B.Sc. BOTANY LAB MANUAL

4th Semester

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B.SC (Honours) in Botany (CBCS)

C8P: Molecular Biology

Preparation of LB medium and raising *E.Coli.*

Lysogeny broth (LB) is a nutritionally rich medium primarily used for the growth of bacteria. Its creator, Giuseppe Bertani, intended LB to stand for lysogeny broth,but LB has also come to colloquially mean Luria broth, Lennox broth, or Luria–Bertani medium. The formula of the LB medium was published in 1951 in the first paper of Bertani on lysogeny. In this article he described the modified single-burst experiment and the isolation of the phages P1, P2, and P3. He had developed the LB medium to optimize Shigella growth and plaque formation. LB media formulations have been an industry standard for the cultivation of Escherichia coli as far back as the 1950s. These media have been widely used in molecular microbiology applications for the preparation of plasmid DNA and recombinant proteins. It continues to be one of the most common media used for maintaining and cultivating laboratory recombinant strains of Escherichia coli. For physiological studies however, the use of LB medium is to be discouraged.

There are several common formulations of LB. Although they are different, they generally share a somewhat similar composition of ingredients used to promote growth, including the following:

i. Peptides and casein peptones, ii. Vitamins (including B vitamins), iii. Trace elements (e.g. nitrogen, sulfur, magnesium), iv. Minerals

Sodium ions for transport and osmotic balance are provided by sodium chloride. Tryptone is used to provide essential amino acids such as peptides and peptones to the growing bacteria, while the yeast extract is used to provide a plethora of organic compounds helpful for bacterial growth. These compounds include vitamins and certain trace elements. In his original 1951 paper, Bertani used 10 grams of NaCl and 1 gram of glucose per 1 L of solution; Luria in his "L broth" of 1957 copied Bertani's original recipe exactly.[6] Recipes published later have typically left out the glucose.

Formulae: The formulations generally differ in the amount of sodium chloride, thus providing selection of the appropriate osmotic conditions for the particular bacterial strain and desired culture conditions. The low salt formulations, Lennox and Luria, are ideal for cultures requiring salt-sensitive antibiotics.

LB –Miller (10g/L NaCl), LB-Lennox (5 g/L NaCl), LB-Luria (0.5 g/L NaCl)

The following is a common method for the preparation of 1 litre of LB:

- Measure out the following: 10 g tryptone, 5 g yeast extract, 10 g NaCl
- Suspend the solids in ~800 ml of distilled or deionized water.
- Add further distilled water or deionized water, in a measuring cylinder to ensure accuracy, to make a total of 1 liter.
- Autoclave at 121 °C for 20 mins.
- After cooling, swirl the flask to ensure mixing, and the LB is ready for use.

Isolation of Genomic DNA from *E. coli*

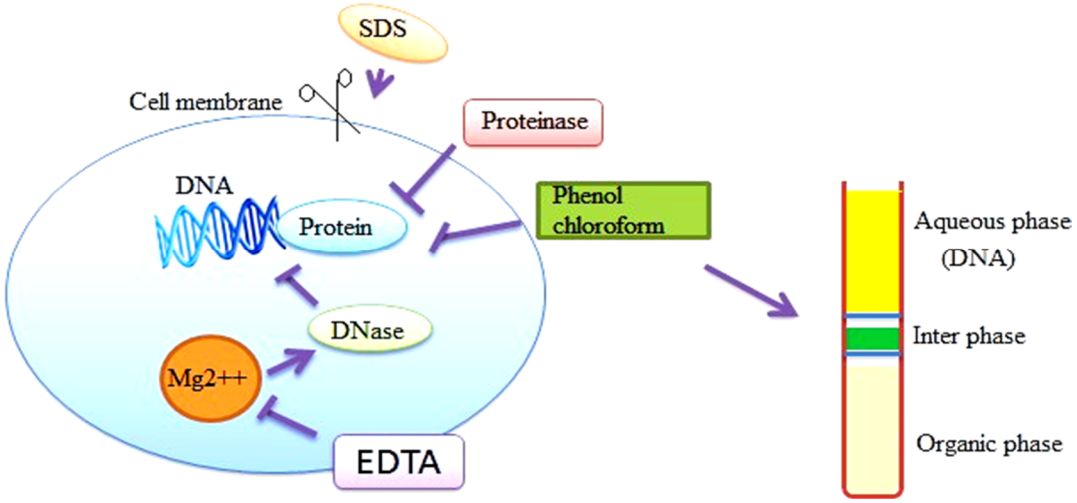
Aim: To isolate the genomic DNA from ***E .coli***

Principle:

The isolation and purification of DNA from cells is one of the most common procedures in contemporary molecular biology and embodies a transition from cell biology to the molecular biology (from *in vivo* to *in vitro*). The isolation of DNA from bacteria is a relatively simple process. The organism to be used should be grown in a favorable medium at an optimal temperature, and should be harvested in late log to early stationary phase for maximum yield.

The genomic DNA isolation needs to separate total DNA from RNA, protein, lipid, etc. Initially the cell membranes must be disrupted in order to release the DNA in the extraction buffer. SDS (sodium dodecyl sulphate) is used to disrupt the cell membrane. Once cell is disrupted, the endogenous nucleases tend to cause extensive hydrolysis. Nucleases apparently present on human fingertips are notorious for causing spurious degradation of nucleic acids during purification. DNA can be protected from endogenous nucleases by chelating Mg^{2++} ions using EDTA. Mg^{2++} ion is considered as a necessary cofactor for action of most of the nucleases. Nucleoprotein interactions are disrupted with SDS, phenol or proteinase K. Proteinase enzyme is used to degrade the proteins in the disrupted cell soup. Phenol and chloroform are used to denature and separate proteins from DNA. Chloroform is also a protein denaturant, which stabilizes the rather unstable boundary between an aqueous phase and pure phenol layer. The denatured proteins form a layer at the interface between the aqueous and the organic phases which are removed by centrifugation. DNA released from disrupted cells is precipitated by cold absolute ethanol or isopropanol.

Schematic diagram showing the principle of isolation of genomic DNA from *E. coli*



Materials Required:

- LB Broth
- *E. coli* DH5α cells
- Reagents
- TE buffer (pH 8.0)
- 10% SDS
- Proteinase K
- Phenol-chloroform mixture
- 5M Sodium Acetate (pH 5.2)
- Isopropanol
- 70% ethanol
- Autoclaved Distilled Water
- Eppendorf tubes 2 ml
- Micropipette
- Microtips
- Microfuge

Preparation of Reagents:

1. **TE BUFFER (pH 8.0):** 10 mm Tris HCl (pH 8.0), 1 mm EDTA (pH 8.0)
2. **10% SDS:** Dissolve 10 g of SDS in 100 ml autoclaved distilled water.
3. **PROTEINASE K:** Dissolve 10 mg of Proteinase K in 1 ml autoclaved distilled water.
4. **PHENOL – CHLOROFORM MIXTURE:** The pH is very important. For RNA purification, the pH is kept around pH 4, which retains RNA in the aqueous phase preferentially. For DNA purification, the pH is usually 7 to 8, at which point all nucleic acids are found in the aqueous phase. Mix equal volume of phenol with chloroform. Keep the mixture on ice and add 20 ml TE buffer, extract by shaking for 15 minutes. Remove the dust on the surface layer using a pipette. Repeat 4-5 times. Add 30-40 ml of TE buffer and store it on ice.

5. **5M SODIUM ACETATE:** Dissolve 41 g of sodium acetate in 100 ml distilled water and adjust pH with dilute acetic acid (pH 5.2).

6. **ISOPROPANOL**

7. **70% ETHANOL**

PROCEDURE:

- 2 ml overnight culture is taken and the cells are harvested by centrifugation for 10 minutes
- 875 µl of TE buffer is added to the cell pellet and the cells are resuspended in the buffer by gentle mixing.
- 100 µl of 10% SDS and 5 µl of Proteinase K are added to the cells.
- The above mixture is mixed well and incubated at 37º C for an hour in an incubator.
- 1 ml of phenol-chloroform mixture is added to the contents, mixed well by inverting and incubated at room temperature for 5 minutes.
- The contents are centrifuged at 10,000 rpm for 10 minutes at 4º C.
- The highly viscous jelly like supernatant is collected using cut tips and is transferred to a fresh tube.
- The process is repeated once again with phenol-chloroform mixture and the supernatant is collected in a fresh tube.
- 100 µl of 5M sodium acetate is added to the contents and is mixed gently.
- 2 ml of isopropanol is added and mixed gently by inversion till white strands of DNA precipitates out.
- The contents are centrifuged at 5,000 rpm for 10 minutes.
- The supernatant is removed and 1ml 70% ethanol is added.
- The above contents are centrifuged at 5,000 rpm for 10 minutes.
- After air drying for 5 minutes 200 µl of TE buffer or distilled water is added.
- 10 µl of DNA sample is taken and is diluted to 1 or 2 ml with distilled water.
- The concentration of DNA is determined using a spectrophotometer at 260/280 nm.
- The remaining samples are stored for further experiments.

PRECAUTIONS:

- Cut tips should be used so that the DNA is not subjected to mechanical disruption.
- Depending on the source of DNA the incubation period of Proteinase K should extended.
- The phenol chloroform extraction should be repeated depending on the source of DNA to obtain pure DNA.
- DNase free plastic wares and reagents should be used.

Results and Discussion:

Aim: To estimate the concentration of DNA by diphenylamine reaction.

Principle: This is a general reaction given by deoxypentoses. The 2-deoxyribose of DNA, in the presence of acid, is converted to ω-hydroxilevulinic aldehyde, which reacts with diphenylamine to form a blue coloured complex, which can be read at 595 nm.

Requirements:

1. Standard DNA solution- Dissolve calf thymus DNA (200µg/ml) in 1N perchloric acid/buffered saline.

2. Diphenylamine solution- Dissolve 1g of diphenylamine in 100 ml of glacial acetic acid and 2.5 ml of concentrated H2SO4. This solution must be prepared fresh

3. Buffered Saline- 0.5 mol/litre NaCl; 0.015 mol/litre sodium citrate, pH 7.

Procedure:

1. Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labeled test tubes.

2. Pipette out 1 ml of the given sample in another test tube.

3. Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled water serves as the blank.

4. Now add 2 ml of DPA reagent to all the test tubes including the test tubes labeled 'blank' and 'unknown'.

5. Mix the contents of the tubes by vortexing / shaking the tubes and incubate on a boiling water bath for 10 min.

6. Then cool the contents and record the absorbance at 595 nm against blank.

7. Then plot the standard curve by taking concentration of DNA along X-axis and absorbance at 595 nm along Y-axis.

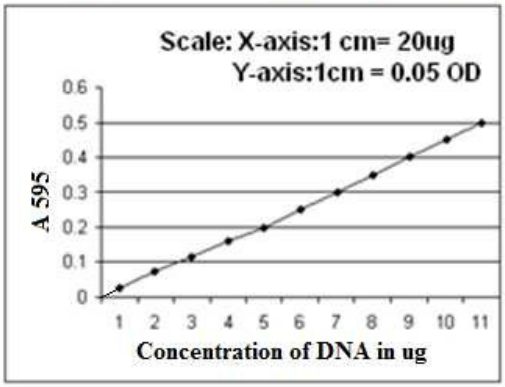
8. Then from this standard curve calculate the concentration of DNA in the given sample.

Result: The given unknown sample contains ----µg DNA

Observations and Calculations

Volume of standard (200 µ g/ml) DNA (ml)	Volume of distilled water (ml)	Concentration of DNA (µ g)	Volume of DPA reagent (ml)	Incubate in boiling water bath for 10 Min & Cool	A595
0.0	1.0	00	2		0.00
0.2	0.8	40	2		
0.4	0.6	80	2		
0.6	0.4	120	2		
0.8	0.2	160	2		
1.0	0.0	200	2		
1.0 Unknown	0.0	To be Estimated	2		

Standard Curve for DNA estimation by DPA method



DNA replication

It is a process by which DNA in living organisms can multiply and make new copies of itself so that it can be passed on to new daughter cells and perpetuate itself for generations. This is how DNA has been transferred from one cell to another in living beings for years. Cells have this unique capability to multiply in numbers by cell division. When the cells divide, in order to survive and live, they also need a DNA molecule. So how can that one DNA in the mother cell be passed to the two new daughter cells that are created when a cell divides? This is accomplished by the mechanism of DNA replication. The two strands of the parent DNA separate and new strands are replicated using the strands of the old DNA. When replication is done, two new DNA molecules are made and each of them is transferred to a new daughter cell.

Requirements for DNA polymerase to catalyse DNA synthesis –

- Template strand that can be read in 5’ to 3’ direction
- Primer to provide 3’-OH end to which new nucleotides can be added
- dNTPs
- Mg^{2+} ions

Process of DNA replication (Theta model) –

- Initiation of replication occurs at a specific region called **origin of replication** where the ds-DNA denatures to form ss-DNA and within which replication commences
- The locally denatured segment of DNA is called the **replication bubble** and the 2 strands in this region using which new complimentary strands are synthesized are called the **template strands**
- As the DNA unwinds, a y-shaped structure is formed at either ends of the replication bubble. It is known as the **replication fork**. In such cases, bidirectional replication occurs
- The fork is generated by a complex of 7 proteins called **primasome** that includes – Dna G primase, Dna B helicase, Dna C helicase assistant, Dna T, Primase A, B and C
- In *coli*, the OriC region spans 245 bp and contains clusters of 3 copies of 13-mer and 4 copies of 9-mer sequences
- To initiate replication, an initiator protein called **Dna A ATP** (encoded by dna A gene) binds to 9-mer sequences and denatures the region connecting it to 13-mer sequences by breaking A-T bonds which are weaker as they are held by 2 hydrogen bonds. This requires energy from ATP. This forms the **initial complex**
- DNA helicase (**Dna B**) is loaded onto the DNA strands by a helicase loader (**Dna C**). DNA helicase untwists the DNA. This forms the **pre-priming complex**
- **SSBP** (Single-stranded Binding Protein) binds the open strands of DNA to prevent rewinding. **Gyrase**, a type of topoisomerase, releases the tension generated by rapid unwinding of the DNA strands at 3000 rpm
- The DNA helicase recruits **primase** enzyme that synthesizes a short segment of 5-10 nucleotides called primer which allows elongation of DNA. This happens because DNA polymerase III can only add nucleotides but cannot initiate synthesis of a new strand.
- Elongation is carried out by **replisome** which is made up of DNA polymerase III and the primasome complex. The DNA pol III enzyme tethers itself to the ss-DNA via its core enzyme. The core enzyme catalyzes the DNA synthesis by adding complementary nucleotides
- DNA synthesis takes place in 5’ to 3’ direction towards the **replication fork**. The strand that is being synthesized in this direction continuously is called the **leading strand** while the strand that is synthesized in the opposite direction is called **lagging strand**
- The leading strand requires just 1 primer whereas the lagging strand requires many such primers. Since, leading strand is synthesized continuously and simultaneously along with the discontinuous synthesis of lagging strand, the entire process of DNA synthesis is a **semi-discontinuous process**
- Fragments of lagging strand are known as **okazaki fragments**. After the strands have been completely synthesized, these okazaki fragments are joined together.
- DNA pol III is removed. DNA pol I removes the primers by its 5’ to 3’ exonuclease activity exposing the template nucleotides. It then adds complementary nucleotides by its 5’ to 3’ polymerase activity to the 3’-OH end of the previous okazaki fragment, thereby replacing the primers

- The nicks that remain behind are joined by **ligase** by creating a phosphodiester bond. This is known as nick translation
- Any errors in base-pairing is removed by DNA polymerase III by its 3' to 5' exonuclease activity immediately before proceeding onto the next nucleotide. This is called **proof-reading**
- Termination of this process occurs when the replication forks reach the **ter sites**. Tus proteins (Terminus Utilization Substance) bind to ter sites and halt progression of forks. In *coli*, there are 10 replication termini (Ter sites) each spanning 23 bp
- Ter B and C terminate the clockwise fork while ter A, D and E terminate anti-clockwise fork
- In circular chromosomes, the daughter chromosomes remain interlocked and are called **catenanes**. Topoisomerase II resolves this problem by breaking some bonds in DNA molecules so as to separate the strands **– Decatenation**

DNA Replication by Rolling Circle Model –

This occurs when a circular ds-DNA genome needs to be made in multiple copies such as in lambda phage

A nick is made at the origin of replication on the outer strand, also called the (+) strand, making 2 ends of the (+) strand – 5' and 3' end

The 3'-OH end is extended by replication enzymes which is the leading strand using the inner or (-) strand as the template. As the 3' end is being lengthened, the 5' end gets displaced and forms an ever lengthening tail

The 5' end of the (+) strand acts as a template for the complementary lagging strand making it double stranded

The leading strand and the template of the lagging strand remain covalently attached to each other. Concatamers are formed. They are then cut apart to give separate genomes

C9P: Plant Ecology and Phytogeography

Purpose: To determine the soil pH.

Materials: Soil sample, distilled water, pH paper/barium sulphate/comparometer/ tintometer, pH indicator etc.

Procedure:

The following methods are commonly used.

[I] pH Paper

1. Add a pinch of soil to 5 ml distilled water.

2. Take a broad range pH paper indicator (a small piece) and dip it in the soil-water suspension. The colour of the paper changes.

3. Match the colour with the colour scale given on a booklet. This gives an approximate pH value.

4. For more correct value, narrow range pH paper indicator of the value indicated by broad range paper is now taken (i.e., if the pervious value comes to 8, now use indicator of the scale varying between 7.5 to 8.5).

5. The colour change is compared with the scale given on booklet and approximate pH value is

determined. (pH papers are those papers on which indicators of various pH ranges are absorbed).

[II] Barium sulphate test

1. A spoonful of soil is added to an equal amount of Barium sulphate.

2. About 10-20 ml of distilled water is added to the test tube containing soil suspension.

3. Now sufficient quantity of soil indicator is added to the test tube and contents are thoroughly shaken.

4. Allow the contents to stand.

5. Match the colour developed with the colour chart and note the pH value.

(For this purpose B.D.H. Barium sulphate soil testing outfit would be very useful).

[III] Comparometers(fintometers.

1. These are boxes with two windows. In one of the windows of the box, test tube containing soil-water suspension is kept, while in another, a tube with standard fiolution is kept (or in tintometers a rotating colour disc is adjusted) and the comparisons are made.

2. To prepare a soil- water suspension, take a tube supplied with the apparatus.

3. Add a little Barium sulphate and almost twice the amount of soil.

4. Fill the tube up to the mark with distilled water and shake thoroughly. Allow the tube to stand till the clear liquid appears.

5. Place the tube in slot of the box and compare the colours to find out the pH value.

Purpose: Test for the presence of carbonate, nitrate and deficiency of replaceable bases.

Materials: Hydrochloric acid, diphenylamine, sulphuric acid, ammonium thiocyanate, hydrogen peroxide,

water, white glazed tiles, test tubes, soil samples, etc.

Procedure:

Following are the methods for determination of contents of carbonate, nitrate and replaceable bases.

[I] Carbonate contents

It can be determined in the field by adding conc. HCL to the soil sample. This produces effervelicence

which indicates the presence of carbonate in the soil. If two samples are analysed, degree of effervescence is compared. More is the effervescence, more would be the carbonate contents.

[II] Nitrate contents

Prepare 1: 5 soil water suspension. Shake it thoroughly. Add diphenylamine [prepared in conc. H_2S0_4 (0.2%)] to the clear solution. Take a few drops of soil suspension on a white tile and add a few drops of diphenylamme. Blue colour developed indicates the presence of nitrate. If two samples are to be compared, the comparison of depth of blue colour indicates the degree of nitrate contents. Darker the blue colour is, more would be the nitrate

contents. (For good results, use soil rich in organic contents, since these are generally rich in nitrate contents as well).

[III] Deficiency of replaceable bases(Ca, K, Mg, Na, etc.)

Take a pinch of soil and add it to a saturated alcoholic solution of ammonium thiocyanate. Shake the contents thoroughly. Allow the solids to settle down and a clear liquid is available. Now add a drop or two of H_20_2 and note the red colour which develops. The degree of colour depth indicates the deficiency of replaceable bases.

Purpose: Test for the presence of inorganic salts in the soil.

Materials: Test tubes, beaker, soil, conical flask, distilled water, barium chloride solution, hydrochloric acid, sulphuric acid, nitric acid, ammonium nitrate, ammonium molybdate, silver nitrate, etc.

Procedure:

Take about 200 g of soil sample in a conical flask. Add 500 rol distilled water to the conical flask and shake vigorously. Keep the flask overnight so that soluble salts dissolve in water. Pour the water slowly and collect the filtrate (henceforth called water extract).

[I] Chloride

Take 20 rol of water extract of the soil in a beaker. Add 10 ml N/IO, H2S04 and thus neutralize carbonate and bicarbonate present in the extract. Now add silver nitrate to the solution. A white precipitate develops to indicate the

presence of chloride.

[II] Sulphate

Take 20 ml of water extract of the soil in a beaker. Add 2-5 ml of conc. HCl and boil. Add $BaCl_2$ solution to the beaker. A white precipitate develops to indicate the

presence of sulphate.

[III] Phosphate

Take 10 ml of water extract of the soil. Add a few drops of ammonium molybdate solution, conc. $HN0_3$ and NH_4N0_3.A yellow colour develops to indicate the presence of phosphates.

Purpose : Demonstration of $C0_2$, 0_2, chlorine and ammonia in water.

Materials: Lime water, nitric oxide, alkaline pyragallate solution, ammoniacal cuprous chloride, iodide paper (paper soaked in potassium iodide solution and starch solution), Hydrochloric acid, glass rod, Nessler's solution, test tubes, water, pH indicator papers, etc.

Procedure and results

[I] Carbon dioxide

Take water sample and add to it freshly prepared lime water. Lime water turns milky. The degree of milky white colour indicates the amount of carbon dioxide. Clear lime water turns milky due to the formation of insoluble calcium.

[II] Chlorine

The presence of chlorine can be detected in water sample by dipping starch-iodide paper which turns blue.

[III] Ammonia

The presence of ammonia in water can be demonstrated by anyone of the following tests.

1. A glass rod dipped in concentrated hydrochloric acid is inserted in the test tube containing water sample .. Dense white fumes of ammonium chloride are produced.

2. The sample turns brown on addition of Nessler's solution (Nessler' solution is prepared by pouring potassium iodide in mercuric chloride solution until the precipitate of mercuric iodide formed dissolves in excess of potassium iodide. The solution is then made alkaline with caustic potash.)

External Morphological features of hydrophytes

The following are some of the common morphological features shown by hydrophytes.

1. Root. *A few major characters are listed below-*

1. It is often very poorly developed. The roots may even be absent e. g. *Ceratophyllum, Wolffia,* etc.

2. In some hydrophytes root system is well developed mainly for the purpose of attachment, e.g. *Nymphaea, Cyperus, 7ypha,* etc.

3. In free floating plants, adventitious roots are developed not for anchorage but for buoyancy e.g. *Eichhomia, Pistia,* etc.

4. Sheath-like root pockets are developed *inAzolla, Lemna, Pistia,* etc. instead of root cap. It helps the plants to float.

5. Spongy roots which are negatively geotropic develop for floating in *Pistia.*

2. Shoot. The stems are spongy, delicate and flexible.

3. Petiole. The following are the hydrophytic characters.

1. Petiole is very long and delicate in plants with roots attached and leaves floating, e.g. *Nymphaea, Sagittaria,* etc.

2. Bulbous petiole of *Eichhomia* helps the plant to float on water surface.

4. Leaves. Leaves of hydrophytes show following characters.

1. The leaves of submerged plants are variously dissected, so that water flows easily without

resistance; e.g. *Ceratophyllum, Hydrilla, vallisneria,* etc.

2. The surfaces of floating leaves possess waxy coating as in *Nymphaea* or leaf hairs as in *Salvinia.*

3. In emergent plants, leaves are heterophyllous. The leaves below the water are narrow, long, segmented and dissected; while the leaves outside the water are broad, small and entire. Such dimorphic leaves are found in *Limnophila heterophylla, Ranunculus scleratus, Sagittaria sagittifolia,* etc.

Purpose: To determine the minimum size of the quadrate by Species- area curve.

Materials: Meter scale, string or cord, nails, paper, pencil, etc.

Procedure:

1. Prepare a L-shaped structure in the field of 1 meter X 1 metre by using 3 nails and tying string

with them.

2. Now measure 10 cm on one side of the arm of L and then the other.

3. Using another piece of string and nails prepare 10 X 10 sq cm area.

4. Count the number of species occurring in this area.

5. Increase this area to 20 X 20 sq.cm and similarly record additional species occurring in this area.

6. Repeat the same procedure till 1 X 1 sq meter area is covered.

7. Note down the observations as follows.

8. Using the above recorded data, prepare a graph. Number of species are plotted on Y axis and sizq of the quadrats on X axis.

Results:

At one point of the graph, curve starts flattening or shows only a gradual increase.

Conclusion:

The point of the graph, at which steep increase of the curve becomes gradual or curve flattens, denotes minimum area of the quadrat suitable for the study of an area under consideration.

*Table 1.*Total number of species and the area.

Area	Total no. of species
10 × 10 sq.cm 20 × 20 sq.cm 30 × 30 sq.cm upto 100 × 100 sq.cm	

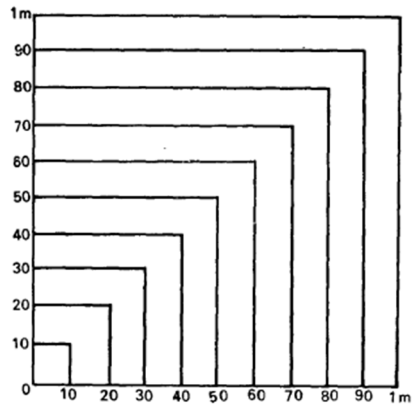


Fig. 16. Procedure to find out minimum required size of the quadrat.

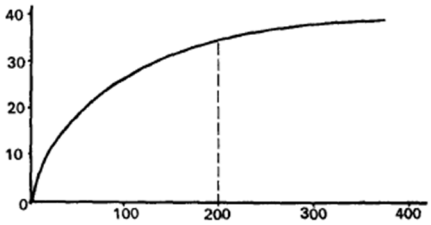


Fig. 17. Species-area curve to determine the size of the quadrat.

Purpose: To determine the frequency of various species occurring in a given area.

Materials: Quadrats of required size. measuring tape, paper, pencil, etc.

Procedure:

The following are some of the common methods.

[I] Quadrat

1. Take a quadrat of suitable size, lay it randomly at number of places.

2. Identify the species or distinguish them as A, B,C, etc.

3. Find out the presence or absence of each of the species in each segment (square) of the quadrat

and tabulate the data.

4. If the species are not identified taxonomically in the field, collect them, glue or fix with cello tape to herbarium sheets, use the same identification marks, e.g., A, B, C, etc. as used in the table and properly preserve the sheets.

[II] Line transect

1. In a grassland, line transect can also be used for determining frequency .

2. A measuring tape or a cord marked into one meter segments is used.

3. Take a tapc or cord across the grassland in North-South direction.

4. Note the presence or absence of plant species in each one meter segment. Only those plant species are considered which touch the cord or tape.

[III] Belt transect

Similar method IS used In belt transects. The plant species occurring in alternate segments or uniform area are recorded.

Results

The record of the observations is kept In the following way (refer to table 2)

Table 2. **To determine the frequency of various species.**

Serial no.	Name of the plant species	No.of quadrats/segments					Total no. of a species	Total no. of quadrats in which species occurred	Total no. of quadrats studied	Frequency %	Frenquency class	Density	Abundance
		1	2	3	4	5							
1.	*Alysicarpus monilifer*	5	5	-	-	-	10	2	5	40	B	2	5
2.	*Convolvulus pluricaulis*	10	-	-	-	-	10	1	5	20	A	2	10
3.	*Cynodon dactylon*	15	10	12	13	15	65	5	5	100	E	13	13
4.	*Cyperus rotundus*	-	6	-	-	-	6	1	5	20	A	1.2	6
5.	*Desmodium triflorum*	-	12	-	-	-	12	1	5	20	A	2.4	12
6.	*Dichanthium annulatum*	12	-	12	10	11	45	4	5	80	D	9	11.25
7.	*Eclipta alba*	5	6	-	-	4	15	3	5	60	C	3	5
8.	*Euphorbia hirta*		-	-	6	-	4 10	2	5	40	B	2	5
9.	*Evolvulus nummularius*	-	-	3	-	-	3	1	5	20	A	0.3	6
10	*Gomphrena globosa*	2	4	3	1	2	12	5	5	100	E	2.4	2.4
11.	*Indigofera linifolia*	-	-	-	6	-	6	1	5	20	A	1.2	6
12.	*Launea nudicaulis*	-	-	-	-	3	3	1	5	20	A	0.6	3
13.	*Phyllanthus niruri*	-	-	-	-	2	2	1	5	20	A	0.4	2
14.	*Rhynchosia minima*	-	-	-	4	3	7	2	5	40	B	1.4	3.5
15.	*Sida cordifolia*		-	-	6	4	2 12	3	5	60	C	2.4	4
16	*Vernonia cinerea*	-	-	11	-	-	11	1	5	20	A	2.2	11

Number of plants in each frequency class. A = 8, B = 3, C = 2, D = 1, E = 2

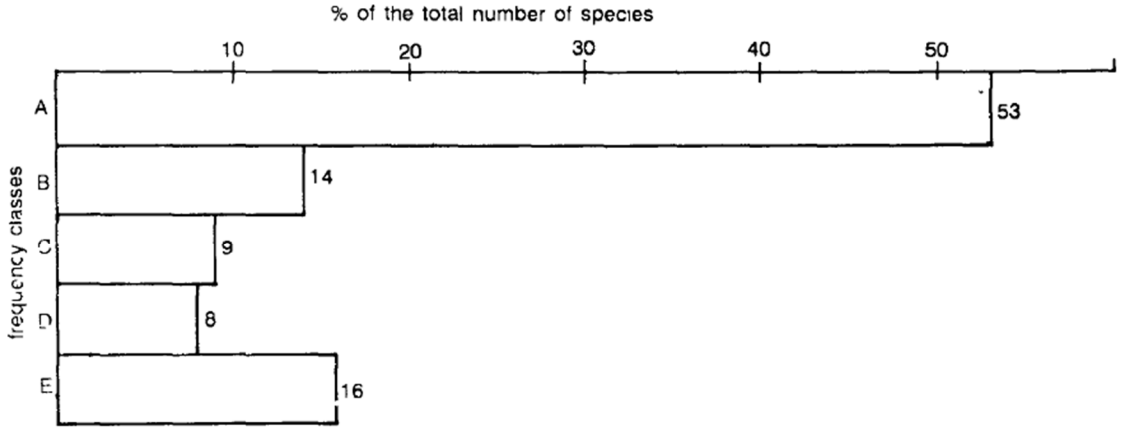


Fig. 20. Raunkiaer's normal frequency diagram.

Calculations

1. Calculate the percentage frequency as follows –

$$\text{Percentage frequency} = \frac{\text{Total number of quadrats / segments in which species occurred}}{\text{Total number of quadrats / segments studied}} \times 100$$

2. Distribute various species into five frequency classes (Raunkaier, 1934) as given below –

Table 3. **Distribution in frequency classes.**

Frequency %	Frequency class
1 - 20 %	A
21 - 40 %	B
41 - 60 %	C
61 - 80 %	D
81 - 100 %	E

Write down the frequency class in appropriate column against each species.

3. The distribution of sixteen species in five frequency classes is A = 8, B = 3, C = 2, D = 1 and E = 2. Find out the percentage of these species falling into different frequency classes as follows out of the total number of species recorded.

$$\frac{\text{No. of species falling in frequency class}}{\text{Total number of species recorded}} \times 100$$

for frequency class A = 8/16 × 100 = 50
frequency class B = 3/16 × 100 = 18.75
frequency class C = 2/16 × 100 = 12.5
frequency class D = 1/16 × 100 = 6.25
frequency class E = 2/16 × 100 = 12.5

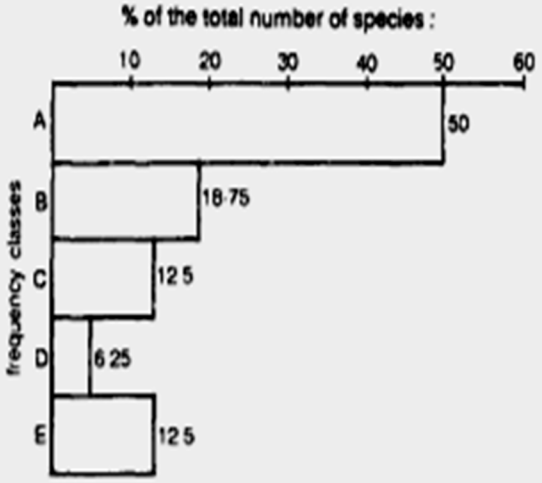


Fig. 19. Frequency diagram of the place studied.

4. Take a graph sheet and show % of the total number of species on y-axis and the frequency classes on x-axis. This is known as frequency diagram.

Conclusions

1. Compare the frequency diagram of the place studied with that of Raunkiaer's normal frequency diagram
2. When values of frequency classes B,C and D are comparatively higher than their values in normal frequency diagram, the vegetation is said to be **heterogenous**, as is the case in the present study. (higher values of class E indicate homogeneity of vegetation).
3. Also compare the figures with frequency figures proposed by Raunkiaer as Law of Frequency given below

$$A > B > C \gtrless D < E$$

Purpose: To determine the density/abundance of various species occurring in given area. Density gives the numerical strength of a species in a community. Abundance, on the other hand, gives the number of individuals of a species in a habitat.

Materials: Quadrat of required size, paper, pencil, etc.

Procedure

1. Take a quadrat of suitable size and lay randomly at number of places in an area under study.

2. Identify the species or distinguish them as A, B, C, ctc. If it is difficult to identify the species taxonomically in the field, collect them, glue or fix by cellotape to herbarium sheets, put the same identification marks e.g., A,B,C, etc. and preserve the sheets.

3. Count the number of individuals of each species from each square of the quadrat.

4. Record observations in a tabular form.

Observation:

Note down your observation III the following table - (refer to table 1 also)

Conslusions:

(1) Density = an average number of individuals of a given species over the total number of samples studied in an area.

Total number of individuals of a species Total number of quadrats studied value in column no. 4 - value in column no. 6

(2) Abundance = the number of individuals of a given species per unit area (quadrat) of occurrence. Total number of individuals of a species Total number of quadrats occurrence value III column no. 4 value in column no. 5

Generally, frequency and abundance are co-related to find out the distribution of a species.

(a) High frequency X low abundance = regular distribution

(b) Low frequency X high abundance = contagious distribution

Purpose: To determine the vegetational cover in a given area.

Materials: Measuring tape, Vernier callipers, Scissors, paper, pencil, etc. Procedure

The following are the two common methods.

[I] Line intercept method A

Take a measuring tape across the grassland. Measure and note the length of the tape, intercepted by individual plants.

[II] Line intercept method B

Cut a few stems of individual species at the ground surface. Measure the diameter of the cut end

by Vernier callipers. Alternatively measure the diameter of the plant at a fixed height above the

ground level.

Conclusion.

I. List the observations in the following way –

Calculate (a) total length of transect covered by all thc species and (b) percentage of total length of transect covered by different species. This gives percentage cover.

Relative cover percentage = (length of one type / total length of all the individuals) x 100

Table 5. **To determine vegetational cover.**

Serial no.	Name of plant species	Length of Individuals					Total %
		1	2	3	4	5	
1	A						
2	B						
3	C						
4	D						
5	E						
Total no. of plant sps.		Total no. of individuals studied.					Total length of all the individuals

II. Tabulate in the following form –

Calculate by using Average basal area = πr^2

Where r = radious= diameter (Average)/ 2

If multiplied by the value of dencity (D) : D x average basal area = ... sq cm/sq meter.

Table 6. **To calculate vegetational cover.**

Serial no.	Name of plant species	Diameter of Individuals					Total	Average
		1	2	3	4	5		
1	A							
2	B							
3	C							
4	D							
5	E							

Total basal area of all species -

Purpose : Determination of local vegetation: frequency and relative frequency, density and relative density and importance value index.

Materials: Ouadrats, measuring tape, paper pencil, etc.

Procedure:

1. Lay thc quadrats, idcntify the species, count their number in each quadrat and record the obscrvations in a tabular form as done earlier.

2. Use line transect or belt transect and record the observe.

Calculations:

Importance value index (IVI) is a measure of dominance and ecological success of a species. It takes into consideration relative dominance, relative density and relative frequency. These are calculated as follows-

1. Relative dominance = (Total basal area of the species / Total basal area all the species)X 100

2. Relative density = (Number of individuals of the species/ Number of individuals of all the species)X 100

3. Relative frequency =(Number of occurrences of the species/ Number of occurrences of all the species) X100

Table 7. To determine frequency, relative frequency, density relative density and importance value index.

1	2	3	4	5	6
Serial no.	Name of plant species	Relative Dominance	Relative Density	Relative Frequency	IVI (3+4+5)
1 2 3 4 5	A B C D E				

Arrange the species in decreasing values of IVI.

Purpose: To study the species composition of an area for analysing biological spectrum and comparison with Raunkiaer's normal biological spectrum.

Materials: Record book, pen, pencil, etc.

Procedure:

1. Visit the area under study.

2. Study the different life forms and their general appearance, spread, etc.

3. Place each one of the plant under different life forms as per the classification proposed by Raunkiaer (1934); as given below

Observations:

1. Record the observations in the following table.

2. Draw the percentage distribution of different life forms in it graph on Y-axis and different classes on X - axis.

3. Compare this biological spectrum with Raunkiaer's normal biological spectrum for the world's phanerogamic flora which shows

(1) phancrophytes 46%

(2) chamaephytes 9%

(3) hemi- cryptophytes 26%

(4) geophytes / or + helophytes and hydrophytes 6%

(5) therophytes 13%

Table 8. **Raunkiaer's classification of life forms**

Symbol	Life form	Characteristics
P	**I. Phanerophyte**	Perennating bud well above the ground
	These are the further sub-divided into following -	
MM	1. Megaphanerophyte	Perennating bud above 30m high.
	2. Mesophanerophyte	..... between 8 m and 30 m
M	3. Microphanerophyte	.... between 2 m and 8 m
N	4. Nanophanerophyte	.... under 2 m
Ch	**II. Chamaephyte**	Herbaceous or low woody plants whose perennating buds borne just above ground level up to 0.3 m.
H	**III. Hemicryptophyte**	Perennating buds close to the ground (rather half hidden in the soil).
	IV. Cryptophyte	Perennating organs below surface or water.
	These are further sub-divided into following-	
G	1. Geophyte	Perennating buds underground.
HH	2. Helophyte	Marsh plants with perennating buds in watterlogged mud.
	3. Hydrophyte	Perennating buds beneath the water.
Th	**V. Therophyte**	Survival in unfavourable season through seeds or spores, annuals.

Table 9. **To determine biological spectrum.**

Life form class	No. of individuals	Total no. in each class		%
MM	卌 卌 卌 |||	18		
M	卌 卌 卌 卌 卌 卌 卌 卌 卌 |||	48	128	47.4%
N	卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 ||	62		
Ch	卌 卌 卌 卌 卌 卌 |||	28		10.3%
H	卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 卌 |||	58		21.4%
G	卌 卌 卌		16	20	7.4%
HH	卌			
Th	卌 卌 卌 卌 卌 卌 卌		36		13.3%
	Total no. of individuals observed	270		

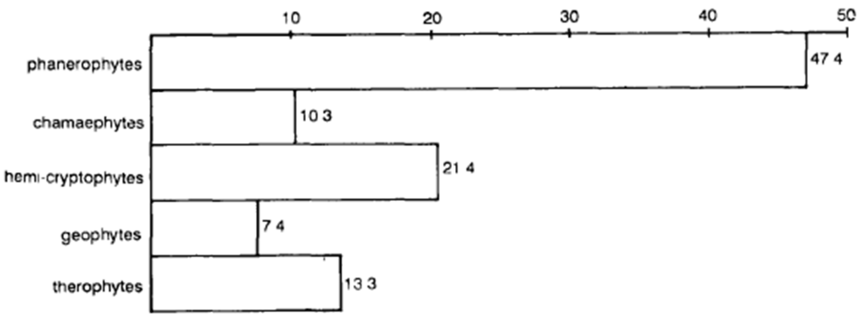


Fig. 22. Biological spectrum for the area studied.

Purpose: To find out bulk density of a given soil sample.

Materials: Soil samples, petri dishes, oven, measuring cylinders, balance etc.

Procedure:

1. Collect soil samples from different places at a depth of 15 cm.

2. Dry the soil in an oven at 105°C till constant weight is attained.

3. Transfer a part of this soil to the measuring cylinder and determine the volume (1).

4. Also determine the weight of soil by first weighing measuring cylinder and the soil (2) and then the

weight of measuring cylinder alone (3).

Observations and Calculations

Record the observations as follows.

5. Volume of the soil (1)

6. Weight of measuring cylinder + soil (2)

7. Weight of measuring cylinder (3)

Calculate by using following formula

Bulk density *(gm/*cm^3) = Weight of soil (gm) / volume of soil [(2 - 3)/ (1)]

8. Place the soil in one of the three following

Soil classes Bulk density (gm/cm3)

(a) Medium to fine textured 1.1 - 1.5

(b) Coarse textured 1.2 - 1.65

(c) Alkaline saline 1.70 -1.85

The bulk density is defined as dry weight of unit volume of soil (in gm/cm3). It is inversely proportional to pore space of the soil.

Purpose: To find out the porosity (per cent pore space) of a given soil sample.

Materials: Soil samples, petri dishes, beaker, measuring cylinders, beakers, balance, etc.

Procedure:

1. Scrape the soil surface to flat.

2. Dig straight walled pit (l0X l0X 10 cm).

3. Collect the removed soil in a beaker.

4. Dry the soil in an oven at 105°C till constant weight is attained.

5. Transfer a part of this soil to measuring cylinder and determine the volume (1).

6. Also determine the weight of soil (2) and then the weight of measuring cylinder alone(3).

Observations and calculations

Record the observations as follows.

7. Volume of the soil (1).

8. Weight of measuring cylinder + soil (2).

9. Weight of measuring cylinder (3).

10. Calculate the bulk density by using following formula

Bulk density *(gm/* cm^3) = weight of the soil /volume of the soil [(2 - 3) /(1)]

11. Now calculate percentage pore space by the formula given below.

Per cent pore space = [(2.6 - Bulk density) / 2.6] X 100

Where 2.6 is the approximate specific gravity of soil.

CP10: Plant Systematics

1. Ranunculacea (*Ranunculus scleratus*)

Habit: Herb.

Root: Tap, branched.

Stem: Herbaceous, aerial, erect, angular, fistular, smooth, nodes and internodes very prominent, green in colour.

Leaf: Cauline and ramal, alternate, exstipulate; simple, petiolate, leaf base cheaving, lamina much dissected, each lobe is ovate, entire, obtuse, glabrous, multicostate reticulate.

Inflorescence: Dichasial cyme.

Flower: Bracteate, bracteolate, pedicellate, complete, actinomorphic, hermaphrodite, pentamerous, hypogynous, thalamus prominent and convex, spirocyclic and yellow.

Calyx: Sepals 5, polysepalous, quincuncial, slightly petaloid and boat shaped.

Corolla: Petals 5, polypetalous, imbricate, yellow, each petal at its base has a pocket-shaped nectary.

Androecium: Stamens indefinite, polyandrous, spirally arranged, filament long, dithecous, basifixed, extrorse.

Gynoecium: Polycarpellary, apocarpous, ovary superior, unilocular, one ovule in each locule, placentation basal, style absent, stigma simple and sticky.

Fruit: Etaerio of achenes.

Floral formula: $\mathrm{Br},\ \mathrm{brl},\ \oplus\ ,\ \male\ ,\ K_5,\ C_5,\ A_\infty,\ \underline{G}_\infty\ .$

Classification and identification.

Class. *Dicotyledonae*

1. Venation reticulate.
2. Flowers pentamerous.

Sub-Class. *Polypetalae*

1. Petals free.

Series. *Thalamij70rae*

1. Thalamus dome-shaped.
2. Flowers hypogynous and ovary superior.

Orders. *Ranales*

1. Stamens indefinite.
2. Carpels free or immersed in torus.

Family. *Ranunculaceae*

1. Usually herbs often with divided leaves.
2. Flowers hemicyclic with one to many generally free carpels.
3. Stamens indefinite and extrorse.

2. Brassicaceae (*Brassica campestris*)

Stem: Herbaceous, aerial, erect, cylindrical, branched, solid, smooth and green.

Leaf: Cauline and ramal, alternate, exstipulate, simple, sessile, lower leaves lyrate with deeply cut margins,
acute, glabrous, unicostate, reticulate.

Inflorescence: Racemose raceme.

Flower: Ebracteate, pedicellate, complete, actinomorphic, hermaphrodite, tetramerous, hypogynous, cyclic
and yellow.

Calyx: Sepals 4 in two whorls of 2 each, polysepalous, slightly petaloid.

Corolla: Petals 4, polypetalous, cruciform, each petal is distinguished into a claw and a limb, valvate.

Androecium: Stamens 6 in two whorls (2 + 4), polyandrous, tetradynamous, 4 inner long and 2 outer short,
dithecous, basifrxed and introrse, glands are present at the base of 4 longer stamens.

Gynoecium: Bicarpellary, syncarpous, ovary superior, unilocular but becomes bilocular later on due to the
development of a false septum (replum), ovules many in each locule, placentation parietal, style short
and stigma is bilobed.

Fruit: Siliqua.

Floral formula: Ebr, $\oplus$, ⚥, K_{2+2}, C_4, A_{2+4}, $\underline{G}_{(2)}$.

Classification and identification

Class. *Dicotyledonae* .

1. Venation reticulate.
2. Flowers pentamerous.

Sub-Class. *Polypetalae*

1. Petals free.

Series. *Thalamiflorae*

1. Flowers hypogynous and ovary superior.

Order. *Parletales*

1. Carpels united to form unilocular ovary with parietal placentation.

Family. *Cruciferae*

1. Herbs with alternate exstipulate leaves.

2. Corolla cruciform.

3. Stamens tetradynamous.

4. Ovary bicarpellary, syncarpous, unilocular but becomes bilocular due to the development of a false septum; fruit siliqua .

3. Malvaceae (*Abutilon indicum* (Linn.) Sweet.)

Stem: Herbaceous, aerial, erect, cylindrical, branched, solid, pubescent and green.

Leaf: Cauline and ramal, alternate, stipulate, simple, petiolate, deltoid, serrate, acute, slightly hairy and rugose above, velvety, multicostate, reticulate, divergent type.

Inflorescence: Solitary axillary.

Flower: Bracteate, pedicellate, complete, actinomorphic, hermaphrodite, pentamerous, hypogynous and cyclic.

Calyx: Sepals 5, gamosepalous, free at the tips, valvate, persistent, green.

Corolla: Petals 5, polypetalous, slightly connate at the base and adnate to staminal tube, twisted.

Androecium: Stamens indefinite, monadelphous forming a tube around the style, the tube being united with the petals (epipetalous). In the upper part of the staminal tube are borne monothecous and extrorse anthers.

Gynoecium: Multicarpellary, syncarpous, ovary superior, multilocular, with one ovule in each locule, placentation axile, style long and stigmas as many as carpels.

Fruit: Capsule.

Floral formula: $\mathrm{Br},\ \oplus,\ \varphi^{\hat{}},\ \mathrm{K}_{(5)},\ \widehat{\mathrm{C}_5},\ \widehat{\mathrm{A}_{(\infty)}},\ \underline{\mathrm{G}_{(5-\infty)}}$.

Classification and identification.

Class. *Dicotyledonae*

1.Venation reticulate.

2. Flowers pentamerous.

Sub-Class. *Polypetaiae*

1. Petals free.

Series. *Thaiamiflorae*

1. Flowers hypogynous and ovary superior.

Order. *Malvales*

1. Stamens usually indefinite and monadelphous.
2. Ovary 3 to many carpellary with axile placentation.

Family. *Malvalceae*

1. Leaves stipulate.
2. Calyx often with an epicalyx.
3. Stamens monothecous and anthers reniform.
4. Fruit - capsule or schizocarp.

4. Myrtaceae (*Callistemon)*

Stem: Herbaceous, lower portion woody, aerial, erect, cylindrical, branched, solid and glabrous, younger
portions puberulous, brown.

Leaf: Cauline and ramal, alternate 2/5, exstipulate, simple, sub- sessile, lanceolate, entire, acute, unicostate
reticulate, leathery, gland dotted.

Inflorescence: Pendent intercalary spike.

Flower: Bracteate, sessile, complete, actinomorphic, hermaphrodite, pentamerous, epigynous and cyclic.

Calyx: Sepals 5, gamosepalous, imbricate or valvate, persistent.

Corolla: Petals 5, polypetalous, imbricate, boat-shaped.

Androecium: Stamens indefinite, polyandrous, filaments bright red and united at the very base forming a staminal sheath, dithecous, versatile, introrse.

Gynoecium: Tricarpellary, syncarpous, ovary inferior, trilocular, placentation axile, many ovules in each locule, style long and stigma capitate.

Fruit: Capsule

Floral formula: $\cdot \mathrm{Br}, \oplus, \varphi, \mathrm{K}_{(5)}, \mathrm{C}_{5}, \mathrm{A}_{\infty}, \overline{\mathrm{G}}_{(3)}$.

Classification and identification.

Class. *Dicotyledonae*

1. Venation reticulate.
2. Flowers pentamerous.

Sub-Class. *Polypetalae*

1. Petals free.

Series. *Calycijlorae*

1. Thalamus cup-shaped.

2. Ovary inferior.

Order. *Myrtales*

1. Leaves simple and entire.

2. Ovary syncarpous, usually inferior.

3. Placentation axile.

Family. *Myrtaceae*

1. Woody, with opposite or alternate, exstipulate leaves.

2. Stamens indefinite sometimes in bundles.

3. Carpels 2 to 8.

5. Umbelliferae – (*Coriandrum)*

Stem: Herbaceous, aerial, erect, angular, branched, solid, glabrous, nodes are very prominent, aromatic smell

present.

Leaf: Cauline and ramal, alternate, exstipulate, compound decompound, petiolate, leaf base sheathing, pinnae

narrow, entire, acute, unicostate reticulate, aromatic smell present.

Inflorescence: Compound umbel consisting of many umbellules.

Flower: Bracteate, pedicellate, complete, central flowers actinomorphic, peripheral flowers zygomorphic due

to unequal size of petals, hermaphrodite, pentamerous, epigynous and cyclic.

Calyx: Sepals 5, polysepalous, valvate, persistent.

Corolla: Petals 5, polypetalous, valvate, each petal is bilobed. In central flowers (actinomorphic) the lobes of

all petals are equal in size. In case of peripheral flowers (zygomorphic) one anterior petal has 2 large

equally developed lobes, two lateral petals have one bigger and one smaller lobe and the rest two

petals have two equal small lobes.

Androecium: Stamens 5, polyandrous, filaments long and slender, dithecous, dorsiflXed and introrse.

Gynoecium: Bicarpellary, syncarpous, ovary inferior, bilocular, with one pendulous ovule in each locule,

placentation axile, styles 2, stigmas 2 and capitate. A disc called stylopodium is present below the

style.

Fruit: Cremocarp splitting into 2 mericarps.

Floral formula.

(a) Central flower. $Br, \oplus, \delta, K_5, C_5, A_5, \overline{G}_{(2)}$.

(b) Peripheral flower. $Br, \circlearrowleft, \delta, K_5, C_5, A_5, \overline{G}_{(2)}$.

Classification and identification.

Class. *Dicotyledonae*

1. Venation retic,:ulate. 2. Flowers pentamerous.

Sub-Class. *Polypeta/ae*

1. Petals free.

Series. *Calyciflorae*

1. Thalamus cup-shaped. 2. Ovary inferior.

Order. *Umbellales*

1. Inflorescence umbel. 2. Ovary inferior with 1, 2, or 8 fused carpels and as many locules. 3. Ovules

solitary, pendulous in each locule.

Family. *Umbeliferae*

1. Stems fistular. Leaves alternate, exstipulate usually much dissected with sheathing leaf base. 2.Carpels 2, fused, with 2 styles on swollen style base (stylopodium). 3. Fruit schizocarp, splitting into 2 mericarps.

6. Asteraceae (*Tridax*)

Stem: *Herbaceous,aerial, erect, cylindrical, branched, solid, glabrous, hairy and light brown.*

Leaf: *Cauline and ramal, opposite decussate, exstipulate, simple, sessile, elliptic-Ianceolate, crenulate, acute, hairy, unicostate reticulate.*

Inflorescence: *Capitulum. It is heterogamous - the peripheral flowers (ray florets) are ligulate and central flowers (disc florets) are tubular, involucre of bracts present.*

[I] Ray florets. *Present on periphery, bracteate, sessile, incomplete, zygomorphic, unisexual, pistillate, tetramerous, epigynous and cyclic.*

Calyx. *Sepals 4, reduced to pappus.*

Corolla. *Petals 4, gamopetalous, valvate, ligulate, the posterior two petals reduced to dentate structures.*

Androecium. *Absent.*

Gynoecium. *Bicarpellary, syncarpous, ovary inferior, unilocular, placentation basal, style short and stigma bifid.*

Floral formula. $Br, \circlearrowleft, \varphi, K_{pappus}, C_{(2/2)}, A_0, \overline{G}_{(2)}$.

[II] Disc florets. *Present in centre, bracteate, sessile, complete, actinomorphic, hermaphrodite, tetramerous, epigynous and cyclic.*

Calyx. *Sepals 4, reduced to pappus.*

Corolla. *Petals 4, gamopetalous, valvate, corolla tubular.*

Androecium. *Stamens 4, syngenesious, epipetalous, filaments long, dithecous, basiftxed, introrse.*

Gynoecium. *Bicarpellary, 'syncarpous, ovary inferior, unilocular, basal placentation, style short and stigma bifid.*

Fruit. *Cypsella.*

Floral formula. $Br, \oplus, ⚥, K_{pappus}, \overset{\frown}{C_{(4)}, A_{(4)}}, \overline{G}_{(2)}.$

Classification and identification.

Class. Dicotyledonae

1. Venation reticulate.

2. Flowers pentamerous.

Sub-Class. Gamopetaiae

1. Petals fused.

Series. Inferae

1. Ovary inferior .

2. Stamens usually as many as corolla lobes.

Order. Asterales

1. Stamens epipetalous.

2. Ovary unilocular with one ovule.

Family. Compositae

1. Leaves generally alternate.

2. Inflorescence capitulum.

3. Calyx reduced to hairy pappus.

4. Stamens epipetalous and syngenesious

7. Solanaceae – (*Solanum nigrum*)

Stem: Herbaceous, aerial, erect, cylindrical, branched, solid, smooth or puberulous and green.

Leaf: Cauline and ramal, alternate, but due to the fusion of the petiole with the stem axis, the leaves at some

places seem to be opposite, exstipulate, simple, petiolate, ovate, entire or slightly lobed or sometimes serrate, acute, glabrous, unicostate reticulate.

Inflorescence: Extra-axillary cyme.

Calyx: Sepals 5, gamopetalous, valvate, persistent.

Corolla. Petals 5, gamopetalous, valvate, rotate, white.

Androecium: Stamens 5, polyandrous, epipetalous, ftlaments broad at the base and hairy, anthers conniving,

dithecous, basifixed and dehisce by apical pores.

Gynoecium: Bicarpellary, syncarpous, ovary superior, bilocular with many ovules in each locule, placentation

axile, septum oblique, placentae highly swollen, style long and hairy, stigma bilobed.

Fruit: Berry.

Floral formula: $\text{Ebr}, \oplus, \varphi, K_{(5)}, \overset{\frown}{C_{(5)}}, A_5, \underline{G}_{(2)}$

Classification and identification.

Class. *Dicotyledons*

1. Venation reticulate.

2. Flowers pentamerous.

Sub-Class. *Gamopetalae*

1. Petals fused.

Series. *Bicarpellateae*

1. 'Carpels two.

2. Ovary usually superior.

Order. *Po1emonillles*

1. Alternate, exstipulate leaves.

2. Flower actinomorphic.

Family. *Solanaceae*

1. Flowers solitary terminal or cymosely umbelled.

2. Septum is oblique and the placentae are highly swollen.

3. Fruit - berry or capsule.

8. Lamiaceae (*Ocimum sanctum* Linn.)

Habit: Herb or undershrub.

Root: Branched tap root.

Stem: Herbaceous, aerial, erect, quadrangular, branched, solid, pubescent, green.

Leaf: Cauline and ramal, opposite decussate, exstipulate, simple, petiolate, ovate, serrate, acute, pubescent,

aromatic smell present, unicostate reticulate.

Inflorescence: Verticillaster.

Flower: Bracteate, pedicellate, complete, zygomorphic, hermaphrodite, pentamerous, hypogynous and cyclic.

Calyx: Sepals 5, gamosepalous, calyx 1/4 bilabiate, valvate, persistent.

Corolla: Petals 5, gamopetalous, corolla 4/1 bilipped, 'valvate.

Androecium: Stamens 4, polyandrous, epipetalous, didynamous, dithecous, dorsiflXed, introrse.

Gynoecium: Bicarpellary, syncarpous, ovary superior, placentation axile, tetralocular with one ovule in each locule, a disc is present below the ovary, style gynobasic and stigma bifid.

Fruit: Carcerulus.

Floral formula:

Classification and identification.

Class. *Dicoty1edonae*

1. Venation reticulate.

2. Flowers pentamerous.

Sub·Class. *Gamopetallle*

1. Petals fused.

Series. *Bicarpellatae*

1. Carpels two.

2. Ovary usually 3uperior.

Order. *Lamia1es*

1. Flowers zygomorphic.

2. Corolla bilipped.

3. Stamens 4, didynamous or 2.

4. Ovary 2 - 4 locular.

5. Fruit drupe or schizocarpic.

Family. *Labiatae*

1. Stem quadrangular. 2. Decussate or whorled exstipulate leaves. 3. Inflorescence verticillaster.

4. Gynoecium generally bilocular with 2 ovules in each locule, sometimes 5. Style gynobasic. 6. Fruit carcerulus.

9. Euphorbiaceae (*Jatropha)*

Habit: Shrub.

Root: Tap, branched.

Stem: Woody, aerial, erect, cylindrical, branched, solid, upper portions red while lower portions green, glandular hairs present.

Leaf: Cauline and ramal, alternate, exstipulate, simple, deeply lobed, petiolate, petiole filiform, covered with many glandular hairs, lobes elliptic-ovate, serrulate, acute, glaucous, venation multicostate reticulate, divergent type, texture coriaceous.

Inflorescence. Panicle cyme.

[I] Male flower. Bracteate, bracteolate, pedicellate, incomplete, actinomorphic, unisexual, staminate, pentamerous, cyclic, 5 nectariferous discs are present alternating to petals.

Calyx: Sepals 5, polysepalous, quincuncial, persistent, margins glandular.

Corolla: Petals 5, polypetalous, twisted, red.

Androecium: Stamens 10 in 2 whorls of 5 each, monadelphous at the very base only, fIlaments short,

dithecous, dorsiflXed, introrse.

Gynoecium: Absent.

Floral formula: $\mathrm{Br, brl, \oplus, \male, K_5, C_5, A_{(5+5)}, G_0}$.

[II] Female flower: Bracteate, bracteolate, pedicellate, incomplete, actinomorphic, unisexual, pistillate, pentamerous, hypogynous, a nectariferous disc present below the ovary.

Calyx: Sepals, 5, polypetalous, quincunical, persistent.

Corolla: Petals 5, polypetalous, twisted.

Androecium: Absent.

Gynoecium: Tricarpellary, syncarpous, ovary superior, trilocular, placentation axile with one ovule in each

locule, styles 3, stigma 3 and each is bifid.

Fruit: Regma splitting into 3 cocci.

Floral formula: $\mathrm{Br, \breve{brl}, \oplus, \female, K_5, C_5\, A_0, \underline{G}_{(3)}}$.

Classification and identification.

Class. Dicotykdonae

1. Venation reticulate.

2. Flowers pentamerous.

Sub-Class. Monochlamydeae

1. Flowers usually with one whorl of perianth, commonly sepaloid or none.

Series. Unisexuals

1. Flowers unisexual.

2. Perianth sepaloid or much reduced or absent.

3. Ovules 1 or 2 per carpel.

Family. Euphotbioceae

1. Alternate stipulate leaves with latex.

2. Perianth usually in one whorl or absent.

3. Stamens 1 to indefinite, free or united or branched.

4. Gynoecium tricarpellary, syncarpous, superior, trilocular with one or two ovules in each locule.

5. Styles three.

10. Fasaceae (*Crotalaria* sp.)

Stem. Herbaceous, aerial, erect, cylindrical, branched, solid, hairy and green.

Leaf. CauIine and ramal, alternate, stipulate, stipules free-lateral, palmately compound, trifoliate, petiolate, leaf base pulvinus, obovate, entire, hairy, multicostate reticulate.

Inflorescence. Racemose raceme.

Flower. Bracteate, pedicellate, complete, zygomorphic, hermaphrodite, pentamerous, hypogynous and cyclic.

Calyx. Sepals 5, gamosepalous, valvate, odd sepal anterior, persistent.

Corolla. Petals 5, polypetalous, vexillary aestivation, corolla papilionaceous.

Androecium. Stamens 10, monadelphous, dithecous, dorsiflXed, ·Iltrorse.

Gynoecium. Monocarpellary, ovary superior, unilocular, ~vules many, placentation marginal, style long and curved, stigma capitate.

Fruit. Legume. .

Floral formula. $\mathrm{Br},\ \oplus,\ ♀,\ K_{(5)},\ C_{1+2+(2)},\ A_{(10)},\ \underline{G}_{1}.$

Classification and identification.

Class. Dicotyledonae

1. Venation reticulate.

2. Flowers pentamerous.

Sub-Class. Polypetalae

1. Petals free.

Series. Calyciflorae

1. Thalamus cup-shaped.

2. Ovary inferior or semi-inferior.

Order. Rosales

1. Alternate, stipulate leaves.

2. Carpels one or more.

Family. Papilionaceae

1. Flowers zygomorphic.

2. Gynoecium usually one.

3. Corolla papilionaceous with descending imbricate aestivation.

4. Ovary monocarpellary .

11. Caesalpineaeceae (*Cassia* sp.)

Stem. Herbaceous, lower portions woody, aerial, erect, cylindrical, branched, solid, smooth and green.

Leaf. Cauline and ramal, alternate, exstipulate, compound, unipinnate and paripinnate, petiolate, petiolulate,

leaf-base pulvinus, ovate, entire, acute, unicostate reticulate, glabrous, coriaceous.

Inftorescence. Axillary or extra axillary, pendant, racemose raceme.

Flower. Bracteate, pedicellate, complete, zygomorphic, hermaphrodite, pentamerous, hypogynous and cyclic.

Calyx. Sepals 5, polysepalous, quincuncial, odd sepal anterior, petaloid.

Corolla. Petals 5, polypetalous, ascending imbricate, yellow.

Androecium. Stamens 10, in two whorls of 5 each, the anterior 3 stamens are reduced to staminodes,

polyandrous, dithecous, dorsifixed, introrse.

Gynoecium. Monocarpellary, ovary superior, unilocular, placentation marginal, ovules many, ovary sickle

shaped, style short, stigma capitate.

Fruit. Legume.

Floral formula. $\mathrm{Br, brl, \oplus, \delta, K_4, C_5, A_{5+5}, \underline{G}_1.}$

Classification and identification.

Class. *Dicoty1edonae*

1. Venation reticulate.

2. Flowers pentamerous.

Sub-Class. *Polypetalae*

1. Petals free.

Series. *Calyciflorae*

1. Thalamus cup-shaped.

2. Ovary inferior or semi- inferior.

Order. *Rosales*

1. Alternate stipUlate leaves.

2. Carpels one or more.

Family. *Caesalpiniaceae*

1. Flowers zygomorphic.

2. Corolla with ascending imbricate aestivation.

3. Gynoecium usually monocarpeUary and semi-inferior.

12. Asclepiadaeceae

Stem. Herbaceous, lower portions woody, aerial, erect, cylindrical, branched, solid, lower portions smooth, upper portions covered with woolly hairs, pale green, milky latex present.

Leaf. Cauline and ramal, acute, hairy, woolly, unicostate reticulate, hermaphrodite, pentamerous,

hypogynous and cyclic.

Inflorosence: Polychasial cyme.

Flower. Bracteate, bracteolate, pedicellate, complete, actinomorphic, hermaphrodite, pentamerous,

hypogynous and cyclic.

Calyx. Sepals 5, polysepalous, quincuncial.

Corolla. Petals 5, gamopetalous, twisted, coloured.

Androecium. Stamens 5, united with the stigma to form gynostegium, each stamen is represented by

two pollinia with their retinaculae. The'pollinia of the adjacent anthers are joined by their retinaculae

to corpusculum in a groove, to form a unit known as translator. A coronary outgrowth is present at

the back of each stamen.

Gynoecium. Bicarpellary, ovaries free but upper portion of style and stigma are fused, superior, placentation marginal, ovules many per locule, stigmatic head pentagonal.

Fruit. Etaerio of follicle.

Floral formula: $\oplus, \varphi, K_5, C_{(5)}, \widehat{A_{(5)}}, \underline{\widehat{G}}_{2}$.

Classification and identification

Class. Dicotyledonae

1. Venation reticulate.

2. Flowers pentamerous.

Sub-Class. Gamopetalo.e

1. 'Petals fused.

Series. Bicarpellllltae

1. Carpels two.

2. Ovary usually superior.

Order. Gentiana/es

1. Leaves opposite.

2. Flowers actinomorphic.

" 3. Stamens epipetalous.

Family. Asc/epiadaceae

1. Flowers solitary or in cymose umbels.

2. Petals usually convolute.

3. Stamens gynandrous, pollen usually in pollinia with translators.

4. Ovaries two, free, but united by the style.

13. Apocynaceae – *Hollorhen, Catharanthus.*

Stem. Herbaceous, aerial, erect, angular, branched, solid, puberulous, purple-red, milky latex present.

Leaf. Cauline and ramal, opposite decussate, stipulate interpetiolar, simple, elliptic-obovate, entire, mucrollate, puberulous, unicostate reticulate, latex present.

Inflorescence. Axillary dichasial cyme or solitary axillary.

Flower. Ebracteate, pedicellate, complete, actinomorphic, hermaphrodite, pentamerous, hypogynous and cyclic.

Calyx. Sepals 5, polysepalous, valvate, persistent.

Corolla. Petals 5, gamopetalous, twisted, corolla hypocrateriform, purple.

Androecium. Stamens 5, polyandrous, inserted at the mouth of the corolla tube, epipetalous, dithecous, dorsifrxed , introrse.

Gynoecium. Bicarpellary, syncarpous, ovaries are free and superior, placentation marginal, style long, stigma drum- shaped and sticky. Two ligulate hypogynous nectaries are present one on the anterior side and the other on the posterior side of the ovary.

Fruit. Etaerio of follicles.

Flora formula. $\text{Ebr},\ \oplus,\ ♀,\ K_5,\ C_{(5)},\ A_5,\ \underline{G}_{(2)}$.

Classification and identification.

Class. *Dicotyledonae*

1. Venation reticulate.

2. Flowers pentamerous.

Sub-Class. *Gamopetalae*

1. Petals fused.

Bicarpellate

1. Carpels two

2. Ovary usually superior.

Order. *Gentianales*

1. Leaves opposite.

2. Flowers actinomorphic.

3. Stamens epipetalous.

Family. *Acocynaceae*

1. Inflorescence cymose.

2. Stamens not gynandrous.

3. Ovules one or two in each locule.

4. Ovaries two, free, but united by the style.

5.Latex present.

14. Rubiaceae (*Oldenlandia)*

Habit. Prostrate herb.

Root. Simple tap root.

Stem. Herbaceous, aerial, weak, trailing, prostrate, angular, branched, solid, puberulous, pinkish green.

Leaf. Cauline and' ramal, opposite decussate, I stipulate, stipule interpetiolar, simple, sessile,

elliptic-Ianceolate, entire, puberulous, unicostate reticulate.

Inforescence. Axillary dichasial cyme.

Flower. Bracteate, bracteolate, pedicellate, complete, actinomorphic, hermaphrodite, tetramerous, epigynous and cyclic.

Calyx. Sepals 4, gamosepalous, valvate, persistent.

Corolla. Petals 4, gamopetalous, valvate, funnel-shapeg, violet.

Androecium. Stamens 4, polyandrous, epipetalous, insetted at the mouth of the corolla, filaments short, anthers sagittate, dithecous, basifIxed, introrse.

Gynoecium. Bicarpellary, syncarpous, ovary inferior, bilocular, ovules many in each locule on T-shaped placenta, placentation axile, style short, stigma simple and capitate.

Fruit-Berry.

Floral formula $\mathrm{Br,\ brl,\ \oplus,\ \delta,\ K_{(4)},\ \overbrace{C_{(4)};\ A_4}^{},\ \underline{G}_{(2)}}$.

Classification and identification

Class. *Dicotyldonae*

1. Venation reticulate.

2. Flowers pentamerous.

Sub-Class. *Gamopetalae*

1. Petals fused.

Series.lnferae

1. Ovary inferior.

2. Stamens usually as many as corolla lobes.

Order. *Rubiaks*

1. Leaves opposite.

2. Stamens epipetalous.

3. Ovary 2-8 locular.

Family. *Rubiaceae*

1. Opposite decussate, entire leaves with interpetiolar stipules.

2. Flowers in cymes.

3. Gynoectium bicarpellary, syncarpous, inferior, each locule with 1-8 ovules.

4. Placentum T.shaped.

15. Liliaceae (*Asphodelus):*

Habit. Herb.

Root. Adventitious.

Stem. Reduced underground.

Leaf. Radical. arising in a rosette-like manner, acicular, acute, cylindrical, venation multicostate paralle1.

Inflorescence. Scapiferous racemose raceme, the scape is aerial, erect, cylindrical, branched, solid, smooth

and green.

Flower. Bracteate, bracts boat -shaped and keeled at the back, pedicellate, complete, actinomorphic,

hermaphrodite, trimerous, hypogynous and cyclic.

Perianth. Tepals 6, present in two whorls of 3 each, polytepalous, valvate, petaloid, white, a prominent

brownish ridge is present in the centre of each tepal.

Androecium. Stamens 6, in two whorls of 3 each, polyandrous, epitepalous, ftlaments of outer whorls are

longer and that of ianer whorl short, ditbecous, versatile, introrse.

Gynoecium. Tricarpellary, syncarpous, ovary superior, trilocular with two rows of ovules in each locule,

palcentation axile, style slender and stigma bilobed.

Fruit. Capsule.

Floral formula. $Br, \oplus, \delta, P_{3+3}, A_{3+3}, \underline{G}_{(3)}$.

Classification and identification.

Class. *Monocotyledonae*

1. Venation paralle1.

2. Flowers trimerous.

Series. *Coronarieae*

1. Inner perianth petaloid.

2. Ovary superior.

Family. *Liliaceae*

1. Inflorescence usually scapiferous racemose type.

2. Perianth in two whorls and petaloid.

3. Stamens also in two whorls and epiptepalous.

4. Gynoecium 2-5 locular and placentation axile.

16. Poaceae (*Triticum vulgare*)

Habit. Herb.

Root. Adventitious, fibrous.

Stem. Herbaceous, aerial, erect, cylindrical, branched, branching is only at the basal region of the stem and is

known as tillering, culm, smooth and green.

Leaf. Alternate, exstipulate, simple, sessile, leaf distinguished into a linear leaf blade and a leaf sheath, and

at the junction of these two a small membranous ligule is present, lamina lanceolate, entire, acute,

minutely hairy, multicostate parallel.

Inflorescence. Spike of spikelets. Each spikelet consists of the following parts-

(1) A pair of glumes present at the base; outer one is called the first glume and the inner one as second

glume. These glumes are barren.

(2) After glume, is present lemma or inferior palea.

(3) There is present superior palea or pale. The essential organs of flower lie between superior palea or

lemma and inferior palea or pale.

Flower. Sessile, complete, zygomorphic, hermaphrodite, hypogynous and cyclic.

Perianth. Represented by two rudimentary free tepals known as lodicules.

Androecium. Stamens 3, polyandrous, filaments long, dithecous, versatile and introrse.

Gynoecium. Monocarpellary, ovary superior, unilocular, with one marginal ovule, style absent, stigma 2 and

feathery.

Fruit. Caryopsis.

Floral formula. . Br, ⦶, ⚥, P_2, A_3, $\underline{G}_1$.

Classification and identification.

Class. *Monocotyledonae*

1. Ventation parallel.

2. Flowers trimerous.

Series. *Glumaceae*

1. Flowers solitary, sessile in the axil of bract.

2. Perianth of scales or none.

3. Ovary usually unilocular and one ovuled.

Family. *Poaceae*

1. Joined stems with alternate 2 ranked leaves with split sheath and ligule.

2. Inflorescence spilelet and each begins with one or two empty glumes then palea with axillary flowers.

3. Stamens usually three.

4. Gynoecium superior with one ovule.

5. Fruit caryopsis.