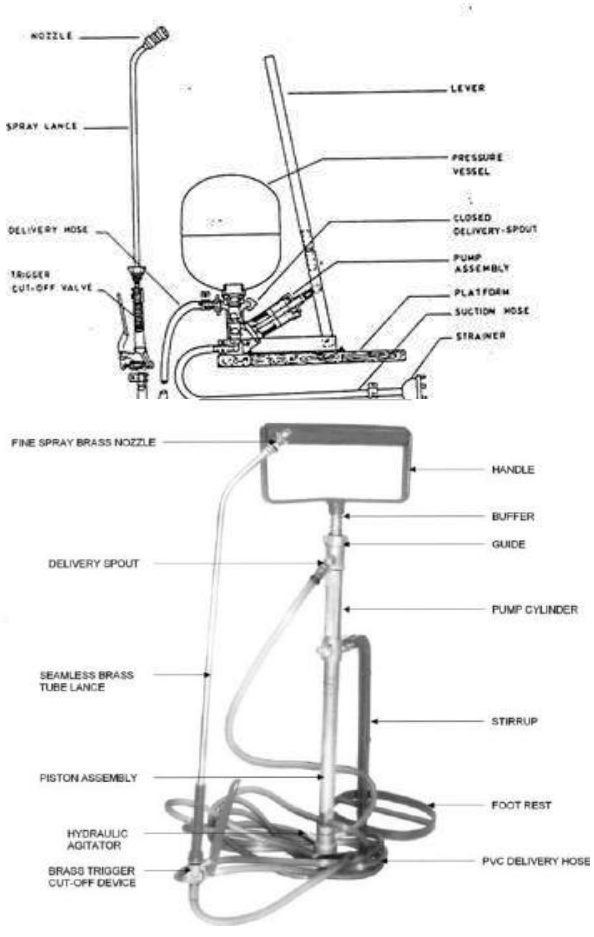
sprayerconsists of liquid tank, hydraulic pump, operating lever, pressure chamber, agitator,delivery hose, spray lance and nozzle. A bean shaped plastic tank of 14-16 liters capacity iscommonly used. It is necessary to operate the hand lever continuously at the rate of 15-20strokes per minute. The normal working pressure is 40 psi. It is user for spraying field cropsvegetables and nurseries. The area covered per day is 0.8 to 1 ha.

e. Rocker Sprayer

It is very much similar to thefoot sprayer.The main differenceis the operation ofpump. Thepump actuation is done by hand of the operator. The sprayer pumpmountedon wooden platform iskept on ground andthe spraysolution is kept in a separate tank or container. It can develop highpressure
10 kg/cm2. For sprayingtall trees, an extension bamboolance can be fitted. The adjustabletype hydraulic nozzle (TripleAction Nozzle) is normally used. It can be used for spraying trees and tall fieldcrops. Itcovers about 1.5 to 2 hectares of areain a day.

f. Foot Sprayer or Pedal Pump:

The pump of the sprayer isworked byoperating a pedal lever bythe foot of the



operator. The sprayliquid is kept in bucket or containerand it is sucked by a suction hosethrougha filter (strainer) due to pistonmovement. A suitable ball valve isprovided in the piston assembly toserve as suction valve. The liquid fromthe pump cylinder is then deliveredinto a pressure chamber where fromthe pressurized liquid reacheshydraulic nozzle. Minimum two person team is required to work on this machine.Hydraulic pressure of 10 kg/cm2 can be achieved which is necessary to project the jet ofspray to tall trees simultaneously from two spray nozzles. The foot operated sprayer isbasically for orchard and tree spraying. The design is strong and sturdy. An adjustable typehydraulic nozzle (Tripple Action Nozzle) is generally used which can generate differenttypes of spray patterns viz., fine spray (hollow cone), medium spray and coarse spray (jet).

The fine and medium spray are suited for low height orchards, jet spray are necessary fortree spraying. The spray jet can reach height of 15 - 20 feet. For spraying taller trees an extraextension like bamboo lance may be used to gain additional height by 8 - 10 feet. It isdifficult to treat field crops by foot sprayers because the sprayer is kept on ground andpesticide solution tank is also kept on ground separately and so movement of the longdelivery hose becomes very difficult. About 1 to 1.5 ha area can be sprayed in a day.

B. Manually Operated air compression Sprayers

These are also known as pneumatic sprayersbecause air pressure is employed for forcing theliquid though the nozzle for atomization. Thecontainers of these sprayers should not be filledcompletely with the spray fluid. A part of thecontainer is kept empty so that adequate air pressurecan be developed over the spray fluid in the tank.They do not have agitators and hence are not usefulspraying materials which settle down quickly.

a. Pneumatic Hand Sprayer

The container for the spray fluid also acts as thepressure chamber. An air pump attached to thechamber inside. The inner end of the discharge pipe runs down to the bottom of thecontainer and its outlet terminates in a nozzle is filled about 3/4th of it and the pump isworked force air into the space to build sufficient pressure upon the spray fluid. Thesesprayers are used extensively in kitchen gardens, in glasshouses and in doors againsthousehold insects. The capacity of tank is up to one liter, if used in field it can cover an areaof 0.1 ha in a day.

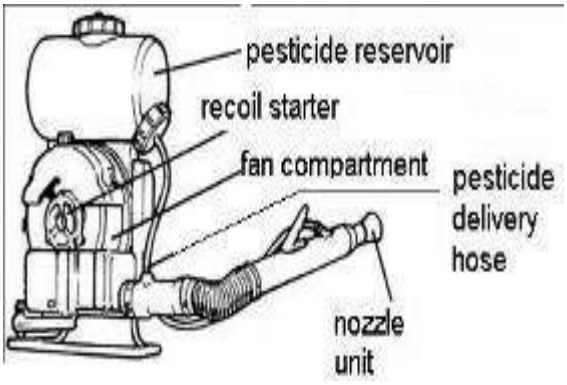
b. Pneumatic Knapsack Sprayer

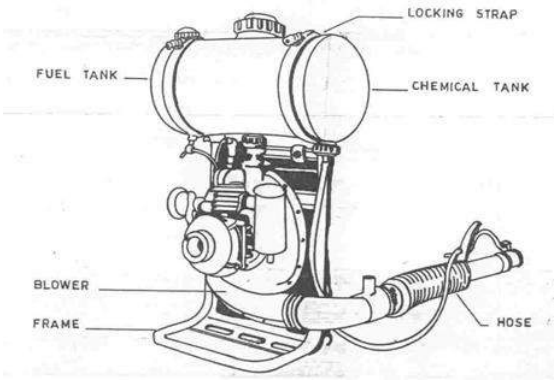
This is similar to compresssion hand sprayer but are used for spraying large quantitiesof liquids (9-10 Litres). It comprises a tank for holding the spray as well as compressed air,

avertical air pump with a handle, filling hole with a strainer, spray lance with nozzle andrelease and shut-off devices. The tank is provided a convenient rest with the back of theoperator and has shoulder straps that allow it to be carried by him. These sprayers are usedagainst agricultural pests and mosquito control operations. This pump covers an area ofabout 0.8 to 1.2 ha in a day.

C. Power Sprayer (Mist blowers cum Duster**)**

Here the spray fluid is blown out by an air produced in the machine. It consists ofchemical tank, fuel tank, carburator, spark plug, engine, blower assembly, delivery system,nozzle system and starter pulley. The power operated spraying system can be converted into a dusting unit by changing certain components. The tank in these is made of a thickpolyethylene and has a capacity of 10 liters. The fuel tank capacity is 1.0 to 1.5 liters. It isprovided with 1.2 to 3.0 hp petrol engine. This can also be used for dusting providedsuitable accessories. The area coveredby these sprayers is about 2 ha in a day.



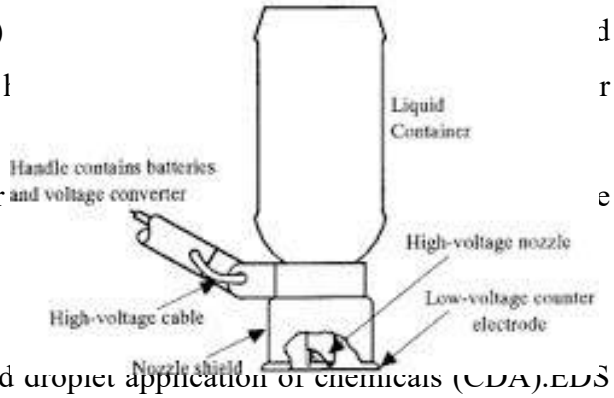


Mist blower Motorized Knapsack sprayer

D. Hand carried, battery operated spinning disc sprayer (Ultra Low Volume Sprayer)

The pesticides are applied assuch or with less than 5 litres sprayfluid produces fine droplets (80µm).These are light weight sprayers(<3kg) d by anenclosed DC motor with a plasticspray l r supply unit.

Liquid is gravity fed frompolyethenecontainer e liquid isflung off by centrifugal force.



E. Electrodyn Sprayers (EDS)

It is new system of spraying for the controlled droplet application of chemicals (CDA).EDS

puts more of active chemical on the target than any other spraying system since thecharged particles are attracted to target crop which ensure coverage on the underside ofleaves where many pests feed and also there is minimal drift to non target areas. The EDSconsists of a spray stick and an unique combination of bottle plus nozzle the bozzle. Thespray stick consists of the batteries and a solid state high voltage generator. The bozzlecontains ready formulated chemical for immediate application to crops. The pesticide inULV formulation is used undiluted at a quantity less than 6 liters/ha and usually at 0.5 to2.0 liters/ha for field crops. The droplet size varies from 20-150 micron with ground sprayingequipment for ULV spray an area of 5 ha can be covered ina day.

DUSTERS

The dusting powders are low concentration ready to use type, dry formulationscontaining 2 to 10% pesticide. The inert material or dry diluents is talc, soapstone,attapulgite, etc., and it is non toxic. The sulphur dust is not diluted with inert material. Thedusts are applied at 20 - 50 kg/ha. It should be noted that the application is done in highlyconcentrated form, as compared to high volume or low volume spraying technique.Therefore, adequate precautions must be taken in handling the dust and during theapplication in field. The dusters are available both manually operated and power operatedmodels. All dusters consist essentially of a hopper which usually contains an agitator, anadjustable orifice and delivery tubes. A rotary fan or a bellows provides the conveying air.

A. Manually operated dusters

a. Plunger duster

They are very simple, low cost machines and useful in a limited way. It consists of adust chamber, a cylinder with a piston or plunger, a rod and a handle. The field applicationcapacity is low. They hold 200 to 400 g of dust in a chamber into which air is pushed by anadjoining piston type air pump operated by hand. The dust cloud is issued from thedischarge outlet. It is useful forsmall scale use in kitchen garden and in household.

b. Bellows duster

This is also a simple design low cost dusting machine. A collapsible bellows pushes airinto a dust hopper of 1-2 kg capacity and dust is discharged from the nozzle outlet.

c. Rotary duster:

This type of duster makes use of a fan or blower to flow large volume of air at highspeed. The dust powder is fed into the stream of air and blown from the outlet tube. The fanor

blower rotatesat high speed by hand cranking handle, which is geared to it. The highergear-ratio and better blower design provide easy cranking and good volume of air isemitted. The dust hoppers are generally cylindrical and are provided with agitator, feedersand dust metering mechanism.Such rotary dusters are either shoulder slung type or belley mounted type. Theshoulder-slung models are better balanced when the dust hoppers are filled. But it becomesinconvenient to operate in crops like sugarcane and cotton. The belley mounted type can beused in such situations. A hand rotary duster can discharge dust powder from 0 – 150 g/minand displace air about one m3/min at35 RPM. Such machine can treat 1 to 1.5 ha /day.

d. Power Duster

These are bigger machines run with the help of engine or electrical motor. Some powerdusters are tractor mounted type and are driven by tractor P.T.O. The equipment ismounted on ironframe (stretcher) and can be carried by 2-3 men. The engine/motor drives acentrifugal fan usually via V-belt drive. The engine is petrol/ diesel run and 3 - 5 H.P. Thefan displaces 20 m3 air/min or more at 100-250 km/hr air velocity. These dusters are good forlarge area treatment and suitable for application on tall trees. In this type of duster design,usually the dust powder is not rotated in the fan-case but dust powder is aspirated in thedelivery channel by air blast. The dust hopper capacity is 10- 20 kg and dust can bedischarged at a rate of 1 to 8 kg/min. A power dustercan cover about 10 ha/day.

e. KNAPSACK DUSTER

The motorised knapsack sprayer can be converted to a duster by replacing some plasticfittings inside the hopper. Almost all mist blowers have provision of converting them fromspraying unit to dusting unit. The two stroke petrol engine runs a blower fan and deliversthe air through a hosepipe system. The dust is agitated and lifted by the blast of air in thehopper (2-5kg capacity) and it is fed into the main air hose or a long dusting hose (40-50 ftlong polythene perforated hose) can also be attached to knapsack duster. Such anattachment is very good for large area treatment in less time. The dust output can beadjusted from 0 to 1.5 kg/min. The motorised knapsack sprayer-cum-duster unit is thereforeuseful for both low volume spraying and dusting operation.

Soil Injector

It is also known as soil gun, which consists of a cylindrical tank for the liquid fumigant,a pump barrel and plunger assembly, injector nozzle, thrust handle and injection handle.The hand operated soil injectors have a capacity of 1 to 3 liters and they can cover about 0.5ha in a day. They are used to apply liquid nematicides to kill soil nematodes.

Granule Applicators:

They are used to apply granular formulations of pesticides uniformly. These are twotypes of granular applicators.

i. There is a plastic hopper 1 liter capacity from which the granules flow by gravity to anozzle.

ii. It is a knapsack type with hopper of 10 liters capacity.

Bird Scarer

It produce loud noise at regular interval and used to scare away the birds. It has threeessential chambers, a chamber to hold calcium carbide, a smaller chamber placed inside theformer to hold water and combustion chamber attached to the main chamber. Water actswith calcium carbide and generates acetylene which explodes producing the noise. Thefrequency of flow of water into calcium carbide chamber. One kg of calcium carbide issufficient for working a machine for 24 hours. One bird scarer is sufficient to cover 1 to 2 ha.

Rat Traps:

Several types of mechanical devices for trapping rats and mice are used in India. Inthese traps baits like dry fish are used for attracting these rats. The cage type wooden boxwith a door closingdevice and spring board types are the more common ones used in thehouses.

Practical- 17: To study about the Sampling techniques for estimation of insect population and damage

Sampling population estimates of insect pests are the fundamental activity in ecologicalentomology. Regular monitoring can answer several important questions such as- Whatkinds of pests are present? Are the pest numbers great enough to do damage and towarrantcontrol? Are bio-agents or natural control present and working? When is the right time tobegin control? and have management efforts successfully reduced the number of pests?

Pest monitoring is the pre-requisite for any successful pest management programwherein, no control measure should be undertaken for a pest unless it is known that- thepest is activelypresent and it is present in sufficient numbers to cause an economic loss.

How to count or measure a species/damage caused in plant, soil or other habitat ?

The sampling method should be: suitable for all key pests, rapid and simple to use, easyintegration into current sampling program, sampling equipment readily available and easyto carry and sampling procedure be simple to understand and conduct.

Sample unit: Single plant, clusters, plants/hill, plant/m2 etc.

Sampling Size: In preliminary studies: sample size will be small and 10% of the mean errorshall be acceptable. Number of samples depends on degree of precision required and chosento minimize the variance and cost..

Types of Sampling

Random sampling: The sample is taken at random with good field coverage to determineinsectnumbers or damage per samples unit. For this purpose, use of random numbers ismade.

Stratified random sampling: It involves the division of population in to different stratabased ondistribution of population.

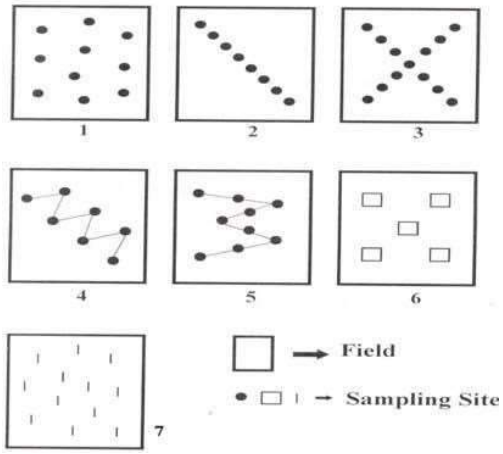
Sequential sampling: It requires continuous sampling until a pre established upper or lowerinfestation level is found.

Trap sampling: This refers to using light, suction, sticky or sex pheromone traps to detectthepresence of insects in an area.

Systematic sampling: It involves sampling ofpopulation at fixed intervals.

Selection of Sampling Site

1. Random
2. Along one diagonal
3. Along two diagonals
4. Zig-zag diagonally
5. Along alphabet _W‘
6. In micro-plots of 1m2
7. Meter row length



Important Sampling Methods

Absolute methods: This method is used to estimatedensity of insects per unit area.

Differenttypes of absolute sampling are denoted by **n.**

Unit of habitat method

- **In situ or direct counts**: e.g. Leafhoppers
- **Knock down:** removing insects form the habitats-drop sheet method e.g. *Helicoverpa*

spp.brushing, washing etc.

- **Netting:** for highly mobile insects
- **Trapping:** Use of different types of traps. Phermone traps, Light traps, suction traps etc.
- **Extraction from soil:** From a fixed volume of soil insects can be counted. e.g. whitegrubs, cutworms, pupae of several lepidopterous larvae.
- **Indirect techniques:** By taking crop samples for example, **dead hearts** in case ofsugarcane shoot borer, **number of plants cut** e.g cutworms, **per cent defoliation** e.g.foliage feeders, **root damage** e.g. termites, root weevils; **shoot damage** e.g. spottedbollworms, **per cent fruiting bodies damaged** e.g. bollworms of cotton, pod borers,**stubble infestation** e.g. in sugarcane.

Absolute sampling method are desirable because they are accurate, however, these methodsare time consuming, often difficult to conduct and are usually expensive compared torelative methods. Relative methods are more economical in terms of time, labour andequipments.

Relative methods: This method provides an identification of insect pests abundance

ordamage relative to other times or location. Different types of relative methods are as followsvisualsearches, use of various traps, plant damage etc.

Remote sensing: Acquiring information through the satellite about pest damage withoutcoming into physical contact. It can be useful in monitoring of certain pests. A radar canmonitor height, speed and direction of insects like locusts, aphids etc.

Components of Remote Sensing

1. Platform

- The vehicle/device on which sensors are mounted
- Carriers or vehicles for the sensors

2. Sensor System

- The device which senses the energy reflected/emitted by the target object

3. Data Products

- Information received from the sensor Packaged as per user requirement

OTHER METHODS

Beat bucket: Requires 20-25 litre capacity plastic bucket (white or light coloured); similar toshake cloth/drop sheet method; top 25 cm of a single plant is bent into the bucket andshaken vigorously (12-15 times during 4-5 seconds); plant is quickly removed andinsects/predators and spiders are counted. It is more effective than shake cloth method;reduces variability due to field scouts.

Vacuum sampling: Sucks into bags most everything from on and around a single plant orplant part; impractical for regular use in sampling and the samples are too messy to process.Further improvements could be made by better initial planning and involvement of thestatistician with the biologist.

Sampling techniques for major insect pests on Paddy crop

Crop	Pest	Economic threshold level	Method of sampling
Paddy	**Green leafhopper**		
	a) At earing stage	5-15 insects/hill	Select 5 micro-plots of 1m2 each in a field andshake vigorously plantsin 5 hills/plot or shake vigorously 25 random plants and count leafhopper fallen on water.
	b) At flowering stage	10-15 insects/hill	Same as above
	Stem borer	5-10% plants with dead-hearts or 2%white ears or one egg mass or moth/m^2.	Count infested and healthy tillers in 25 random plants.
	Leaf-folder	2 damaged leaves/ plant or onelarva/hill	Count infested and healthy plants among 25 random plants or count
	Rice gundhi bug	1-2 insects/hill	Count the insect on 25 random
Gram	**Gram pod borer**	One larva/meter row length	Count larvae in one meter row length from 10-20 random sites in a field.
Okra	**Leafhopper**	2-5 nymphs/leaf	Count leafhopper nymphs from underside of three fully developed leaves in the upper canopy of each of 20 random plants or count leaves showing yellowing and curling from margins and healthyleaves of 20 random plants in a field

FUNDAMENTALS OF AGRICULTURAL EXTENSION EDUCATION- AGS-208

PRACTICAL 1 : ACQUAINTANCE WITH UNIVERSITY EXTENSION SYSTEM

Extension has been traditionally funded, managed and delivered by the public sector all over the world. Agricultural extension in India has grown over last six decades. It is supported and funded by the national government—through its Ministry of Agriculture (MoA) and other allied ministries. The share of agriculture in Gross Domestic Product (GDP) has declined from over half at the time of independence to less than one-fifth this year. Indian agriculture sector has an impressive long-term record of taking the country out of serious food shortages despite rapid population increase, given its heavy reliance on the work of its pluralistic extension system.

There are five major agricultural extension systems devoted to extension work in India:

(i) the Ministry of Agriculture at central level, including the Indian Council of Agricultural Research (ICAR) and the Directorate of Extension (DoE); (ii) State Departments of Agriculture (DoA), as well as the State Agricultural Universities (SAUs); (iii) the Departments of Agriculture (DoA), Animal Husbandry (DAH), Horticulture (DoH) and Fisheries (DoF), as well as the Krishi Vigyan Kendra (KVKs) and, more recently, the Agricultural Technology Management Agency (ATMA) at the District level; (iv) also, there are a wide variety of producers groups, including cooperatives and federations of milk, fruits, cotton, oilseeds, coconut, spices etc.; as well as (v) civil society organizations, such as the Non-governmental Organization (NGOs). The major activities of agricultural extension at the district level are the assessment, refinement and demonstration of technology/products through a network of Krishi Vigyan Kendras (KVKs), the departments of agriculture, animal husbandry, horticulture, fisheries, etc. and the Agricultural Technology Management Agency (ATMA). Also, there are 44 Agricultural Technology Information Centres (ATIC) established under ICAR institutes and SAUs. There is one Directorate of Research on Women in Agriculture (DRWA) located in Bhubaneswar (Odisha). The Division is headed by Deputy Director General (DDG), Agricultural Extension supported by 2 Assistant Director Generals (ADGs). DARE, through the ICAR and the SAUs front-line extension system, plays a catalytic and supportive role by developing extension methodologies, refines and transfers front-line technologies and provides feedback to scientists. Achievements (http://www.icar.org.in/en/agricultural-extension.htm) of the division till December, 2012 are:

- Established a network of over 630 KVK.
- Conducted 4,189 on-farm trials (OFT) on 537 technologies to identify their

location specificity under different farming systems.

- Organized 53,974 Front Line Demonstrations (FLD) to demonstrate production potential of newly released technologies on the farmers' fields.
- Trained more than 1.0 million farmers and extension personnel in agriculture and allied fields.
- Conducted large number of extension activities benefiting about 4.19 million farmers and other end users.
- Production of more than 82,000 qt. of seeds and 10.2 million sapling/seedlings/livestock strains, besides various bio-products for availability to the farmers.
- Identified gender issues in agriculture at DRWA for Women in Agriculture.
- Continued functioning of 44 ATICs in ICAR institutes and SAUs.

Organized 334 interface meetings involving scientists and development officials at district level.

DEPARTMENT OF AGRICULTURAL RESEARCH AND EDUCATION (DARE)

The DARE under MoA was established in December 1973 as a nodal department for all scientific and development related activities as well as bilateral scientific collaborations with other countries. The ICAR- a society registered under the Societies' Registration Act, 1860, is an organization under the DARE, with its headquarters in New Delhi, and a vast network for research all over the country. The Director General (DG) is the principal executive officer and is also the Secretary of DARE. The Governing Body, the chief executive and decision making authority, is chaired by the DG, which consists of eminent agricultural research and extension specialists.

DARE provides the necessary government linkages for ICAR, the premier research organization with a scientific strength of about 25,000 and a countrywide network of 49 institutes including 4 deemed to be of university-status, 6 national bureaus, 18 national research centres, 24 project directorates, 89 All-India Coordinated Research Projects (AICRPs) and 45 agricultural universities spread all over the country. Also providing agricultural education facilities through a very strong network of agricultural education system consisting of 3 Central Agricultural Universities (CAUs), another 2 CAUs have been proposed to be set up in the 12th Five Year Plan (FYP), 45 SAUs and 5 national institutes of ICAR, deemed to be universities including National Academy of Agricultural Research and

Management (NAARM) for catering quality research and education in agriculture.

National Institute of Agricultural Extension Management (MANAGE)

MANAGE was established in 1987, as the National Centre for Management of Agricultural Extension at Hyderabad (Andhra Pradesh), by the MoA, Government of India as an autonomous institute, from which its acronym 'MANAGE' is derived. In recognition of its importance and expansion of activities all over the country, its status was elevated to that of a National Institute in 1992 and re-christened to its present name i.e., National Institute of Agricultural Extension Management. MANAGE is the Indian response to challenges of agricultural extension in a rapidly growing and diverse agriculture sector. The policies of liberalization and globalization of the economy and the level of agricultural technology becoming more sophisticated and complex, called for major initiatives towards reorientation and modernization of the agricultural extension system. Effective ways of managing the extension system needed to be evolved and extension organizations enabled to transform the existing set up through professional guidance and training of critical manpower. MANAGE is the response to this imperative need.

The mandate of MANAGE vests the institute with the responsibility to work in the following directions:

- Developing linkages between prominent state, regional, national and international institutions concerned with agricultural extension management
- Gaining insights into agricultural extension management systems and policies
- Forging collaborative linkages with national and international institutions for sharing faculty resource
- Developing and promoting application of modern management tools for improving the effectiveness of agricultural extension organizations
- Organizing need based training for senior and middle level agricultural extension functionaries
- Conducting problem oriented studies on agricultural extension management
- Serving as an international documentation center for collecting, storing, processing and disseminating information on subjects related to agricultural management.

PRACTICAL 2: GROUP DISCUSSION EXERCISE

Group Discussion

Definition: It is that form of discourse which occurs when two or more persons, recognizing a common problem exchange and evaluate information and ideas, in an effort to solve that problem. Their effort may be directed towards a better understanding of the problem or towards the development of a programme of action relative to the problem. Discussion usually occurs in a face – to – face or co-acting situation, with the exchange being spoken. And when more than two people are involved, it usually occurs under the direction of a leader.

Purposes

1. To solve a problem (decision – making).

2. To exchange information (improve understanding)

3. To motivate.

4. To plan a programme of action

5. To elect or select a person for a position etc.

6. To entertain.

7. To hear and discuss a report.

8. To form attitudes.

9. To release tensions.

10. To train individuals.

Procedure

1. Understand and adopt the proper technique. The technique of a problem – solving group discussion consists of the following six steps based on the “reflective thinking” pattern.

a. Recognition of the problem as such by the group.

b. Definition of the problem, its situation and diagnosis.

c. Listing of as many solutions as possible.

d. Critical thinking and testing of these hypotheses to find the most appropriate and feasible solution or solutions.

e. Acceptance or rejection of the solution or solutions by the group.

f. Lastly, considering how to put the accepted solution into practice.

2. See that one of the group members takes up the role of the discussion leader (or chairman). Extension worker should avoid this role as far as possible, because in such a case, a situation is likely to develop where the group listens and the chairman does all the talking.The size of group should never exceed 30 persons.

Advantages

1. It is a democratic method, giving equal opportunity for every participant to have his say.

2. It appeals to the practical type of individuals.

3. It creates a high degree of interest.

4. The strength of group discussion lies in the fact that the discussants approach the problem with an open mind and suspended judgment in a spirit of enquiry.

5. It is a co-operative effort and not combative or persuasive in nature.

6. Combined and co-operative thinking (Pooling of wisdom) of several persons is likely to be superior to that of isolated individuals.

7. A small group can think together on a problem in an informal fashion and work out solutions better and faster by using this method than by following rigid parliamentary procedure. (Even parliament and legislatures recognize this when they appoint adhoc committees)

8. Develops group morale. When a group discusses a question and then comes to a decision that is “our” decision for the group and they will see that our decision is carried out. (Group action encouraged)

9. It is a scientific method (employing the reflective thinking pattern).

10. Participants need not be good speakers or debaters.

11. Continued experience with such group discussions improves one's capacity for critical and analytical thinking.

Limitations

1. Factions in villages may hinder the successful use of this method

2. The ideal discussants with self-discipline (open mind and suspended judgment) are difficult to find. So, also, it is difficult to find an ideal chairman or leader for group discussion.

3. It is not suitable for dealing with topics to which discussants are new.

4. In large groups especially, and even in small groups to some extent, it is difficult to achieve group homogeneity or cohesion.

5. The size of the group has to be limited, because the success of the method is perhaps inversely proportional to he size of group other factors being constant.

6. It is not a good method for problems of fact.

7. It is not suitable for taking decisions in times of crisis or emergency, as it is a slow process.

8. Due to its informal conversational style, the scope for orderly or coherent arrangement of ideas is limited.

PRACTICAL 3: AUDIO-VISUAL AIDS PREPARATION AND USE

Audio-Visual Aids

- An audio aid is an instructional device in which the message can be heard but not seen.
- A visual aid is an instructional or communicating device in which the message can be seen but not heard.
- An audio-visual aid is an instructional device in which message can be heard as well as seen.

Strictly speaking, no teaching method is complete without talk/speech. It must be remembered that audio-visual aids can only supplement the teacher, but cannot supersede & replace/supplant him.

Important Audio-Visual Aids

Audio-Visual aids are supporting materials & they alone cannot generate learning. They should be considered only a tool that helps to do a job in a better way. Visual aids are of different types. The following are the more commonly used ones in India:

1. Posters:

A good poster creates awareness & interest among the people. It inspires & takes people towards action. It consists of 3 main parts. The first usually announces the purpose or the approach, the second sets out conditions, & the third recommends action. A poster should be bold enough to attract attention of the people, & should communicate only one idea at a time. It should have simple letters which are clear & forceful. The size of a poster should not be less than 50 X 75 cm.

2. Flannel graphs

Flannel graphs serve as a good teaching aid when a piece of sandpaper is fixed to the back of a picture, a photograph, a letter, etc. They can be made to adhere easily to a piece of thick flannel cloth, fixed on a board. They are used as an aid for group methods like informal talks or lectures.

3. Flash cards

Flash cards are a set of small compact cards approximately 30 to 45 cm. in size, and are used to bring home an idea, such as the benefits of a smokeless chulha, the rearing of cross breed cows, compost-making and other practices. Pictures on the theme are drawn on these cards in a logical sequence, which are then flashed before the audience. Upon seeing them, the villagers are able to follow a story more easily.

4. Puppets

Puppets are very popular and especially suitable for village situations. Puppet shows can be effectively organised to gather the rural people. For a puppet show, a short story, brief scenes and quick dialogues are necessary. Such shows can teach a lesson about any specific topic like animal health, literacy, or home-making.

5 Models

Models create a sense of realisation in a person. Models of new farm equipments, compost pits, sanitation devices and animals are mostly prepared for those people who are not in a position to see them in the actual form. They are used to create interest, promote understanding and influence the people to adopt a certain practice.

6 Bulletin-boards

A bulletin-board can serve the purpose of making announcements, displaying events of short duration & photographs of local activities. The information should be written in simple language.

7. Photographs

They are a very simple visual aid. Good photographs show some action & catch the feelings & emotions of the people. They are so arranged that they tell a story. They are displayed on a bulletin-board at a common meeting-place where a large number of people can see them. They should be clear & bold in composition with proper captions. One good picture is perceived as equivalent to 1000 words.

8 Cultural programmes

Local cultural programmes, such as folk-songs & dramas, are used as an effective medium of communicating the message of development programmes. Dramatization of a theme or story

creates a lively interest among the audience. Folk-songs & dances related to the subjects of local interest & importance, when acted on the stage, bring them home more forcefully.

For an effective use of extension teaching methods, it is not enough to know these methods and their techniques. What is more important is the appropriate selection of a method or combination of methods for a particular situation. In fact, when a farmer is exposed to a new idea for several times by different methods or a combination of methods, he is likely to accept it more quickly. Farmers learn about new practices through several stages. These stages are known as:

- Awareness stage - when a person comes to know of a new practice but lacks the complete information.
- Interest stage - when he becomes interested in a new idea & wants to know more about it.
- Evaluation stage- when he mentally applies the new idea to his present situation & evaluates it.
- Trial stage- when he applies the new idea or practice on a small scale in order to determine its utility under his own situation.
- Adoption stage- when he decides to continue the full use of the practice. Thus, it is the cumulative effect on people through exposure to an idea repeatedly that result in action.

PRACTICAL 4: PREPARATION OF EXTENSION LITERATURE – LEAFLET, BOOKLET, FOLDER, PAMPHLET NEWS STORIES AND SUCCESS STORIES

In addition to the personnel contact methods and the face- to- face group teaching methods mass media enable extension workers to greatly increase their teaching efficiency.

Literatures / Publications

Literature are write ups or written materials about an idea or a thing. In extension teaching literature plays an important role in the message dissemination process. Some of the common literature, that forms the part of extension teaching learning process are,leaflet, folders, pamphlets, bulletins, circular letters, newspapers,magazines, journals and newsletters. The literature serves the purpose of communicating precise and reliable scientific information in a simpler language easily understandable to a common man. A brief explanation of various literature used in extension are given below.

Leaflet: A leaflet is a single sheet of printed matter. It is made to give an accurate or specific information on a specific topic.

Folder: A single sheet of printed information in a folded form. There can be any number of folds in a folder. Like leaflet, folder is also primarily meant for dealing a specific topic.

Pamphlet: A pamphlet consists of3 to 12 pages and deals with a specific topic in a detailed manner.

Bulletin: The number of pages fora bulletin ranges from 12 to 20. A bulletin is a written piece of information about a number of related topics presented in a detailed manner.

Booklet: When the number of pages exceeds 20 then it is called as a booklet. Usually a booklet deals with number of topics and the discussions are carried-out more elaborately with illustrations, pictures, figures and tables.

Circular letters : The letter sent to a group of people by passing it out from one man to other like a circular to pass on certain information or messages. Circular letters helps to maintain a continuous contact with farmers.

Newspaper/ Newsletter/Magazine/ Journal: Periodicals give a wide range of information about what is going on in the next door and around. It is mass media which can be of immense use in message dissemination. It helps to serve as a forum for extension activity in an area.It plays the role of communicating the information to people of various level and acquaint the public about programmes activities and progress made in an area.

Circular letter

It is a letter reproduced and sent to many people by the extension worker, to publicize an extension activity or to give timely information on farm and home problems.

News articles (News stories)

News is any timely information that interest a number of persons. It is an accurate, unbiased account of the main facts of a current event that is of interest to the readers of the news papers.

Radio

It is a medium of mass communication, a tool for giving information and entertainment.

Television

It is one of the important mass media for dissemination of information in rural areas. It has an unique advantage over other mass media because it provides words with pictures and sound effects like movies. The messages will reach a large number of people at the same time

Campaign

It is an intense teaching activity undertaken at an opportune moment for a brief period; focusing attention in a concerted manner on a particular problem with a view to stimulate the widest possible interest in a community, block, geographical areas. Ex: FMD Vaccination campaign.

Media forums

Are small organized groups of individuals who meet regularly to receive a mass media programme and discus its contents.

The most media linked to the forum may be a radio as in India - charcha mandals, or radio phonics schools of Latin America –or television as in the Italian telescuola.

It is a combination of mass media inter-personal channels. Here the advantage of group psychology/pressure is used to motivate the individual.

PRACTICAL 5: PRESENTATION SKILLS EXERCISE

Poster is a visual combination of bold design, colour and message to catch and hold the attention of the passerby long enough to implant or to reinforce the significant idea in his mind.

Example: Detail from the poster showing a village that practices good animal husbandry practices.

Aim

- To create awareness about the extension programme.
- To communicate to a passerby.
- To implant very quickly in a viewers mind or to make him recall a single important idea.
- To motivate people to act by repeated reminders.

Principle

Posters are simple graphic representations with the greatest possible impact. As an advertising medium, they arrest the eye and the mind, remind the public of a message. Posters generally contain three main features namely

- They announce a purpose,
- They set conditions and
- They recommend action.

Posters should be attractive, brief and clear and this is the ABC principle of poster. A hand drawn poster may be used in training programmes, group meetings, farmers day, etc. printed posters may be used in large numbers in campaigns, exhibitions, etc.

Materials / facilities required

For hand made posters, paperboards, poster colours, pencil, eraser, scale and good brushes are needed.

For printed posters computer, litho offset press are required.

Planning

- Decide on the theme of the poster and identify the key points.
- Decide on the size of the poster (10”x 15”, 15”x 20”, 18”x 22”, 20”x 30”, 22”x 28”),

caption, illustrations and the colours to be used.

- Based on the availability of funds decide on the number of copies to be produced.
- Keep in mind the date by which the posters are to be made ready.

Preparation

- Prepare a number of dummies, small but proportional to the actual size
- Ensure a balance between words and picture. The pictures should be bold without much detail.
- Put the caption in one line.
- Follow optical spacing of the letters.
- Select the best layout and make some sets of colour combinations. The picture and letters must make a contrast with the background.
- Select the best colour combination. Engage an artist if required.
- Consult a press manager and decide on the economic production of the posters.
- If only a few hand drawn posters are needed make pencil sketches and then colour the poster on a thick chart paper.

Presentation

- Put hand drawn posters in well-lighted places where the visitors are likely to assemble or pass through.
- For printed posters, ensure timely dispatch.
- Display printed posters at prominent places in time. In important places put up a number of posters closely to produce mass effect.

Follow-up

Watch reaction of the audience.

Ensure availability of inputs and services and commensurate with the message in the posters.

PRACTICAL 6: MICROTEACHING EXERCISE

Microteaching is a concentrated, focused form of peer feedback and discussion that can improve teaching strategies. Microteaching is a system of controlled practice that makes it possible to focus on specific teaching behaviors and to practice teaching under controlled conditions. Competence in one skill is developed before proceeding to another skill. Microteaching has been defined in a number of ways.

Skills applicable for micro-teaching:

1. **Lesson planning** with clear-cut objectives and an appropriate planned sequence. The content should be concise, appropriate, relevant, and could cover the specified duration.
2. **Introduction skill (Set induction)** - The process of gaining pupil's attention at the beginning of the class by establishing rapport with pupils, promoting their attentions, exposing them to essential contents, and linking their previous knowledge with the topic.
3. **Presentation and explanation skills** – Teacher enthusiasm, explanation, narration, giving appropriate illustrations and examples, planned repetition, and encouraging group discussion wherever necessary. The trainee teacher should be able to rightly explain the concept by simple, relevant, and interesting examples to increase pupils' understanding.
4. **Skill of stimulus variation** – Securing and sustaining the attention of the pupil is imperative for a good teacher. The effective components of the skill are Gestures (Hand, facial, body)

- Change in the speech pattern
- Voice variation and modulation (Pitch, volume,
- speed)
- Change in the interaction pattern
- Focusing
- Pausing movement
- Emphasis on significant points

5. **Proper use of audio-visual aids** – The increased awareness of the audiovisual aids and other equipment is important for this skill. Neatness, readability, adequate spacing, distinct size, proper spacing between words and lines, and use of relevant words or phrases are the key components for this skill.

6. **Reinforcement** - Recognizing pupil's difficulties, listening, encouraging pupil participation and response. The use of positive verbal and non-verbal cues would be the key components for this skill.

7. **Skill of probing questions** - Probing questions are those which help the pupils to think in depth about the various aspects of the problem enabling pupils to understand the subject deeply. It is important to allow and encourage the fellow trainee teachers to ask structured questions and clarify doubts. Redirection, refocusing, and increasing critical awareness are important components of this skill.

8. **Silence and nonverbal cues** (Body language)

10. **Classroom management** – Providing proper instructions, restricting inappropriate behavior, and calling the pupils by name are essentials of this skill.

9. **Skill of achieving closure** - Method of concluding a teaching session so as to bring out the relevance of what has been learnt, its connection with past learning and its application to future learning. Questions and statements by the teacher by consolidation of the major points covered during the lesson and ability for applying the knowledge gained by pupils during the lesson to new situations. Closure should be timely! Prepare to start and end in time.

PROCEDURE OF MICROTEACHING (MICROTEACHING CYCLE)

Microteaching is a system of controlled practice that makes it possible to focus on specific teaching behaviors and to practice teaching under controlled conditions. Competence in one skill is developed before proceeding to another

Defining the skill: A particular skill to be practiced is defined and explained to trainee teachers in terms of the purpose, teaching behaviors to provide the knowledge and awareness of the component teaching skills with examples.

2. **Demonstrating the skills:** The specific skill in microteaching is demonstrated by the experts/ supervisor in simulated conditions or through video to the trainee teacher.

3. **Planning the lesson:** The trainee teacher selects a topic and plans a short lesson (Micro-lesson) for five to 10 minutes with the help of his/her supervisor, on the basis of demonstrated skill for his/her practice. The lesson planning should be in a logical sequence where maximum application of the components of a skill is possible.

4. **Teaching the lesson:** The trainee teacher teaches the lesson to a micro-class (A small group of pupils, five to 10) and uses the components of skill as per the planning of the lesson. If the situation is different and not as visualized in the planning of the lesson, the trainee

teacher should modify his/her behavior as per the demand of the situation in the class. The trainee teacher should have the courage and confidence to handle the situation arising in the class effectively.

5. The lesson is observed by the supervisor or peers or video recorded or televised at close circuit television (CCTV).

6. **Feedback:** This term refers to giving information to the trainee teacher about his performance (Points of strength as well as weakness) so that trainee teacher can improve his/her performance in the desired direction. The feedback should be constructive and based on observation. Commenting on observable behavior also leads to suggestions for improvement. Thus the teaching is followed by discussion to provide the feed-back to the trainee on the basis of observation of the lesson. The teaching is evaluated by students, peers and the supervisor. Colleagues and post-graduate students can act as peer evaluators. The supervisor reinforces the instances of effective use of the skill and draws attention of the teacher trainee to the points where he/she could not do well. he trainee teacher can observe his/her teaching activities on video or CCTV8. The awareness of his/her own teaching performance provides the reinforcement of the trainee teacher.

7. **Re-planning:** In the light of the discussion, suggestions and feedback given by the supervisor, the trainee teacher re-plans the lesson incorporating the points of strength in order to practice the small skill effectively in the second trial for improvement.

8.. **Re-teaching**: The revised lesson is re-taught to another small group of pupils of same class for the same class duration to practice the small skill. This involves teaching to the same group of pupils if the topic is changed or to a different group of pupils if the topic is the same. This is done to eliminate boredom or monotony of the pupil. The trainee teacher teaches the class with renewed courage and confidence to perform better than the previous attempt.

9. **Re-feedback**: The supervisor observes the re-teach lesson and gives re-feed back to the trainee teacher with convincing arguments and reasons. Thus the re-teaching is again followed by discussion, suggestions and encouraging the teaching performance by the re-feedback provided to the trainee teacher. This is the most important component of microteaching for modification of behavior of trainee teachers in the desired direction in each and every skill practice.

10. **Repeating the cycle**: The “teach-re-teach” cycle may be repeated several times till desired level of skill or adequate mastery is achieved. Such repeated cycles of teaching, feedback and re-teaching help the teacher to improve his teaching skills one at a time.

PRACTICAL 7: UNDERSTANDING PRA TECHNIQUES

Participatory rural appraisal (PRA) is an approach used by non-governmental organizations (NGOs) and other agencies involved in international development. The approach aims to incorporate the knowledge and opinions of rural people in the planning and management of development projects and programmes.

PRA can be described as a family of approaches, methods and behaviours that enable people to express and analyse the realities of their lives and conditions, to plan themselves what action to take, and to monitor and evaluate the results. Its methods have evolved from Rapid Rural Appraisal (RRA). The difference is that PRA emphasises processes which empower local people, whereas RRA is mainly seen as a means for outsiders to gather information

Origins of participatory rural appraisal :

The roots of PRA techniques can be traced to the activist adult education methods of Paulo Freire and the study clubs of the Antigonish Movement. In this view, an actively involved and empowered local population is essential to successful rural community development. Robert Chambers, a key exponent of PRA, argues that the approach owes much to "the Freirian theme, that poor and exploited people can and should be enabled to analyze their own reality."[

PRA provides a structure and many practical ideas to help stimulate local participation in the creation and sharing of new insights. The emphasis on ensuring community feedback broadens the group of people involved. It is increasingly linked to participatory planning processes (e.g. using adapted forms of logical framework analysis). Although PRA was not intended to collect statistically significant information, it is increasingly used in combination with other methodologies to fulfil more scientific information needs and is easily made complementary.

There is no single way to ‘do’ PRA, although there are core principles and over 30 methods available to guide teamwork, do sampling, structure discussions and visualise analysis. The combination and sequence of methods will emerge from the context. Optimal ignorance and triangulation of findings guide the fieldwork in recognition of the need to know enough without knowing it all and to ensure that the qualitative insights are cross-checked by

different sources using different methods. Principles of PRA Different practitioners would find different principles but most would agree to include the following :

1. Using optimal ignorance: this refers to the importance of knowing what it is not worth knowing. It avoids unnecessary details and irrelevant data. It does not measure more precisely than is needed. It optimizes trade off between quality, relevance, accuracy and timeliness.
2. Offsetting biases: especially those of rural development tourism, by being relaxed and not rushing, listening not lecturing, probing instead of passing on to the next topic, being unimposing instead of important , and seeking out the poorer people and their concerns.
3. Triangulation: using more than one, and often three, sources of information to cross-check answers.
4. Learning from and with rural people: directly, on the site, and face-to-face, gaining from indigenous physical, technical and social knowledge.
5. Learning rapidly and progressively: with conscious exploration, flexible use of methods, opportunism, improvisation, iteration, and cross-checking, not following a blueprint program but adapting through a learning process.

RRA	PRA
Extractive in nature Elicited Information owned by outsiders	Learning with local people Information owned by rural people but shared with outsiders.

Methods :

PRA employs a wide range of methods to enable people to express and share information, and to stimulate discussion and analysis. Many are visually based, involving local people in creating.

For example:

maps showing who lives where and the location of important local features and resources such as water, forests, schools and other services; flow diagrams to indicate linkages,

sequences, causes, effects, problems and solutions; seasonal calendars showing how food availability, workloads, family health, prices, wages and other factors vary during the year; matrices or grids, scored with seeds, pebbles or other counters, to compare things - such as the merits of different crop varieties or tree species, or how conditions have changed over time.

PRA activities usually take place in groups, working on the ground or on paper. The ground is more participatory, and helps empower those who are not literate. Visual techniques provide scope for creativity and encourage a frank exchange of views. They also allow crosschecking. Using a combinations of PRA methods a very detailed picture can be built up, one that expresses the complexity and diversity of local people's realities far better than conventional survey techniques such as questionnaires.

A selection of participatory methods and their uses :

Participatory method	**Brief description**	**Examples of particular use**
Timelines	Historical profiles of longer-term events or trends	Fish catch over time, productivity changes, policy changes
Seasonal calendars	Graphical representation of seasonal events or trends	Labor availability, hydrographic changes
Transect walks and through particular areas	Land- and water-use maps based on walking capital, local knowledge of microhabitat, current use of aquatic resources	Quality and quantity of natural resource maps
Social maps	Maps locating key social features	Access to services and infrastructure
Wealth ranking	Socio-economic categorization of households	Assets, income
Preference ranking	Ordinal ranking, e.g. based on pairwise comparisons, based on defined criteria with scoring	Livelihood strategies, assets and matrix ranking access to services (e.g., fish for

		conservation)

Behaviour and attitudes :

PRA depends on facilitators acting as convenors and catalysts, but without dominating the process. Many find this difficult. They must take time, show respect, be open and self-critical, and learn not to interrupt. They need to have confidence that local people, whether they are literate or not, women or men, rich or poor, are capable of carrying out their own analysis.

The use and abuse of PRA :

Unfortunately, there has been much abuse of PRA by outsiders keen only to extract information quickly, and use it for their own purposes. Such practice is unethical because local people are brought into a process in which expectations are raised, and then frustrated, if no action or follow- up results. To avoid this, those wishing to use PRA methods in a purely extractive way need to be transparent about their intentions, and refrain from calling what they do PRA.

In PRA, facilitators act as a catalyst, but it is up to local people to decide what to do with the information and analysis they generate. Outsiders may choose to use PRA findings - for example, to influence policy or for research purposes. In all cases, however, there must be a commitment on the part of the facilitating organisation to do its best to support, if requested to do so, the actions that local people have decided on.

Some tips for doing PRA :

It is easier to give advice than to take it. So one has to be cautious. Here is a personal list of some practical tips

1. Do not lecture. Look, listen and learn.

2. Facilitate; do not dominate; do not interrupt or interfere; once a task is initiated, let people get on with it; give them time to think or discuss among themselves.

3. Embrace error. We all make mistakes, and do things badly sometimes. Never mind. Do not hide it.Share it.

4. Try to obtain opinions from all groups.

5. Relax, do not rush.

6. Meet people when it suits them.

7. Use six helpers – what, why, who, how where, and when

Practical applications :

Since the early 1990s, PRA approaches and methods have evolved and spread with astonishing speed. Originating mainly among non-government organisations (NGOs) in East Africa and South Asia, they have since been adopted by government departments, training institutes, aid agencies, and universities all over the world. They are now being used in at least 100 countries, with PRA networks existing in over 30. PRA has been applied in almost every domain of development and community action, both urban and rural.

Examples include :

- Natural resources management
- Establishing land rights of indigenous people
- Slum development
- HIV/AIDS awareness and action
- Anti-poverty programmes
- Disaster management
- Negotiation and conflict resolution
- Adult literacy

COMMUNICATION SKILLS AND PERSONALITY DEVELOPMENT- AGS-209

PRACTICAL 1: INTERACTIVE LECTURE WITH AV AIDS FOR IMPROVING LISTENING SKILLS AND NOTE TAKING OF STUDENTS

What is listening?

Listening is the ability to accurately receive and interpret messages in the communication process.Listening is key to all effective communication. Without the ability to listen effectively, messages are easily misunderstood. As a result, communication breaks down and the sender of the message can easily become frustrated or irritated.

Purpose of listening:

1. To specifically focus on the messages being communicated, avoiding distractions and preconceptions.
2. To gain a full and accurate understanding into the speakers point of view and ideas.
3. To critically assess what is being said. (See our page on Critical Thinking for more).
4. To observe the non-verbal signals accompanying what is being said to enhance understanding.
5. To show interest, concern and concentration.
6. To encourage the speaker to communicate fully, openly and honestly.
7. To develop an selflessness approach, putting the speaker first.
8. To arrive at a shared and agreed understanding and acceptance of both sides views.

Often our main concern while listening is to formulate ways to respond. This is not a function of listening. We should try to focus fully on what is being said and how it's being said in order to more fully understand the speaker.

What is note taking?

Note-taking is the practice of writing down or otherwise recording key points of information. It's an important part of the research process. Notes taken on class lectures or discussions may serve as study aids, while notes taken during an interview may provide material for an essay, article, or book.

Guidelines for note taking

1. Look over your notes from the previous lecture to provide continuity with the lecture you are about to hear.

2. Record your notes clearly and as completely as possible. You cannot write down the lecture word-for-word, but try to be as complete as possible.

3. Write down the key concepts or terms given before the lecture. Be sure you can adequately define and describe these important names, events, or ideas.

4. Re-read your notes after every lecture. In your free time you can clarify them or go over points you may not have fully digested during the lecture. If you fall behind in note-taking, skip lines on the page and leave room to fill in later. If problems still exist, make sure you ask your instructor about them the next class meeting.

5. It might be advisable to keep a "flashcard" system for your key terms and concepts. An index card (3X5) for each term or concept may assist you in learning the information. Certainly, rewriting your notes in any form will help you retain the material.

6. Keep up with the reading assignments. Read and study all text assignments before class so that the material will seem less mysterious to you.

7. Use abbreviations in your notes, but be consistent in order to avoid confusion. Example: American = Am; Civil War = CW; railroads = r/r; President = Pres; without = w/o; ex = example; i.e. = in other words.

9. Allow plenty of room for taking notes. Depending on your supply of paper, you might even consider skipping a line and/or writing on only one side of a page. Separate important key names, places, or events to avoid confusion. Get in the habit of being neat so that you escape the frustration of not being able to read your own writing.

10. If in doubt about your note-taking expertise, ask your instructor to go over them with you. It is important to develop these skills. Good note-taking will help you in all your classes.

PRACTICAL 2: WRITING SKILLS

What is writing skills?

Writing skills are an important part of communication. Good writing skills allow you to communicate your message with clarity and ease to a far larger audience than through face-to-face or telephone conversations.

Guidelines for good writing:

1. Appropriate or need based from audience's point of view.
2. Use of simple language
3. Use of shorter sentences.
4. Use of comprehensible words.
5. Accuracy and clarity.
6. Use of relevant examples for making things clear.

Steps for good writing:

1. Planning- First plan and design every detail. Rearrange the ideas in order.
2. Put down your ideas.
3. Check and recheck your writing.

PRACTICAL 3: ORAL PRESENTATION SKILLS

What is oral presentation?

Oral presentations, also known as public speaking or simply presentations, consist of an individual or group verbally addressing an audience on a particular topic. The aim of this is to educate, inform, entertain or present an argument. Oral presentations are seen within workplaces, classrooms and even at social events such as weddings. An oral presentation at university assesses the presenter's ability to communicate relevant information effectively in an interesting and engaging manner.

Guidelines for oral presentation:

1. **Review the subject outline**. Look for all relevant detail that you will need to understand the requirements of the task, including when it is due, the weight of the assessment, and the length of time you have to present. **Review the assessment criteria**. What are you are being assessed on?
2. **Analyse the task**. Determine the purpose of the presentation. Do you need to answer a specific question?
3. **Consider the audience**. What are their expectations of your content and delivery?
4. **Brainstorm**. Map out everything you already know about the topic. Write out any ideas you can use to interact with the audience, or engage them, and jot down what questions, explanations and information you want the audience to be provided with.
5. **Do the research**. Find relevant material, take notes, and remember to keep the references you used.
6. **Organise your ideas**. Create a logical presentation so the information flows well.
7. **Pay attention to the language you are using**. Presentations should be delivered in spoken or conversational language rather than written language. Spoken language is much easier for your audience to follow.
8. Arouse interest of audience.
9. Maintaining eye contact.
10. Use proper gesture and body movement.
11. Use of audio-visual aids.
12. Actively involving your audience
13. Use repetitions, pauses and variations in voices.
14. Have a smooth ending.

PRACTICAL 4: READING AND COMPREHENSION SKILLS

What is reading and comprehension?

Comprehension refers to the ability to understand written words. It is different from the ability to recognize words. Recognizing words on a page but not knowing what they mean does not fulfill the purpose or goal of reading, which is comprehension. Imagine, for example, that a teacher gives a child a passage to read. The child can read the entire passage, but he or she knows nothing when asked to explain what was read. Comprehension adds meaning to what is read. Reading comprehension occurs when words on a page are not just mere words but thoughts and ideas. Comprehension makes reading enjoyable, fun, and informative. It is needed to succeed in school, work, and life in general.

Strategies for reading comprehension:

1. Use prior knowledge.
2. Predicting.
3. Identification of main idea and summarization.
4. Questioning.
5. Making inferences.
6. Visualizing.